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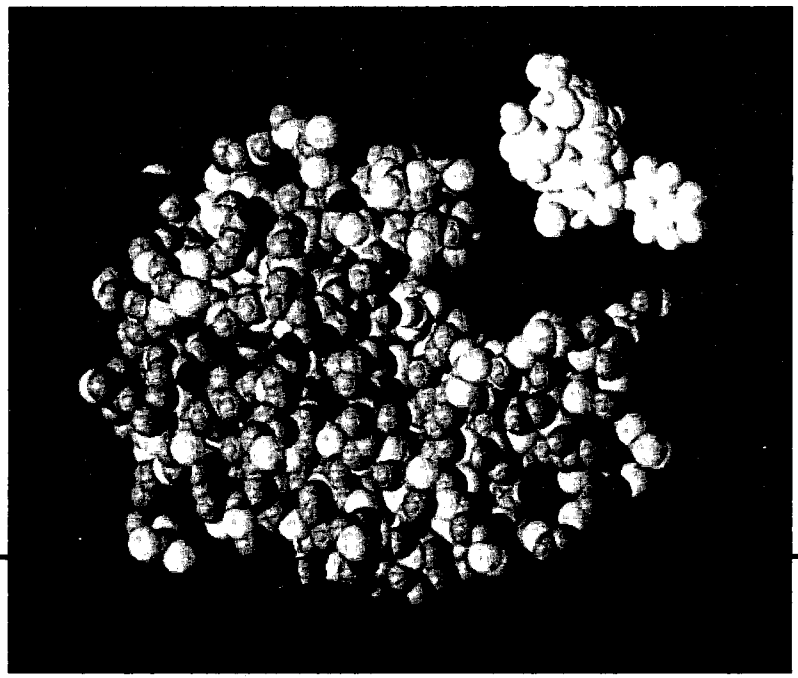
Center for Advanced Materials

CAM ANNUAL REPORT

1989

Polymers and Composites

Anisotropic Polymeric Materials • Polymer-Surface Interactions
Enzymatic Synthesis of Materials



Center for Advanced Materials
Materials and Chemical Sciences Division
Lawrence Berkeley Laboratory • University of California

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Center for Advanced Materials

Annual Report • 1989

POLYMERS AND COMPOSITES

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Cover and page 3: The enzyme lysozyme binds its substrate and then synthesizes its product. (XBC 890-9744)

Enzymatic Synthesis of Materials
Anisotropic Polymeric Materials
Polymer-Surface Interactions

Polymers and Composites Program

The CAM Polymers and Composites Program is concerned with the prediction and control of structure development in high-performance polymers and the enzymatic synthesis of novel polymeric and related types of materials. The program focuses on three areas.

ENZYMATIC SYNTHESIS OF MATERIALS—Enzymes catalyze reactions in a manner that makes them ideally suited to the synthesis of materials. They produce highly uniform and regular products at low temperatures and pressures and leave no side products that might be toxic or wasteful. The CAM program on enzymatic synthesis of materials seeks to explore the application of recently developed techniques in molecular biology, biochemistry and bioorganic chemistry to the synthesis of materials and to understand and exploit the expected enhanced properties of these materials. Research is focused on using enzymes as they are found in nature, on engineering enzymes through genetic and chemical means, and on creating new enzymes—all for the purpose of allowing them to catalyze reactions with non-biological substrates and to produce materials with interesting and important properties. Target products include modified polyamides and polysaccharides, non-biological materials including those polymerized through carbon-carbon linkages, and metal or semiconductor surfaces modified for surface properties or for applications in devices such as sensors.

Significant highlights of the past year include:

- Synthesis of novel polymers through chemical polymerization of enzymatically modified monomers.
- Enzymatic synthesis and polymerization of fluorinated and thiolated carbohydrate monomers.
- Engineering of an enzyme for further enhanced thermal stability.
- Engineering of an enzyme for alteration of its substrate specificity.

ANISOTROPIC POLYMERIC MATERIALS are characterized by a "domain" structure in the melt— micron-scale regions of high local order, interconnected by submicron regions of rapid change in local orientation. The orientation and flow of the molecules in a given domain have a major effect on the macroscopic properties of the polymer product. During the past year:

- Rheological and NMR spectroscopy experiments established that the flow properties of liquid crystalline polymers are dominated by a three phase microstructure: the nematic liquid; small, high-melting crystallites of homopolymer; and a temperature-dependent non-periodic crystal phase with the stoichiometry of the bulk polymer.
- Differential scanning calorimetry experiments on processed, filled liquid crystalline polymer indicated that compounding at temperatures below the normal melt processing temperature induces structural changes that can be annealed out at processing temperatures.

POLYMER-SURFACE INTERACTIONS play a significant role in the development of bulk structure during processing and of the mechanical properties of the manufactured object, failure and fatigue of composite materials, the properties of thin films for microelectronics and food packaging applications, and in performance areas such as tribology and biomedical applications such as implant wear. During the past year:

- A Molecular Mechanics simulation of thin films of a glassy polymer sandwiched between smooth solid surfaces was completed.
- Density functional theory calculations were used to study details of interfacial bonding at the interfaces of oxygen- and nitrogen-containing oligomers with aluminum.
- The theory of adhesive failure between non-crystalline polymers and a non-polymeric surface was used as a framework for the analysis of processing instabilities in melts at high stress levels.

ENZYMATIC SYNTHESIS OF MATERIALS*

The concept of using purified enzymes in the synthesis of materials is a relatively new one. Its validity rests on the demonstration that recent advances in molecular biology and biochemisty allow the manipulation of the substrate and reaction specificity of enzymes. Considerable research remains to be done, however, before these advances can be routinely applied to the synthesis of useful materials. In support of this goal, the CAM program has focused on:

- understanding the fundamental properties of enzymes and enzyme-catalyzed reactions that could be involved in the synthesis of materials.
- engineering enzyme structure, activity and reaction conditions to allow the synthesis of materials from novel substrates.
- determining the structure/function relationships of enzyme-synthesized materials, predicting properties from structure, and designing structures to achieve target properties.

ENZYMATIC SYNTHESIS OF POLYSACCHARIDES

M. Bednarski, H. Blanch, D. Clark, J. F. Kirsch, M. Corey, S. Edwards, C. Hobbs, A. Michiels, L. Oehler, A. Wong

Polysaccharides perform a wide range of functions and there is therefore a great deal of interest in the properties of unusual derivatives that might be produced enzymatically. Fluorinated polysaccharides may, for example, find commercial use as lubricants, adhesives or plastic additives. One of the most important obstacles to be overcome in using enzymes in the synthesis of polysaccharides lies in the fact that in many cases, degradative hydrolysis rather than synthesis is thermodynamically favored. Phosphorylases are enzymes that catalyze the reversible cleavage of the polymer glycogen to form glucose-1-phospate (Figure 1) (MB, LO). In this case, synthesis of polymer can be driven through the coupling of this reaction with others that involve the consumption of the phosphate formed as a byproduct. In an attempt to force the enzyme to make novel polymers using nonnatural monomers, a variety of glucose derivatives were synthesized, including those with fluorines in the 2, 3 and 6 position, thiols in the 3 and 5 position, azide in the 6 and amines in the 2, 3 and 6 position. To date, fluorinated (6 position) and thiolated derivatives (3 and 5 position) have been shown to be substrates for the enzyme and likely to

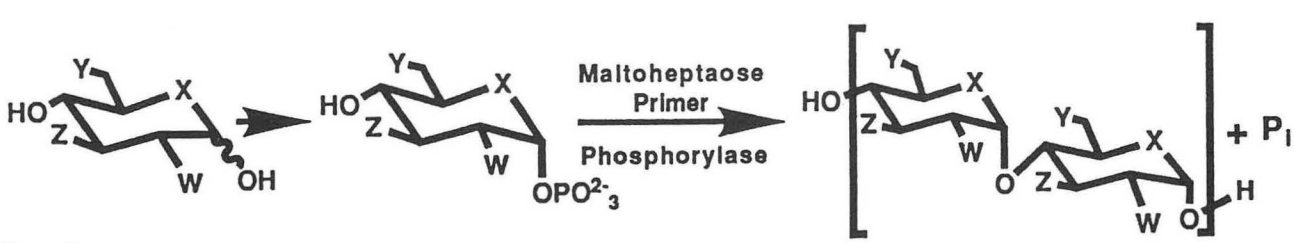

Figure 1
The enzyme phosphorylase catalyzes the polymerization of phosphorylated glucose monomers to make the polysaccharide glycogen. Inorganic phosphate is released in the process and removed enzymatically to pull the reaction towards synthesis. (XBL 901-262)

* Additional support from the Division of Energy Biosciences, U.S. Department of Energy.

