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A Re-examination of the Zeolite-promoted, Clay-mediated Peptide Synthesis

The abiogenic synthesis of amino acids, nucleic acid bases, sugars, and nucleotides has been well documented in recent years.[1-5] However, attempts to produce long chain polypeptides and polynucleotides under simulated aqueous prebiotic conditions have generally been unsuccessful. Inspired by the suggestion of Bernal that aqueous prebiotic synthesis might be facilitated by absorption of reactants on clays,[6] Katchalsky and co-workers demonstrated that amino-acyl adenylates condense in the presence of the clay crystallite, montmorillonite, to yield polypeptides exhibiting a discontinuous range of molecular weights.[7] Although amino-acid adenylates undergo polymerization in basic aqueous media in the absence of montmorillonite,[7-10] the clay-mediated polycondensation results in a substantially larger yield and an increase in polymer lengths.[7]

A recent modification of this procedure by Paecht-Horowitz and Katchalsky[11] utilizes a synthetic ion-exchange zeolite, Decalso F, as the "catalyst" for the formation of amino-acyl adenylate from ATP and amino acids. From their studies on a simple aqueous mixture of Decalso F, montmorillonite, ATP, and alanine (pH 7.0, 37°C), the Israeli workers reported the disappearance of free alanine over a period of ∿ 70 h, with the concomitant formation of polymeric alanine. The polymer products were reported to be similar to those obtained, using pre-formed adenylate, from the montmorillonite-aided polymerization. However, as is reported below, we have been unable to substantiate the Paecht-Horowitz and Katchalsky report concerning the synthesis of either amino-acyl adenylates or peptides utilizing a zeolite catalyst.

Polymerization trials were conducted according to the prescription given recently.[11] Briefly, the reaction mixture typically contained 10^{-4} M DL-alanine (including 10 μCi DL-alanine-2-^{14}C; autoradiography of this labeled compound revealed no detectable radio-impurity), 5 x 10^{-2} M ATP, 500 mg/ℓ montmorillonite, and 100 g/ℓ Decalso. The polymerization mixture was permitted to incubate for 70-100 h at 37°C and the pH was maintained at 7.2 by a pH stat (Radiometer Co., Copenhagen). When alanyl adenylate (labeled with 10 μCi alanine-2-^{14}C) was utilized as the precursor for polymerization, the procedure of Paecht-Horowitz et al.[7] was adopted. Synthesis of DL-alanyl-2-^{14}C adenylate followed the method of Meister and Scott.[12]

After completion of the polycondensation trial, the clay suspension was subjected to a 2 h centrifugation (25,000 x g); the clay pellet was again washed and centrifuged, followed by ultrafiltration of the supernatant through a XM-50 filter (Amicon). Less than 0.1% of the alanine-containing material (radioactivity) was trapped by the filter. This procedure effectively removed small clay or zeolite particles. The resulting supernatant was lyophilized and resuspended into a minimal amount of distilled water (2-4 ml) and stored at -15°C until subsequent analysis for polymeric alanine.

The supernatants from the reaction mixtures were assayed for alanine and low molecular weight polypeptides by means of a Beckman amino acid analyzer. Generally, a sample of each reaction mixture was hydrolyzed and analyzed for free alanine (taken as the initial concentration). Subsequently, the supernatant from the polymerization trial was analyzed for alanine and the value obtained compared with the initial concentration.

Additionally, low polymers of alanine (n approx. 2-5) could be detected by the amino acid analyzer and eluted after the monomer. To detect polymers of alanine which were not detected by the amino acid analyzer, the supernatant was assayed for radioactivity by liquid scintillation counting. This procedure provided an independent check on the amino acid analysis.

Additional characterization of the polymerization samples was obtained by thin-layer chromatography on Macherey-Nagel, Polygram CEL 300 UV cellulose-coated plastic strips. Buffer systems employed were either phenol-water (5:1 w/w) or n-butanol-acetic acid-water (5:2:3 by vol). The chromatograms were sprayed with ninhydrin solution and were additionally analyzed for radioactive components by X-ray film autoradiography.

Aliquots (1 ml) of samples containing polymeric alanine were layered on a Sephadex G-15 column (2.5 x 90) and eluted with 0.05 M HCl. The eluted fractions (5 ml) were monitored for alanine-containing components either by liquid scintillation counting or by ultraviolet absorption at 216 nm.

