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DETERGENT EFFECTS ON THREE NUCLEOTIDE POLYMERASE ACTIVITIES

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SUMMARY

Non-ionic detergents stabilize the activities of three nucleotide polymerases but have varied effects on four other enzymes.

Bovine serum albumin (BSA) has been used for many years to stabilize some enzymes in reaction mixtures and solutions which would otherwise have very low protein concentrations. More recently, non-ionic detergents and lipids have been used to solubilize, stabilize or activate enzymes (1). In particular, it is becoming apparent that non-ionic detergents effect the activity of viral reverse transcriptase.

We have previously reported that the concentration of various non-ionic detergents in the assay strongly influences the activity of an RNA-instructed DNA polymerase (RDP) function from MLV-transformed UC1-B cells (2). This RDP function was shown to be present in the cells only after viral infection and to have the template preferences characteristic of viral reverse transcriptase (3).

Some information about the detergent effect on the RDP activity can be obtained by assaying activity as a function of time. As shown in

Figure 1, the initial activity was nearly independent of the detergent concentration. In the presence of 0.01% Triton DN-65, the reaction continued at a constant rate for more than 60 minutes. However, at low detergent concentrations, the activity decreased with time, and the rate of activity loss was greatest for the lowest detergent concentrations. Thus, the effect of detergent appears to be primarily one of stabilization rather than activation. Increasing the detergent concentration from 0.00067% to 0.01% after the reaction has proceeded for 60 minutes appeared to stabilize the remaining activity but did not recover any of the activity which had been lost. As also shown in Figure 1, 30 μg/ml of BSA resulted in nearly complete stabilization of the polymerase activity.

The volume of the reaction was also found to be very important. In another experiment when the reaction at 0.00067% (0.012 mM) Triton DN-65 was run in a total volume of 1.8 ml from which 100 μl samples are withdrawn at the appropriate times, the activity remains constant for at least 60 minutes, incorporating 43 pmoles at 60 minutes. These results are very different from those shown in Figure 1 for the same detergent concentration in individual 100 μl assays where the surface-to-volume ratio is very much larger. The different results obtained by the two assay methods indicate that at least a portion of the stabilizing effect of detergents is a protection of the enzyme from irreversible surface inactivation on the glass walls of the test tube or at the air-water interface.

Although 0.012 mM detergent was sufficient to stabilize the polymerase activity in large volumes under assay conditions (as discussed in the preceding paragraph), 0.014 mM detergent was not sufficient to stabilize the activity in enzyme stock solutions which did not contain template and substrate. An active extract was diluted to 0.00085% Triton X-100 in 1.36 ml and kept at 0°C. Samples of this diluted extract were then assayed as a function of time at a constant, near optimal, Triton X-100 concentration (0.004%). As shown in Figure 2, about 50% of the polymerase activity was irreversibly lost after 30 minutes, with a half-time of approximately 10 minutes followed by a slow loss of activity in the ensuing 24 hours. The lost activity could not be recovered even when the diluted extract, which was still in the original vessel, was brought to 0.005% Triton X-100 concentration and allowed to stand 30 minutes before an aliquot was taken for assay. The appropriate control, also shown in Figure 2, indicated that the loss of activity was prevented if the detergent concentration was maintained at 0.0048%. These results suggest that the template and/or substrate are also stabilizers of the polymerase activity and yet neither would be a surface active agent. Since higher concentrations of detergent can substitute for the stabilizing effect of template and/or substrate, it may be that the detergent stabilizes the enzyme not only by protecting the RDP from surface inactivation but also by interacting with the enzyme itself.

This detergent effect may, in fact, be a general phenomenon particularly for enzymes which are not always fully exposed to the aqueous

environment of the cell. We have examined the effect of detergent on the stability of several other enzymes.

Three other nucleotide polymerase preparations were tested for detergent effects -- the reverse transcriptase from AMV purified through the DEAE cellulose step as described by Kacian, et al. (4), highly purified E. coli DNA-dependent DNA polymerase (DDP) and E. coli DNA-dependent RNA polymerase (DRP). As expected, the results from the AMV reverse transcriptase are very similar to those shown in Figure 1. Since the purity of the AMV enzyme was greater than for the RDP partially purified from cells, the similarity of the results indicated that the effect of detergents is not an artifact caused by the contaminants in the cellular preparations of the RDP. The results for the two E. coli polymerases are shown in Figure 3. There is a striking similarity in the non-ionic detergent effects for these two enzymes and the RDP. The only difference in the curves of Figures 1 and 3 is in the rate of decrease of enzyme activity in the absence of additional protein or detergent. These differences are explainable in terms of the different protein concentrations in the assays -- the DDP contained only 2.3 ng/ml; the RDP, 3.1 μg/ml; and the DRP, 12 μg/ml.