POSITION	SUBSTITUTION: NH_3	F	S(H)	N_3
W	✔	✔		
X			✔✔	
Y	✔	✔✔		✔
Z	✔	✔	✔✔	

Table 1
Glucose phosphate monomers have been synthesized with the indicated substitutions (✔). Positions W, Y, and Z, as shown in figure 1, are normally OH, X is O in the ring form, OH in the straight chain form. Monomers with substitutions (✔✔) were shown to be active substrates of the enzyme phosphorylase, with actual polymerization demonstrated for 6-flouro derivative.

be polymerizable into unnatural polysaccharides (Table 1). The polymer with flourine in the 6 position has been made. Scale-up of these syntheses is progressing as is the modeling of the fit of these substrates in the active site to determine how controlled alterations of the enzyme active site through mutagenesis might allow it to polymerize others.

Another solution pursued for the problem of synthesis rather than degradation involves the modification of the enzyme so that its solubility in organic solvents is increased. Reactions run in those solvents, which, of course, have a significantly reduced concentration of water, would have their equilibrium shifted substantially to the synthetic direction. In one such study, the enzyme lysozyme was modified by treating it with cyanuric chloride-activated polyethylene glycol (JFK, SE). The modified protein was shown to be as active as native lysozyme in aqueous solution. Its activity in organic solvents is now being determined.

A third solution is being pursued through mutagenesis of the enzyme and custom design of a new substrate (JFK, MB, CH, MC). Based upon the known crystal structure and mechanism of action of lysozyme, substitutions of individual active site amino acids were designed to prevent a key enzyme-polymer interaction that is necessary for activity. New oligomer substrates were designed so that the the mutant enzyme could react with them to allow synthesis. The product polymer would be the same as that made with "normal" oligomers and thus resistant to degradation. Construction of the mutant enzyme has been achieved. One substrate has been synthesized and synthesis of the others are in progress.

Another enzyme whose catalytic ability has been explored for polymer synthesis is dextransucrase (HB, DC, AW). This enzyme is different from the others studied in that it naturally acts in the synthetic mode converting sucrose into $\alpha(1\rightarrow6)$ "polyglucose" and fructose. Since glucose-1-fluoride is a known substrate of dextransucrase, a number of derivatives were synthesized (MB, LO, AM) and assayed (HB, DC, AM, AW) as substrates, including the 2- and 3-fluoro, and the 3- and 5-thio-. Surprisingly none led to polymer synthesis. All the fluorides were, however, found to be competitive inhibitors, suggesting that although they could bind to the enzyme, their added fluorines interfered with the polymerization reaction.

The fluoronated derivatives were also tested as substrates for the enzymes cyclodextrin glucosyl transferase (CGT) and α-amylase. Although both enzymes have transfer activity with underivatized glucose polymers, neither exhibited transferase activity with the derivatized glucose substrates.

These results, combined with the work of others on glycosidases, polymerases and transferases, suggest that these enzymes are far more specific in binding their substrates than the degradative enzymes such as phosphorylase. Site-directed mutagenesis should, however, allow modification of their active sites, permitting them to accept new substrates. Since these enzymes are, by their nature, synthetic enzymes, they might be easier to use than those whose reactions favor degradation.

ENZYMATIC SYNTHESIS OF POLYAMIDES

J. F. Kirsch, C. H. Wong, J. A. Bibbs, J. Petithory

Polypeptides with well defined secondary structure have potential for the development of novel materials. Structures such as helices, sheets, and turns exist in many proteins and are involved in the formation of higher-order protein structures and biological activities. Polypeptides which mimic these regular structures may thus have properties useful for a number of applications. Incorporation of functional groups into such regular polypeptides will create a new class of molecules which could find use in materials science, biology, and chemistry.

Chemical synthesis of polypeptides has traditionally been dependent on the anhydride method using phosgene. The need for the milder, stereoselective, racemization-free, catalytic procedure achievable through the use of enzymes is timely and important. Work in this area is currently focused in two areas: the cloning and expression of one such enzyme, papain; and the modification of another such enzyme, subtilisin, and of its reaction conditions.

The extended amino acid recognition site of papain should facilitate its use in the synthesis of rationally designed polymers. Considerable success has been achieved over the last year in that the DNA coding region for this protein has been successfully cloned from the genome of the papaya plant and its nucleotide sequence has been confirmed (JFK, JP). The last remaining hurdle is to express this cloned gene (i.e., to synthesize the protein in an easily manipulated host organism such as E. coli).

Monomers	Degree of Polymerization	Yield (%)
1. *Single Amino Acids*		
Met-OMe·HCl	40	10 -15
Homo-Phe-OEt·HCl	N.D.	N.D.
Gly-OEt·HCl	20	3
2. *Dipeptides*		
Gly-Gly-OEt·HCl	9.5	20
Glu(Bzl)-Glu(Bzl)-OBzl·TFA	ND*	2
Leu-Met-OMe·TFA	ND*	10
Ala-Met-OET·TFA	11	15
Phe-Met-OMe-TFA	3	10
Ala-Tyr-OBzl·TFA	ND*	ND
3. *Tripeptides*		
Ala-Tyr-Met-OEt·TFA	ND*	10
Ala-Val-Phe-OMe·TFA	8	22

ND*: Insoluble precipitate is formed. The degree of polymerization was not determined due to the insolubility of the product.

Table 2
Synthesis of polyamides using the enzyme subtilisin in a mixture of 50% dimethylformamide/water. Substrates are amino acid esters or the esters of di- or tri-peptides.

Enzymatic procedures using proteases have been developed for the synthesis of small peptides. Again, however, proteases tend to cleave peptide (amide) bonds once they are formed. Work in this project (JAB, CHW)* is focused on two strategies to overcome this problem, both using the enzyme subtilisin.

The first method uses water-miscible organic solvents to selectively inhibit the amide cleavage activities of the enzyme yet to retain significant ester cleavage activity, which can form the basis for synthesis. The enzyme does catalyze the polymerization of several amino acid and peptide esters to make polyamides in 50% DMF/H_2O (Table 2).

The second method involves selective methylation of the active-site histidine residue of subtilisin to alter its activity. A new reagent, methyl (β-cinnamoyl) sulfonate, has been developed which is structurally similar to phenylalanine methyl ester, a natural substrate for subtilisin. This structural similarity allows the reagent to specifically bind to the enzyme active site where it transfers the reactive -CH_3 group to the histidine, which has been shown to play a major role in the catalytic process. The methylated enzyme is severely damaged with regard to its ability to break amide bonds, although it still binds to the normal substrates and has esterase activity, which should allow it to catalyze the condensation of amino acid or peptide esters to form polypeptides (Figure 2).

These results and those with phosphorylase in the synthesis of polysaccharides demonstrate that the concept of protein engineering to force normally degradative enzymes to become synthetic enzymes is a valid one and that this is a viable route to the synthesis of polymeric materials. Detailed knowledge of the structure and function of enzymes (for instance, of the reactive amino acids and the shape of the active site) can allow the rational design of reagents to alter the active site and thereby alter the functioning of the enzyme. Successful engineering of subtilisin, papain, phosphorylase and lysozyme will be followed by the controlled synthesis and the characterization of interesting polyamides and polysaccharides.