DL-Alanine (A grade) and adenosine-5'-triphosphate, disodium salt (A grade) were products of Calbiochem. DL-Alanine-2-^{14}C was obtained from Mallinckrodt, Labeled Compounds Division. Decalso (Permutit Company) was repetitively ground and sieved through a sieve of 100 μ. (We have been unable to find any supplier of the Decalso "F" that was used by the Israeli group.) Final particle diameters were on the average less than 20 μ. Montmorillonite #25 (John C. Lane Tract, Upton, Wyoming) was procured from Wards Natural Science Establishment and prepared according to the method of Posner and Quirk.[13] In addition, we differentially centrifuged the montmorillonite and our polymerization was conducted with samples having average platelet diameters < 2000 Å.

Although the report by Paecht-Horowitz and Katchalsky indicated significant alanine polymerization, we have been unable after nine trials to demonstrate any detectable polymerization utilizing Decalso as the template for adenylate synthesis. Analytical determinations for free alanine, before and after a polymerization trial, supporting this assertion are presented in Table 1. We estimate that our analytical precision for these determinations was within 5%, and that 1% of the alanine converted into low molecular weight polymer should have been observable. Furthermore, TLC analysis of the supernatant revealed only alanine and AMP; no polymeric species was observed. Increasing the concentration of Decalso or montmorillonite two or threefold did not lead to detectable alanine polymerization. Likewise, lengthening the incubation time for the reaction mixture also had no effect on the final free alanine content; neither did substituting sodium tripolyphosphate for the ATP.

Assuming that the polymerization might be thwarted because the intermediate adenylate was not formed, we attempted to monitor adenylate formation utilizing the hydroxamic acid-$FeCl_3$ color test.[14] No evidence for alanyl adenylate was observed, thus indicating that our Decalso did not promote adenylate formation.

To confirm our hypothesis that the ineffectiveness of Decalso in promoting adenylate production was the cause for failure in our polymerization experiments, we repeated the polymerization trials using alanine-2-^{14}C adenylate and the heterogeneous catalyst, montmorillonite. Approximately 30% of the initial alanine (as determined by liquid scintillation counting and amino acid analysis) was converted into polymer in the presence of montmorillonite. Thin-layer chromatograms yielded a characteristic streaking typical for polymers with $n > 2$. In the absence of the

catalyst only ∿ 10% polymer was obtained. The elution pattern from Sephadex G-15 of the montmorillonite catalyzed reaction products is illustrated in Fig. 1. These results support the assertion by Paecht-Horowitz et al.[7] that clay-mediated polymerization provides both a significant increase in yield and degree of polymerization.

It is hard to understand the ineffectiveness of Decalso as a template for production of the amino-acyl adenylate in the light of the earlier reports by Paecht-Horowitz and Katchalsky. Despite our attempts to vary the treatment of the zeolite, in hopes of finding the key to activating the catalytic activity, we have not been able to demonstrate Decalso-mediated adenylate synthesis. It may be that the Decalso utilized by the Israeli group differs chemically or physically from that available for our investigations. This suggestion is supported by a recent disclosure that the Polymer group in Israel has also experienced difficulty with the Decalso-catalyzed production of amino-acyl adenylates, utilizing Decalso from a different supplier than previous (personal communication from Dr. Paecht-Horowitz). Dr. Paecht-Horowitz suggests that the lower cation-exchange capacity of the recently acquired zeolite is responsible for the diminished effectiveness of this material. However, our experiments indicate that increasing Decalso concentration fivefold does not stimulate adenylate formation. Although the Decalso-mediated procedure would be of great utility for prebiotic research, the experiments presented here indicate that further investigation of the properties of the synthetic zeolite is warranted.

The Israeli group have also made the interesting suggestion that their amino acid polymerization might also be accompanied by polynucleotide formation.[15] Like them, we have unsuccessfully searched for evidence

of polynucleotide formation concomitant with the polymerization of alanine from alanyl adenylate. Although polynucleotide synthesis may occur in this reaction, we made no efforts in our initial experiments to eliminate nucleases from our glassware or the reaction mixture. Experiments, conducted in a nuclease-free environment, are now in progress to ascertain whether simultaneous polycondensation of amino acids and nucleotides is feasible in this system.