Four enzyme systems which are not nucleotide polymerases were also examined for the effects of a non-ionic detergent. The results are summarized in Figure 4. Lactate dehydrogenase is not unstable at low protein concentrations and is not affected by the detergent. Hexokinase is a mixed assay with more than a five-fold excess of glucose-6-phosphate dehydrogenase, appears to have a much higher activity in the presence of 100 μg/ml BSA and detergent can partially substitute for the BSA.

Glutamate dehydrogenase is stabilized by 100 μg/ml BSA, but not by detergent. Instead, detergent is an inhibitor of the enzyme activity. Deoxyribonuclease I is stabilized by 100 μg/ml BSA and by 0.01% detergent. However, in addition to stabilizing this enzyme, detergent also inhibits its activity. These detergent effects are obviously quite different from the effects on the polymerases and also different for each enzyme.

These results demonstrate that there are no completely general non-ionic detergent effects on enzymes. The similarity of the results on the three different polymerases, however, indicates that these enzymes have some characteristics in common which the other enzymes tested do not have. It is not subunit structure since the DDP is a single subunit enzyme (5) and the DRP is a multisubunit enzyme (6). It is probably not related to the binding of polynucleotides since the deoxyribonuclease also binds polynucleotides. It may be that the polymerase enzymes have hydrophobic sites, e.g., membrane attachment sites, which are protected in aqueous solutions by the detergents. The soluble enzymes which prefer an aqueous environment would tend to be unaffected or inhibited by detergents. Guidotti has recently described work in which detergents show little binding to soluble enzymes but bind extensively to nonsoluble proteins (7). Not all nonsoluble enzymes would necessarily have their activity positively affected by detergents since some might require a more extensive hydrophobic environment (8) or the spacial orientation provided by intact membranes.

Future work in this laboratory will be directed toward separating the solubilizing aspects of the detergents from other possible effects.

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FIGURE CAPTIONS

Figure 1. Detergent Effect on RDP Activity. Duplicate assays were done in 100 μl total volumes which were 90 mM Tris-HCl (pH 7.8), 4% glycerol, 100 mM KCl, 0.3 mM dithiothreitol (DTT) / 0.02 mM [^{3}H]dTTP (1 C/mmole), 10 μg/ml poly-rA:oligo-dT and 0.1 mM $MnCl_2$. Activity is measured as the incorporation of [^{3}H]dTTP into acid insoluble material. Each assay contained 0.31 μg (3.1 μg/ml) protein from the RDP extract. For addition to the assays, the RDP extract was diluted from 0.1% to 0.0033% Triton DN-65 with buffer A (0.05 M Tris-HCl, 1 mM DTT, 0.5 M KCl and 20% glycerol).

(△) Triton DN-65 was added to assays to yield a final concentration of 0.01%.

(□) BSA was added to assays to yield a final BSA concentration of 30 μg/ml with 0.00067% Triton DN-65.

(x) Triton DN-65 was added to assays to yield a final concentration of 0.0013%.

(o, ●) Two experiments with a final Triton DN-65 concentration of 0.00067%.

(⊖) 1 μl 1% Triton DN-65 added to solid circle assays at 60 min (arrow).

(⊖) 1 μl H_2O added to solid circle assays at 60 min (arrow).

Figure 2. Loss of Polymerase Activity at 0.00085% Triton X-100. Assays were done as described in Figure 1, and the activity is given in pmole/hr per μg protein. Triton X-100 was added to each assay to a final concentration of 0.004%. The same enzyme extract was used for both curves and each 100 μl assay contained 0.36 μg protein.

Figure Captions, page 2

(o) 100 parts 0.006% Triton X-100 were added to 49 parts buffer A and 1 part enzyme extract containing 0.127% Triton X-100. Final detergent concentration in the enzyme solution was 0.0048%.

(●) 100 parts water were added to 49 parts buffer A and 1 part enzyme extract containing 0.127% Triton X-100. Final detergent concentration in the enzyme solution was 0.00085%.

Figure 3. Detergent Stabilization of Two E. coli Nucleotide Polymerases. Both DDP and DRP assays were done in a total volume of 100 μl. Assays were done in duplicate and activity was measured as incorporation of the radioactive nucleotide triphosphate into acid insoluble material.

DDP assays were 60 mM potassium phosphate buffer (pH 7.4), 6 mM $MgCl_2$, 0.45 mM DTT, 0.03 mM dATP, 0.03 mM [^{3}H]dTTP (0.5 C/mmole), 10 μg/ml poly-d(AT) duplex, 3% glycerol and 2.3 ng/ml enzyme.

(o) no additions

(□) 100 μg/ml BSA

(Δ) 0.01% Triton DN-65

DRP assays were 40 mM Tris-HCl (pH 7.4), 10 mM $MgCl_2$, 0.1 mM DTT, 0.1 mM EDTA, 150 mM KCl, 0.15 mM each ATP, CTP and GTP, 0.15 mM [^{3}H]UTP (0.1 C/mmole), 0.15 mg/ml calf thymus DNA and 12 μg/ml enzyme.

(o) no additions

(□) 400 μg/ml BSA

(Δ) 0.008% Triton DN-65

Figure Captions, page 3

Figure 4. Detergent and Protein Effects on Activities of Four Enzymes.