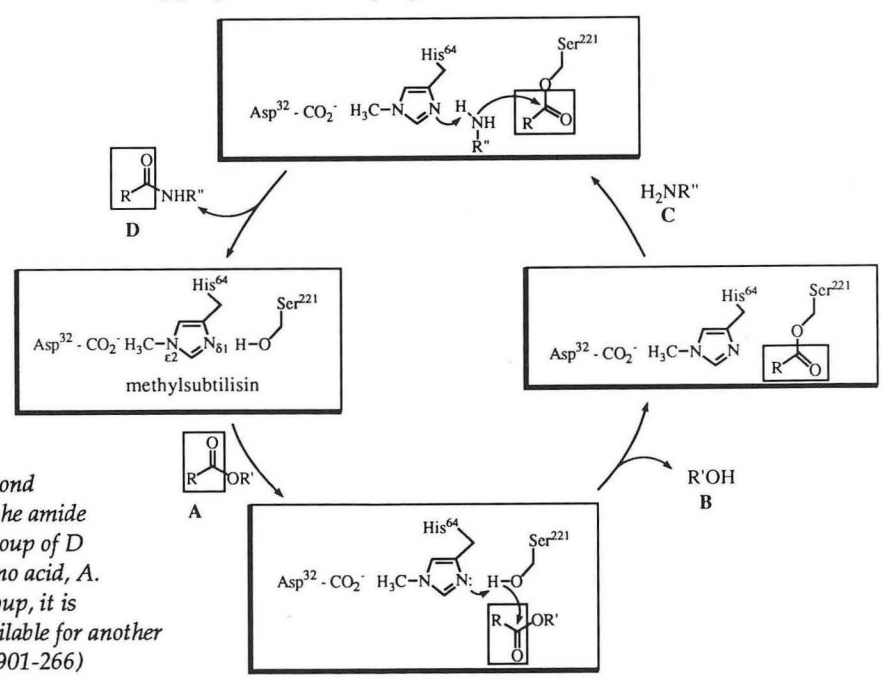

Figure 2
The enzyme subtilisin contains three amino acids, aspartate (asp), histidine (his) and serine (ser), in its active site that are directly involved in the making or breaking of amide bonds. When the active site histidine is methylated, as shown here, conditions can be found to limit amide bond degradation yet allow synthesis. Starting at left and proceeding counterclockwise, the serine of the enzyme binds an amino acid ester, A, splitting off the alcohol group B. A second amino acid ester is brought in, C, and the amide bond is made, D, between the amino group of D and the carbonyl group of the first amino acid, A. Since the R" in D includes an ester group, it is structurally analogous to B, and is available for another round of amide bond synthesis. (XBL 901-266)

*Research in this CAM Project is performed at the Research Institute of Scripps Clinic (RISC), La Jolla, CA, where C.H. Wong is a professor in the Department of Chemistry. J.A. Bibbs is a CAM postdoctoral fellow working with Wong at RISC.

ENZYMATIC SYNTHESIS OF "NONBIOLOGICAL" POLYMERS

M. BEDNARSKI, H. BLANCH, M. CALLSTROM*, D. CLARK, P. SCHULTZ, A. BRAISTED, A. WONG

Traditional views of materials synthesized enzymatically restrict the products to polyamides, polysaccharides, polyesters, polynucleotides and the like. Two projects in the program are focused on expanding the applicability of enzymes to the synthesis of polymeric materials that are not "biological" in nature. The goal here is the use of enzyme to make "classical" polymers with properties that are enhanced by the nature of the control of structure or the low temperature synthesis achieved only through the use of enzymes.

Enzymatic Synthesis of Monomers with Chemical Polymerization

M. BEDNARSKI, M. CALLSTROM*, H. BLANCH, D. CLARK, A. WONG

One approach involves the "coating" of chemically polymerized materials with enzymatically synthesized derivatives that would have significant influence on the properties of the material as a whole. The scheme involves the enzymatic synthesis of surface active groups attached to a polymerizable monomer. A focus of effort in this area has been the use of the enzyme hexokinase in the synthesis of phosphorylated polymers (MB, MC) (Figure 3)†. The primary physiological role of hexokinase is to catalyze the phosphorylation of glucose to give glucose-1-phosphate. This enzyme, however, has been shown to accept a variety of other substrates including aminosugars. It has been used here to synthesize derivatives of glucosamine-1-phosphate. An acrylamide group is then chemically attached to the phosphorylated glucosamine and the compound polymerized using ammonium persulfate as a catalyst. Using this methodology a variety of unnatural carbohydrate-based polymers have been synthesized. Preliminary characterization suggests that these polyanionic materials exhibit interesting rheological properties and are very soluble in water.

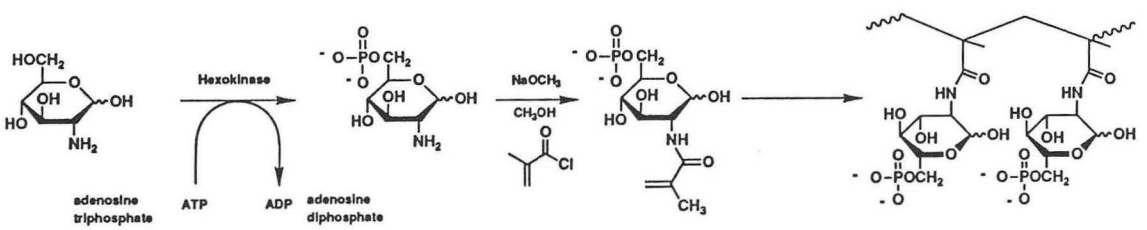

Figure 3
The enzyme hexokinase can specifically attach a phosphate group to the 6-position of the glucose ring. Chemical or enzymatic atttachment of methacrylate or acrylamide produces a polymerizable monomer with, in this case, polyanionic character. The degree of charge on the polymer can be controlled through the copolymerization of unphosphorylated monomers. Use of enzymes insures absolute control of the degree and position of the derivatization of the sugar residue. (XBL 901-263)

* Department of Chemistry, The Ohio State University.
† This work was supported through a contract with the Cargill Corporation.

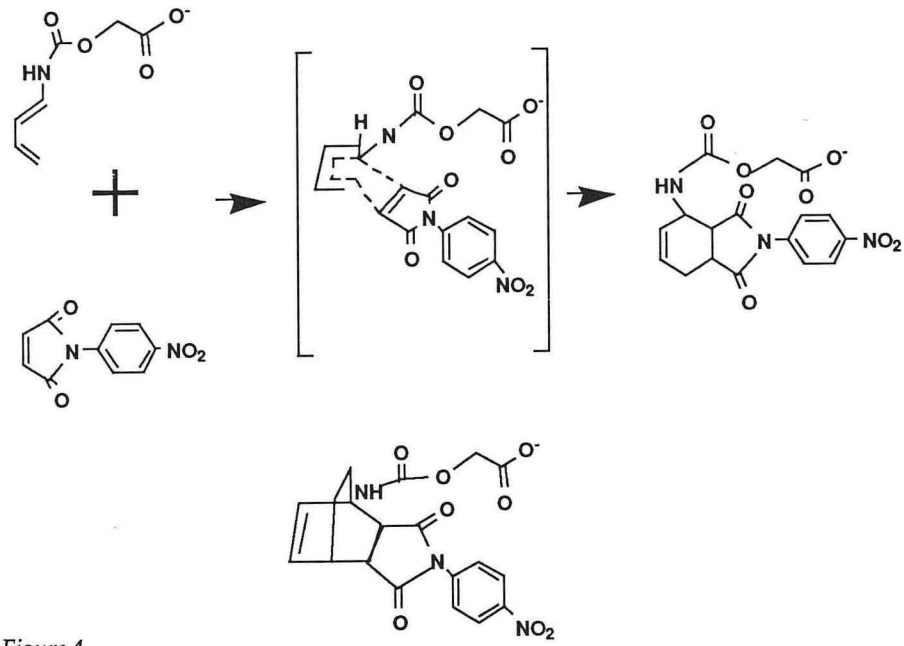

Figure 4
The Diels-Alder reaction involves the making of carbon-carbon bonds between a diene (above left) and a dienophile (below left). The reaction has been determined to proceed through the very short-lived transition state shown in the brackets. Synthesis of a stable transition-state analog (shown below the transition state) allows for the preparation of antibodies to the transition state These antibodies can act as catalysts for the reaction, thus producing "nonbiological" molecules through biological processes. (XBL 901-264)

A twist in this procedure involves the successful use of the transferase activity of glycosidases such as β-galactosidase to enzymatically link the carbohydrate residue to the polymerizable backbone, in this case hydroxy-ethyl methacrylate (HEMA) (HB, DC, AW). This material has applications, for example, as contact lenses, in part because it is a very low protein adsorbing biopolymer. Since this property would be enhanced by attachment of sugars, these modified poly-HEMA's might become an important class of biocompatible materials.