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Table 1

Trial	Ala (initial) (μM)	Ala (final) (μM)	Decalso (g/ℓ)	Montmorillonite (mg/ℓ)
1	145	146	100	500
2	364	370	100	500
3	201	199	300	500
4	166	167	100	1000

Figure Caption

Fig. 1. Elution pattern from Sephadex G-15 of a montmorillonite mediated polymerization, utilizing pre-formed alanyl adenylate.

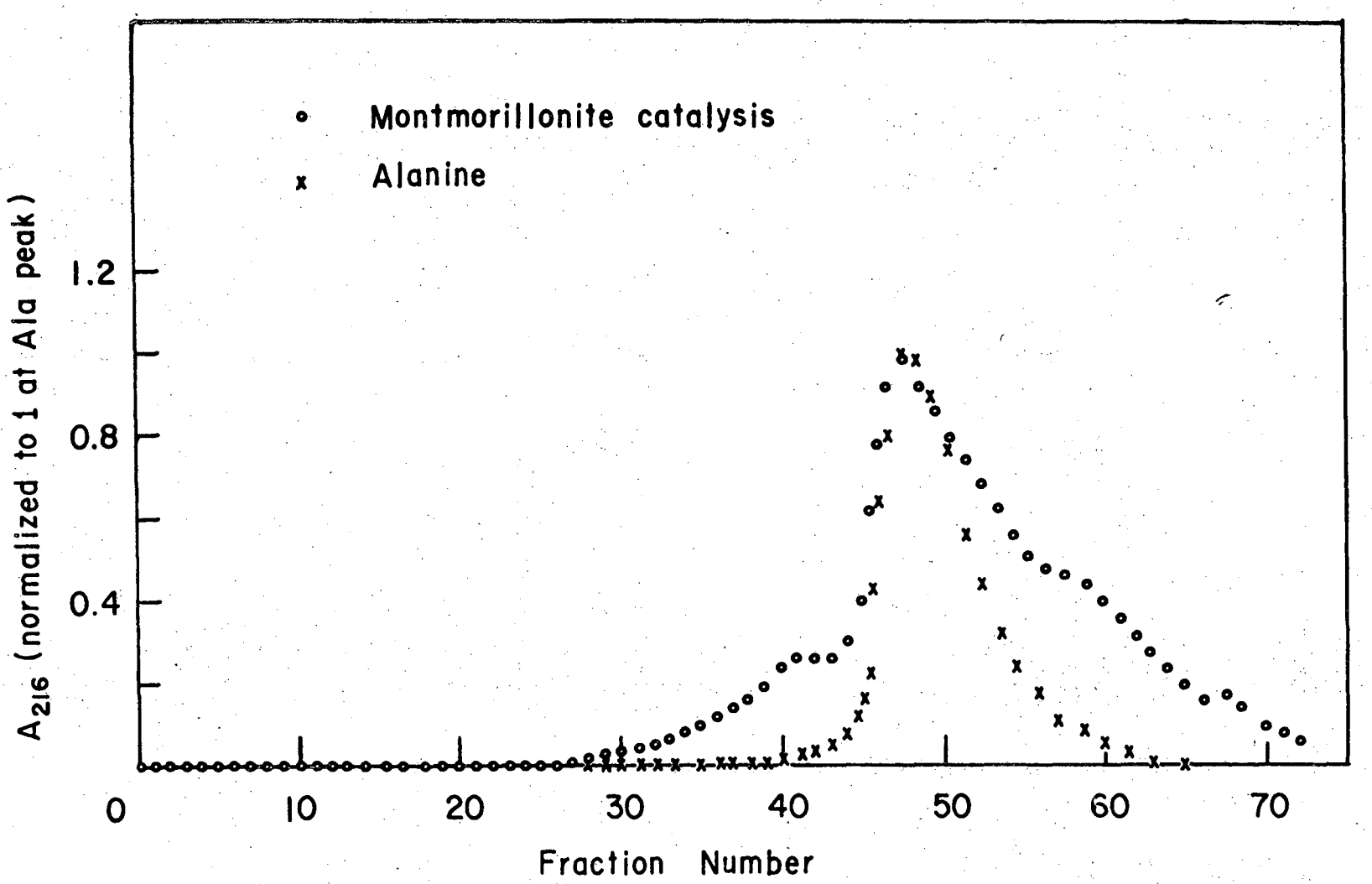
Montmorillonite catalysis
Alanine
A_{216} (normalized to 1 at Ala peak)
1.2
0.8
0.4
0
10
20
30
40
50
60
70
Fraction Number

Fig. 1

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