Catalytic Antibodies

P. SCHULTZ, A. BRAISTED

With the advent of technologies to produce monoclonal antibodies, it has become possible to generate proteins that selectively recognize virtually any given target substrate. Research described here last year demonstrated the ability of certain of these antibodies to catalyze reactions. Thus it now is reasonable to expect that catalytic antibodies can be created even for those reactions that involve substrates that are not "biological" in nature. These antibodies could then be used in an almost unlimited range of reactions leading to the synthesis of the full breadth of organic materials.

Work is progressing on the development of catalytic antibodies for polymer synthesis involving sequential Diels-Alder reactions for the making of carbon-carbon bonds. Chemical synthesis of analogs of the transition state for the linkage reaction has been achieved (Figure 4) and this material has been coupled to a carrier protein for antibody production. Purification of the first group of antibodies is in progress.[§]

[§] Note added in proof: Antibodies with this catalytic activity have been produced.

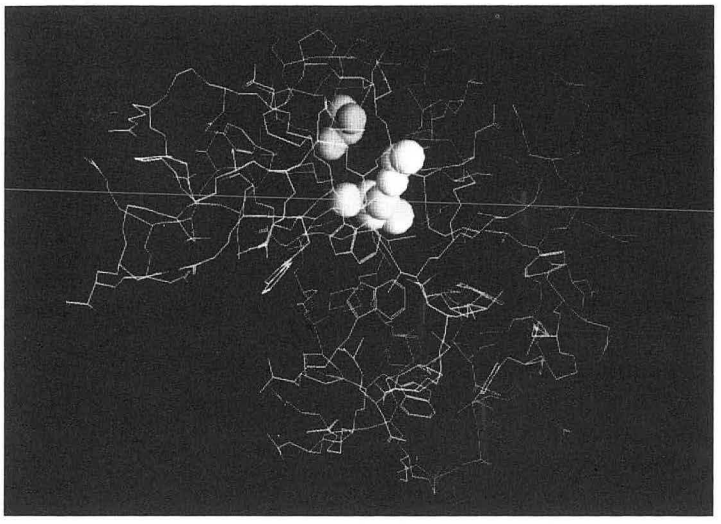

Figure 5
Three dimensional structure of the carbon chain backbone of the enzyme lysozyme shows the position of the active site cleft at top right. Three amino acids at the base of the active site, at positions 40, 55, and 91, are shown in space-filling form. They have been replaced by other, larger amino acids, leading to a 7°C increase in the thermal stability of the enzyme as measured by T_m. Mutations at positions 68, 101 have also made the enzyme more stable at higher temperatures, and mutants with all these changes are being made to examine the additivity of the effects. (BBC 8911-10248)

ENZYME ENGINEERING

Thermostability of Enzymes

J. F. Kirsch, B. Malcolm, P. Shih

Reaction rates approximately double with a 10°C increase in temperature. Thus, the stabilization of enzymes so that they can function effectively at elevated temperatures will be an important advantage in bioreactors. Site-directed mutagenesis technology coupled with x-ray crystallographic structure analysis offers the opportunity to deal with this problem by explicit protein design, even though much remains to be learned about the relationship between protein structure and thermostability.

As discussed here last year, a collaboration with evolutionary biochemist Allan Wilson at Berkeley led to the identification of a set of three amino acids at the base of the active site of the enzyme lysozyme (positions 40, 55, 91) (Figure 5) which, when replaced by other amino acids, led to a 7°C increase in the thermostability of the enzyme. This stabilization corresponds to an almost 3-fold increase in enzyme lifetime at 37°C. During the past year, the x-ray structural analysis of the wild-type and mutant proteins has been completed in collaboration with Brian Matthews and his student Keith Wilson at the University of Oregon. Their work has demonstrated that the large changes in thermostability induced by the genetic engineering of these modest perturbations in structure, i.e., the addition of a single methylene ($-CH_2$) group, can be clearly attributed to hydrophobic packing. The simple conclusion is that increases in hydrophobic packing brought about without concomitant introduction of strain in the rest of the molecule can lead to greater thermostability. This finding has far reaching implications for the design of enzymes for use in reactors.

In view of this, further investigations have been undertaken in order to define rigorously the conditions leading to enhanced thermostability of proteins. By substituting phenylalanine for the naturally occurring isoleucine at position 55, a large increase in the mass of this position was introduced. The x-ray structure suggested that such a large change cannot be accommodated without seriously perturbing the structure. Indeed, a significantly lower melting point was found (67°C vs 74°C) for wild-type enzyme. Substitution of alanine for serine in position 91, however, removes the β-hydroxyl group, but increases the hydrophobicity of the amino acid, giving an increase in stability from 74° to 76°C. This raises

further possibilities for tinkering for greater thermostability, i.e., by the replacement of hydrophilic with hydrophobic amino acids of smaller size. Also in accord with prediction, is the observation that substitution of alanine, a smaller hydrophobic amino acid, for isoleucine, a larger one in position 55 reduces the T_m from 74° to 70°.

Further, almost every substitution introduced for aspartic acid at the 101 position has yielded an enzyme of greater thermostability than wild-type. It thus appears that the wild-type enzyme is selectively destabilized at position 101. Since this residue is involved in substrate binding, this destabilization might have evolved for reasons having to do with efficient catalysis. The mutants, however, are all active. Yet another locus for mutations giving enhanced thermostability has been tentatively identified at position 68. Experiments are now underway to see if mutations at remote positions are additive.

Engineering of the Substrate Specificity of Enzymes

J. F. Kirsch, P. Cioni

Enzymes of living organisms evolved to bind and catalyze reactions with naturally occurring substrates of use to those organisms. They cannot, then, usually bind nonnatural substrates that might react to produce interesting materials. Synthesis of catalytic antibodies is one route to an enzyme for these substrates. Another route is synthesis of specifically engineered enzymes. To study whether this rational redesign is possible, a model system, involving the enzyme aspartate amino transferase, has been investigated. Earlier success, reported here last year, on converting this enzyme (through site-directed mutagenesis) from one that preferentially catalyzes the transamination of negatively charged substrate amino acids to one that transaminates a positively charged substrate (i.e., the complete reversal of substrate charge specificity) encouraged efforts to attempt to find second generation mutants where the catalysis of the new substrate was enhanced further (Figure 6). Crystallographic investigations by collaborators at MIT (Dagmar Ringe) implicated asparagine 142 as a possible target for further mutagenesis studies because it had the potential to hydrogen bond with asp 192 and, therefore, weaken the density of charge around the carboxyl group of that substrate-binding aspartate. Accordingly, asparagine 142 was mutated to alanine. Catalytic

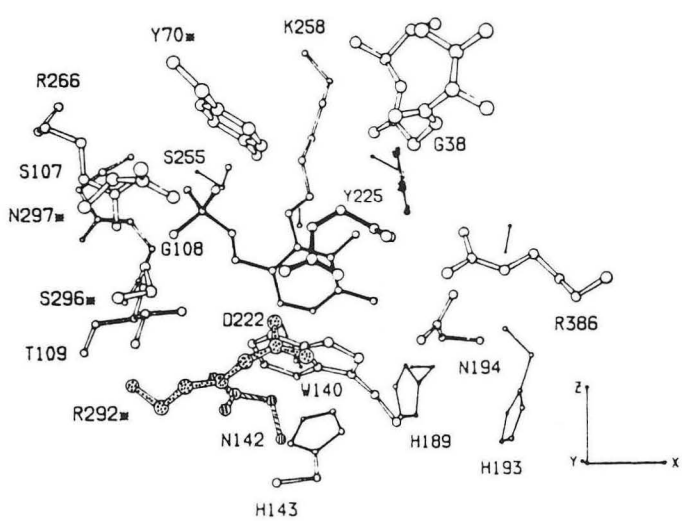

Figure 6
Structure of the active site of the enzyme aspartate aminotransferase. The figure shows 20 amino acids that make contact with the substrate or with another amino acid that makes contact with the substrate. Selective substitutions of these amino acids should produce a new enzyme that can catalyze reactions with a new selected substrate. A substrate analog is shown with filled circles. Substitution of arginine by aspartate in position 292 (stippled) gave a 10^5 fold reduction in activity on the original substrate and a 15 fold increase in activity on the new one. Substitution of asparagine 142 by alanine (partially shown, hatched) increased the activity on the new substrate to 50 fold over that of the original enzyme. Additional substitutions should further enhance the desired selectivity. (XBL 888-3008)

activity was enhanced an additional three-fold giving a total of 50 fold increase with the two mutations. This second success in amino acid substitution for a defined purpose is encouraging. Eleven amino acids make some contact with the substrate, and many more contact those residues and can affect their action. In continuing work more mutations surrounding the active site of the enzyme are being made to enhance activity on the new substrate. This is one of the few successful model systems in which a significant change in substrate specificity has been achieved, and it augurs well for the the long range goal of this work: the achievement of sufficient understanding of protein structure so that site directed mutagenesis can be used in such a way that substrate specificity can be treated as an easily maneuverable experimental parameter.

Semi-Synthetic Enzymes

P. SCHULTZ, C. NOREN

Site-directed mutagenesis, although promising in its potential to help redesign enzymes, is restricted to the use of the 20 naturally occurring amino acids, thus limiting the fine tuning of geometry and functionality. As reported last year, this problem has been overcome by the development of a technique that allows the site-specific substitution of virtually any alpha-amino acid that can be synthesized in the laboratory. Technical aspects of the process have been significantly improved during the past year. The first system developed used a chemically modified and acylated tRNA to carry the unnatural amino acid. A far simpler technique for tRNA preparation has now been developed, involving the synthesis of a synthetic tRNA gene redesigned for this process, and its use in a transcription system to produce the tRNA. Further, the amino-acylation step has been improved through the direct synthesis of the terminal nucleotides of the tRNA linked to the unnatural amino acid. This group can then be enzymatically linked to the rest of the tRNA. This new tool clearly provides a vastly increased potential for specific modification of enzymes to tailor their activities for desired reactions. It is now being pursued with enzymes of interest in materials synthesis, including T4 lysozyme.

CHEMICAL AND ENZYMATIC SYNTHESIS OF BIOELECTRONIC AND OPTICAL DEVICES

M. BEDNARSKI

As reported last year, methods have been developed to chemically and enzymatically modify silicon oxide surfaces. The goal in this area of research is to develop materials for new bioelectronic and optical devices to be used as molecular sensors. The combination of chemical and enzymatic methods has allowed derivatization of silicon chips with carbohydrate molecules with specifical binding properties, in this case, for certain pathogenic bacteria. The nonspecific adhesion of bacteria that might be similar to the target can be prevented by the use of specifically designed

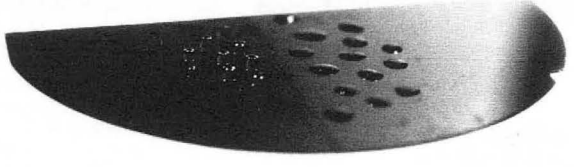

Figure 7
Attachment of hydrocarbon chains to a silicon wafer can change its surface properties from hydrophilic to hydrophobic. Untreated silicon surface (right) is wet by water. Water beads on treated surface. (XBB 8910-9344)

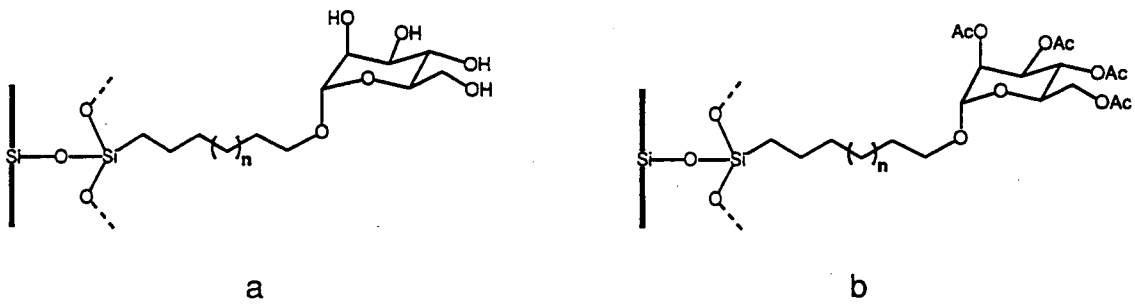

Figure 8
Enzymatic attachment of specific sugars to the end of the hydrocarbon chain can control the binding of selected agents, here bacterial cells, to the surface. Mannose residues act as specific receptors, allowing binding (a). Acetlyation of the mannose prevents binding (b). (XBL 901-267)

mixed monolayers. Hydrophobic groups, for example, prevent the non-specific adhesion of bacteria such as Salmonella. The altered character of the surface can be seen in the change of the shape of water droplets on the surface (Figure 7). Attachment of specific carbohydrate derivatives such as mannose to the ends of hydrophobic groups allows receptor mediated adhesion of the bacteria to occur (Figure 8a). Modification of the mannose can eliminate that binding (Figure 8b).

These coatings have been transferred on to a surface acoustic wave sensor device (Figure 9) to allow detection of the presence of the target structure through its binding to the synthetic surface. This device relies on the generation and modification of an acoustic wave by a piezoelectric material on a silicon nitride membrane. The binding of the receptor molecules to the device can cause a shift in the resonance frequency of the wave, leading to an assay of the binding event. The feasibility has been demonstrated using Salmonella and E. coli, and these devices are currently being refined for sensitivity and versatility. Further applications include the detection of small molecules. The process of chemical and enzymatic surface modification will also be used to synthesize polymers on the surface of the custom designed electro-optical materials.

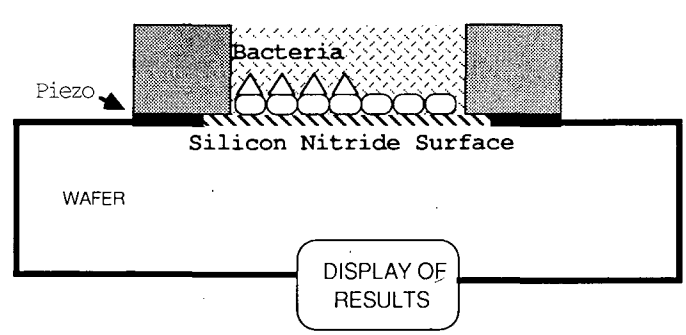

Figure 9
Mounting of a self assembling hydrocarbon chain/sugar derivatized structure on a silicon nitride surface through which a standing acoustic wave has been generated can form the basis for a sensing device. Binding of bacterial cells to the receptors alters the from of the wave in a manner detectable by the attached electronics. (XBL 901–265)

ANISOTROPIC POLYMERIC MATERIALS

The processing of liquids which are anisotropic at rest is common in the manufacture of advanced polymeric materials. The anisotropy might be a consequence of the presence of fibers, as in fiber-filled composite materials, or of the molecular structure of a liquid crystalline polymer. Prediction and control of orientation development of anisotropic liquids during processing remains a major outstanding technical problem.

Liquid crystalline polymers contain rigid elements in the backbone which cause them to exist in a highly oriented ("mesophase") state in the melt. Melt-processible nematic liquid crystalline polymers are currently molded for use as interconnects in electronics applications; the low resistance to flow enables easy filling of small, complex shapes. The rigid polymer backbone, which causes the high degree of local order in the liquid state, is also the cause of other properties of these materials that are considered to be exploitable in high-performance applications: high melting point, high modulus and strength, small in-plane coefficient of thermal expansion, and excellent resistance to chemical solvents. The high degree of local order has stood in the way of the development of shaping processes (other than fiber formation, which is well-established commercially) which can exploit these other desirable properties, however, because it leads to poor transverse physical properties and poor adhesion (and self adhesion).

We have studied the properties of several melt-processible liquid crystalline polymers using rheology and nuclear magnetic resonance spectroscopy as complementary tools. We find that the unusual flow behavior of these materials, and the sensitivity to prior thermal history, are a consequence of the presence of as many as two crystal-like phases at normal processing temperatures: one phase consists of small crystallites of homopolymer, while the other is a non-periodic structure with a stoichiometry very close to that of the bulk melt. Possible transesterification reactions at compounding temperatures may account for some of the unusual properties of filled materials. During the past year we have initiated work on solution-processed (lyotropic) liquid crystalline polymers.

As part of our continuing work on the simulation of structure development in anisotropic liquids, we have extended our computational work on hyperbolic methods to include the theory of fiber suspensions. We have simulated some recent experiments reported in the literature on the flow of fiber suspensions with reasonable success.

THERMOTROPIC LIQUID CRYSTALLINE POLYMERS

J.A. Reimer, M.M. Denn, K. Amundson, D. Giles, D. Kalika

As described last year, we have obtained complementary data using three rheometers and high-temperature NMR on the wholly nematic copolymer of 80 mol % p-hydroxybenzoic acid and 20 mol % poly(ethylene terephthalate) manufactured by Tennessee Eastman. The viscosity data are inconsistent with any available theory for anisotropic liquids. The NMR data indicate the presence of a small fraction of crystallites of poly(hydroxybenzoic acid), which will have a melting point that is higher than the normal processing temperatures. The NMR data at melt temperatures also indicate a slowly decaying component of the proton spin relaxation spectrum with a stoichiometry that is very close to that of the bulk polymer. We have now identified this structure, which comprises a fraction greater than 10%, as likely to be "non-periodic crystals" as

discussed by Windle and co-workers. This crystal-like structure is undoubtedly associated with the thermal history-dependent transients observed in rheological experiments; the structure induced during these transients can be melted out reversibly by brief excursions to higher temperatures.

The picture that emerges has considerable significance for processing, since the multiphase nature of the system clearly needs to be taken into account in any analysis. The small crystallites undoubtedly act as tie elements to produce a structure whose rheological properties are reminiscent of those commonly associated with suspensions of submicron particles like carbon black, rather than the properties normally associated with classical nematic liquids.

FILLED LIQUID CRYSTALLINE POLYMERS

M.M. Denn, L. Nuel

Thermotropic (melt-processible) liquid crystalline polymers are often used with particulate fillers, in part because the fillers appear to improve the isotropy of properties in molded parts. We reported last year that the compounding in a twin-screw extruder appears to induce a gap-dependence of the viscosity that is much greater than that observed in the unprocessed polymer, and that the polymer shows an apparent viscosity decrease in a small capillary but not in a larger one upon addition of small amounts of carbon black.

We have carried out differential scanning calorimetry (DSC) experiments on filled and unfilled polymers, the latter with and without compounding. DSC traces at the compounding temperature show marked differences between compounded and uncompounded polymers, with the compounded polymer showing an apparent phase transition that does not exist in the uncompounded material. Annealing to processing temperatures for a sufficiently long time removes the second peak, however. It is likely that the difference between compounded and uncompounded material observed rheologically is affected by the residence time in the rheometer, and the thermal history associated with individual data points is again shown to be a relevant parameter. The changed DSC behavior of the compounded material may be associated with transesterification reactions that have been reported for these polymers.

FLOW SIMULATION OF FIBER-FILLED LIQUIDS

R. Keunings, M.M. Denn, J. Rosenberg

Some years ago we developed a procedure for simulating the flow of fiber-filled liquids in converging geometries at low fiber loadings.

Excellent agreement was obtained between our theoretical predictions and experiments on flow through a 4:1 contraction, where the presence of the fibers effected a major change in the flow structure; this work demonstrated that predictions of fiber orientation distributions based on flow fields ignoring the presence of the fiber could be grossly in error.

That work was limited, in that it required certain characteristics of the flow field to insure that an approximation roughly equivalent to assuming that the fiber orients with the flow could be utilized. We have extended our algorithms for hyperbolic viscoelastic constitutive equations, described last year, to include the theory of fiber suspensions in a Newtonian fluid, and we have simulated several flows. The simulations include a series of experiments carried out by a group at the Los Alamos and Sandia National Laboratories on falling-sphere rheometry in a tube containing

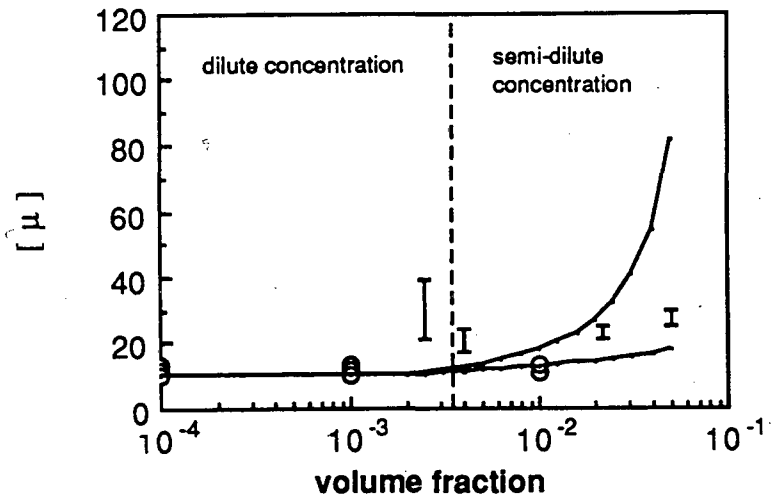

Figure 10
Intrinsic viscosity (i. e. , fractional contribution per unit volume of fibers to the suspension viscosity) as a function of volume fraction for a sphere falling in a tube filled with the suspension. The data, indicated by error bars, are from experiments by Milliken and co-workers for randomly distributed fibers. Experiments by the same group on fibers initially aligned with the tube axis show an intrinsic viscosity of about 10. The circles are computed points for random and aligned orientations. The two lines in the semi-dilute range reflect the fact that the theory is ambiguous here, with the value of crucial coefficients depending on the bulk orientation of the fibers. Overall, the theory captures the large intrinsic viscosity observed experimentally, but it does not agree quantitatively with some of the data. The difference is probably due to the fact that the spheres used in the experiments were comparable in size to the fiber length, while the theory assumes that the fibers are much smaller in all dimensions than the sphere. (XBL 901-119)

fiber suspensions. We found that in the case of this confined flow, the velocity field is approximately that of an unfilled system, although the stress distribution is quite different. The apparent viscosity of the suspension can be computed and expressed as an intrinsic viscosity; the intrinsic viscosity, when multiplied by the fiber volume fraction, gives the fractional correction to the suspension viscosity.

The computed and experimentally measured intrinsic viscosities are shown in Figure 10 for the case of a random initial distribution of fibers. The theory predicts that the intrinsic viscosity is insensitive to the initial fiber orientation distribution, but the experiments showed a large effect.

The difference is probably a consequence of the fact that the theory assumes that the fibers are small relative to the size of the sphere, while the experiments were carried out with spheres of a size comparable to the fibers. Theoretical calculations are in good agreement with an asymptotic solution of the model equations.

POLYMER/SURFACE INTERACTIONS

The physical or chemical interactions between polymer segments and a non-polymeric surface results in conformational changes of the polymer in the neighborhood of the interface, making it different from that in the bulk.

Interaction during melt processing between the melt and the metal shaping surface is known to affect adhesion and the onset of flow instabilities, and hence the throughput and bulk material properties. The properties of polymer composites are determined by the nature of the interaction between the matrix and the particulate or fiber filler; the interaction affects not only interface adhesion, but such properties as failure and fatigue as well. The interactions of thin polymer films with metal and metal oxide

interfaces are of importance in a variety of applications in the microelectronics industry; in polymer/metal interactions only certain functional groups of the organic polymer may interact chemically with the substrate. The interaction of a polymer with a non-polymeric surface is also of significance in other areas, including tribology and such biomedical applications as implant wear.

Because of the industrial significance of polymer/surface interactions, we initiated research in this area in 1987. We have made significant progress in identifying the apparent interfacial basis of certain processing instabilities. Our theoretical work has been successful in moving towards the goal of predicting macroscopic thermodynamic and mechanical behavior at interfaces from the chemical constitution of chains and solid surfaces. Experimental studies using nuclear magnetic resonance spectroscopy and attenuated total reflectance Fourier transform infrared spectroscopy are currently underway and should produce useful results during the coming year.

MOLECULAR MODELING

D. Theodorou, K. Mansfield

This work aims at the development of statistical-mechanics-based simulation methods for the prediction of polymer interfacial properties from chemical constitution. The motivation is to provide a foundation for the rational, "molecular engineering" design of multiphase materials containing polymers, such as composites, adhesives, and coatings.

Recently, we designed an efficient Molecular Mechanics simulation approach for glassy amorphous polymers. During this year we applied this approach to thin films of a glassy polymer (atactic polypropylene) sandwiched between smooth solid substrates (graphite basal planes). The film thickness was chosen large enough so that each half of the sandwich is representative of a single bulk polymer/solid interface. Our objective was to understand the organization of macromolecules in the interfacial region, and to test our ability to predict interfacial thermodynamic properties from first principles.

Our model "composite" system is represented as an ensemble of atomistically detailed microstates, each in mechanical equilibrium. To create a microstate, polymer chains are first grown between the confining

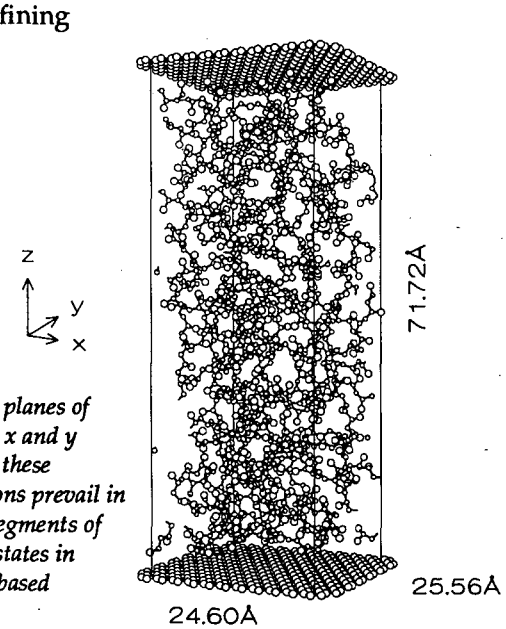

Figure 11
Model microstate of glassy atactic polypropylene sandwiched between two basal planes of graphite. The microstate is characterized by periodic boundary conditions in the x and y directions; it can thus be considered as part of a film which extends infinitely in these directions. The film thickness has been chosen large enough so that bulk conditions prevail in the middle, yet small enough to keep computations tractable. The box contains segments of seven polymer chains, each of molecular weight 3,214. Ensembles of such microstates in detailed mechanical equilibrium are useful in providing quantitative molecular-based information on the properties of composite interfaces. (XBL 901-114)

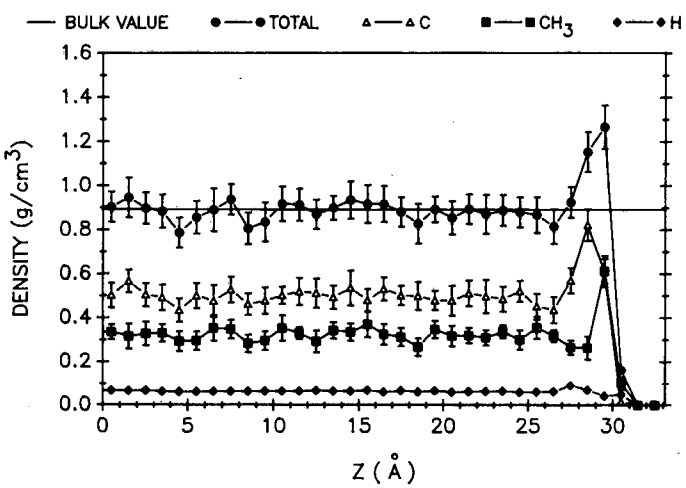

Figure 12
Local mass density distribution at a glassy polypropylene/graphite interface. The centers of carbon atoms of the graphite surface are located at z=33.1Å. The region at small z values is representative of unperturbed bulk polymer. The solid line indicates the experimentally observed macroscopic density at the simulation temperature (20°C below T_g). Individual contributions to the mass density profile from carbon, hydrogen, and methyl groups are also shown. The local density displays a strong maximum next to the highly attractive graphite surface. Chains adsorb on the surface through their pendant methyl and hydrogen groups. (XBL 8910-3660)

solid surfaces through a Monte Carlo procedure that resembles polymerization. The microstate is subsequently relaxed through an energy minimization technique on a Cray X-MP supercomputer. Potential parameters are based on recommendations of Flory and Steele, and are not adjusted in the simulation. A model microstate is illustrated in Figure 11.

From the microstates we have generated so far, we were able to obtain an estimate for the work of adhesion between glassy polypropylene and graphite which is within 4% of available experimental values. In addition, we have elucidated details of chain organization and conformation at the interface. Local segment density is enhanced, and bond orientation deviates from isotropy over a 10Å-thick region near the graphite. Chains adsorb on the graphite through their pendant methyl and hydrogen groups (Figure 12). There is a distinct tendency for adsorbed hydrogens to locate themselves preferentially over the centers of hexagons in the graphite surface honeycomb. The segment clouds of individual chains orient with their longest principal axes parallel to the solid substrate. Local structure assumes its bulk characteristics at distances larger than two radii of gyration from each graphite surface.

NEAR-SURFACE STRUCTURE OF POLYMER-METAL/ METAL OXIDE INTERFACES

A. Chakraborty, J.S. Shaffer

This work focuses on the use of quantum and statistical mechanical methods to study the details of interfacial bonding and chain conformations at the interfaces of oxygen- and nitrogen-containing polymers with aluminum, nickel, and their oxides. We have made significant progress in studying the segment-level interactions of polymethyl methacrylate (PMMA) oligomers and 4-4'-n-propylcyclohexyl cyanocyclohexane with aluminum surfaces. We use two models to represent the surface; one is the so-called jellium model, and the other is one wherein the surface is represented as a finite cluster of metal atoms.

Density functional theory has been our method of choice to study the interfacial interactions under consideration. We have calculated both the energetics and the changes in the electronic properties of the surface as the oligomers approach the metal surface. In our calculations with the jellium representation of the aluminum surface, we use linear response theory, and we construct the exchange-correlation energy functional by using the local density approximation and the Perdew-Zunger expression for the

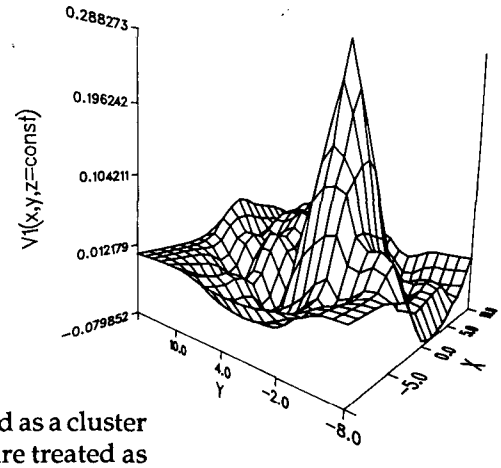

Figure 13
Spatially variant potential induced on a "jellium" representation of the aluminum surface due to a PMMA dimer unit approaching the surface (the closest carbonyl group is 4.0 bohr from the surface). The X-Y plane is the metal surface. The spatial variation is directly related to the electronic and stereochemical properties of the organic oligomer. This induced potential can be related to STM currents for adsorbed oligomers. Furthermore, calculating this potential is crucial for calculating the energetics of oligomer-metal surface interactions. Our calculated energetics using these induced potentials reflect the strength and specificity of chemical interactions at the interface. (XBL 901-115)

exchange-correlation energy. When the surface is represented as a cluster of metal atoms, the organic oligomer and the metal cluster are treated as one supermolecule and the full Kohn-Sham equations are solved. Pseudopotentials are used to represent the metal core electrons in the cluster calculations, and again, we use the local density approximation.

We find that the potential induced on the surface as a consequence of interacting with the organic oligomers is spatially variant (Figure 13). Furthermore, the spatial variation of the induced potential is directly related to differences in the electron density distribution around various functional groups of the organic molecule. This spatial variation of the induced potential has been related to recent scanning tunnelling microscope studies of adsorbed layers of 4-4'-n-propylcyclohexyl cyanocyclohexane by Spong and co-workers at IBM. We have also calculated the energy hypersurface of PMMA oligomers interacting with aluminum surfaces. The energy hypersurface calculated in this way reflects the strength and specificity of the interfacial interactions, and cannot be represented by a simple potential.

ADHESION AND MELT FRACTURE

D. Hill, T. Hasegawa, M.M. Denn

There is evidence that the onset of melt flow instabilities at high stress levels ("melt fracture") is affected by interactions between the polymer

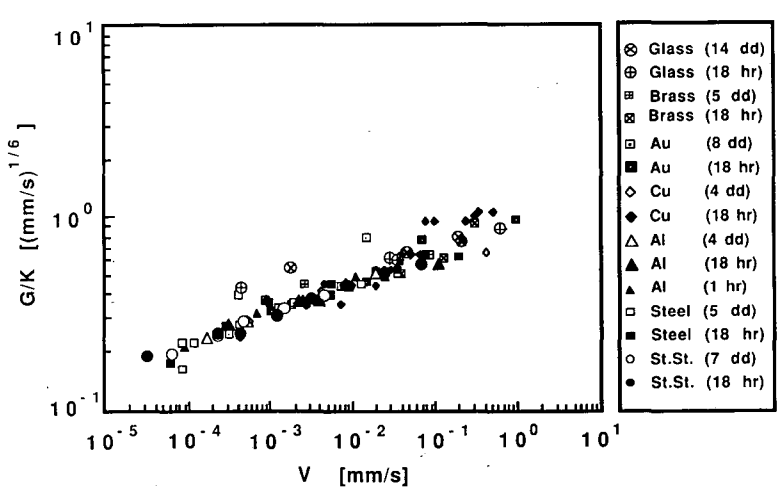

Figure 14
Normalized peeling force plotted versus the speed of propagation of the interface for cast films of linear low density polyethylene on a variety of substrates. The times in parentheses indicate the aging of the film between sample preparation and testing. The best fit through the data is a line with slope of 1/6 on logarithmic coordinates. (XBL 901-117)

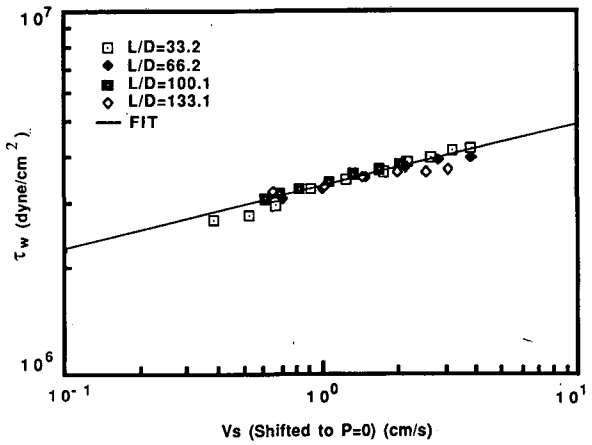

Figure 15
Wall stress versus inferred wall slip velocity data of Kalika and Denn for the same linear low density polyethylene used in the peel experiments, adjusted to atmospheric pressure using the pressure dependence derived from the bulk rheological properties. According to the adhesion theory the slope of these data should be the same as the data in Fig. 14, and this is the case. The theory predicts the magnitude of the data to within to about 40% without any adjustable parameters. (XBL 901-118)

and the metal surface of the extrusion die. We have used the theory of adhesive failure between non-crystalline polymers and a non-polymeric surface to provide a framework for the analysis of melt fracture. Data from adhesion "peel" experiments of cast films of linear low-density polyethylene on a variety of metal and metal-oxide substrates, which were mentioned in a previous report, were shown to superimpose on a master curve of strain energy release rate versus the velocity of the interface, as shown in Figure 14, with a slope on logarithmic coordinates of 1/6. The theory predicts that inferred slip velocities for melt flow of this polymer at high stress levels should show the same functional dependence between the wall stress and the slip velocity after adjustment for high-pressure free-volume effects. Data of Kalika and Denn are shown in Figure 15; the best-fit line through the data has a slope of 0.17, as predicted by the theory. The theory predicts the magnitude of the stress corresponding to a given slip velocity to within 40%.

The results of this study are by no means conclusive, but they do make a strong case for the fact that melt fracture is a consequence of an adhesive failure between the melt and the metal or oxide surface of the die. It is particularly striking that both the onset of extrudate surface distortions and the functional form and magnitude of the melt slip velocity can be obtained solely from peel tests on the polymer below the melt processing temperature and a knowledge of the temperature and pressure dependence of the bulk rheological properties.

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AWARDS

- Peter Schultz received the ACS Award in Pure Chemistry and the ACS Arthur C. Cope Scholar Award.

INDUSTRY INTERACTIONS

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Industrial Collaborations

- Dr. Patrick Gruber of the Cargill Corporation is working with the Enzymatic Synthesis of Materials project on the development of a new class of carbohydrate-based polymers derived for monosaccharides or oliosaccharides functionalized with polymerizable side groups.

- Dr. Steven Rosenberg of Chiron Corporation is continuing his work on cloning and gene expression with the Enzymatic Synthesis of Materials project.

- Dr. Hans Ribi, Biocircuits, Inc., is collaborating with the Enzymatic Synthesis of Materials project on the use of enzymes in the fabrication of sensors.

- Affymax Inc. is collaborating with the Enzymatic Synthesis of Materials group in the production of catalytic antibodies.

- Dr. A.E. Zachariades, IBM Almaden Research Center, worked with the Anisotropic Polymeric Materials project on the mechanisms by which structure develops in liquid crystalline polymers.

- Dr. Peter Pierini, Dow Chemical Company, is collaborating with the Anisotropic Polymeric Materials project on a study of structure development in lyotropic liquid crystalline polymers.

- Dr. Mohammud Zaidi, Alcoa, is collaborating with the Anisotropic Polymeric Materials Project on a study of the rheology (deformation characteristics) of liquid crystalline polymers at temperatures where the material can be deformed and shaped in the solid phase..

Gifts

- Gifts from Alcoa, E.I. du Pont de Nemours & Company, and Raychem supported general studies in the Anisotropic Polymeric Materials project and the Polymer-Surface Interactions project.

- Gifts from BP America, Cray, Exxon, Shell, and Union Carbide supported studies in the Polymer-Surface Interactions Project.

- A gift from Dow Chemical Company supported studies in the Anisotropic Polymeric Materials project.

Contracts

- The Cargill Corporation is working with the Enzymatic Synthesis of Materials project on the study of new carbohydrate-based polymers.

- IBM Almaden is working with the Polymer-Surface Interactions project on polymer-surface interactions.

Technology Transfer

- Biocircuits, Inc. is using techniques developed in the Enzymatic Synthesis group in the fabrication of sensing devices.

- Affymax, Inc. is using techniques developed by the Enzymatic Synthesis group in the production of catalytic antibodies.

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