Lactate Dehydrogenase (beef heart, type III, from Sigma): One ml assays were 25 mM Hepes (pH 7.0), 0.5 mM $NADH_2$, 1.0 mM pyruvate and 2.4 ng/ml enzyme protein.

Hexokinase (yeast, type III, from Sigma): One ml assays were 10 mM Hepes (pH 8.0), 0.5 mM NADP, 10 mM $MgCl_2$, 1.5 mM ATP, 1.5 mM glucose, 2.5 μg/ml glucose-6-phosphate dehydrogenase, and 2 μg/ml hexokinase.

Glutamate Dehydrogenase (bovine liver, type II, from Sigma): One ml assays were 12.5 mM α-ketoglutarate and 12.5 mM NH_4Cl at pH 7.0, 0.5 mM $NADH_2$ and 1 μg/ml enzyme protein.

Deoxyribonuclease (bovine pancrease, type I, from Worthington): One ml assays were 25 mM Hepes (pH 7.0), 6 mM $MgCl_2$, 4 mM KCl, 0.06 mg/ml calf thymus DNA and 2 ng/ml enzyme protein.

(o) no additions

(Δ) 0.01% Triton DN-65

(□) 100 μg/ml BSA

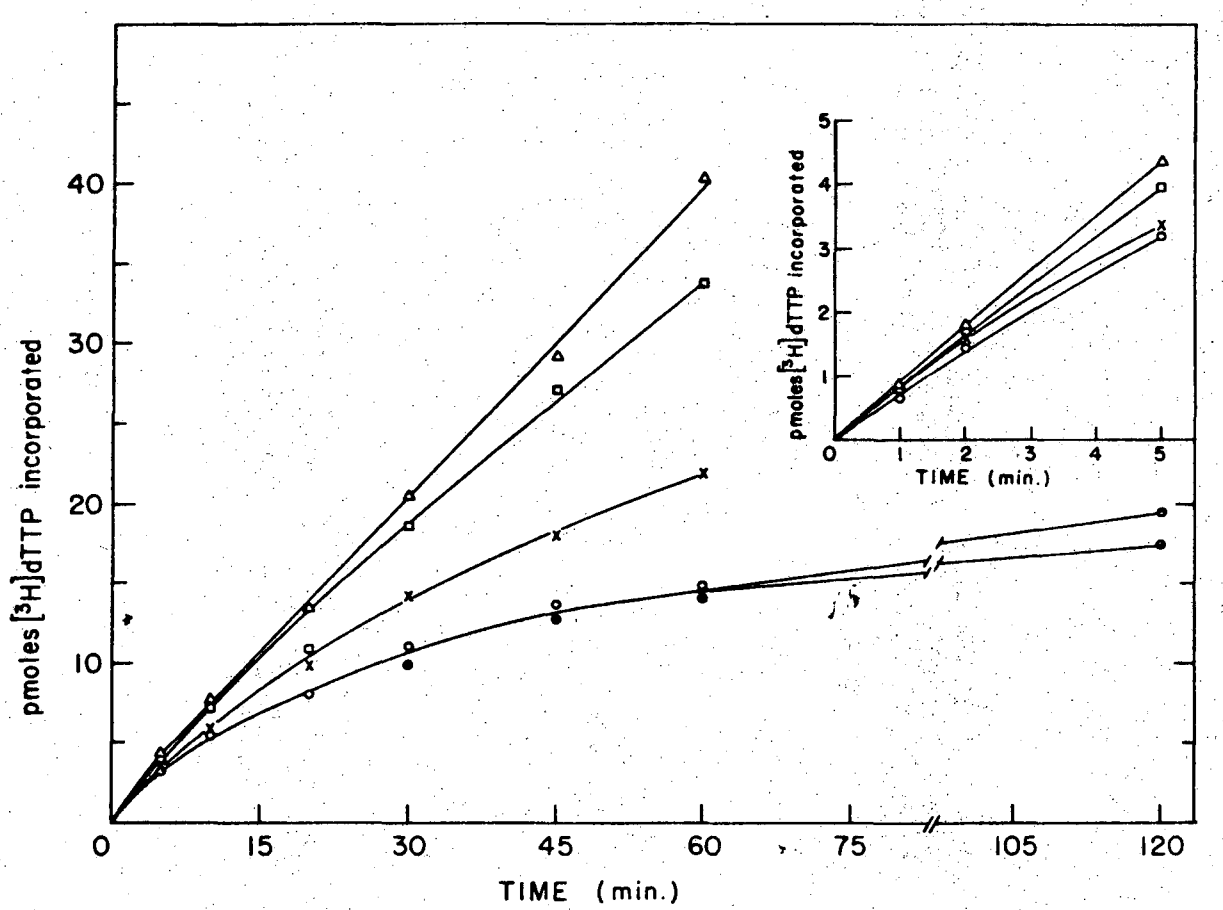

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Figure 1

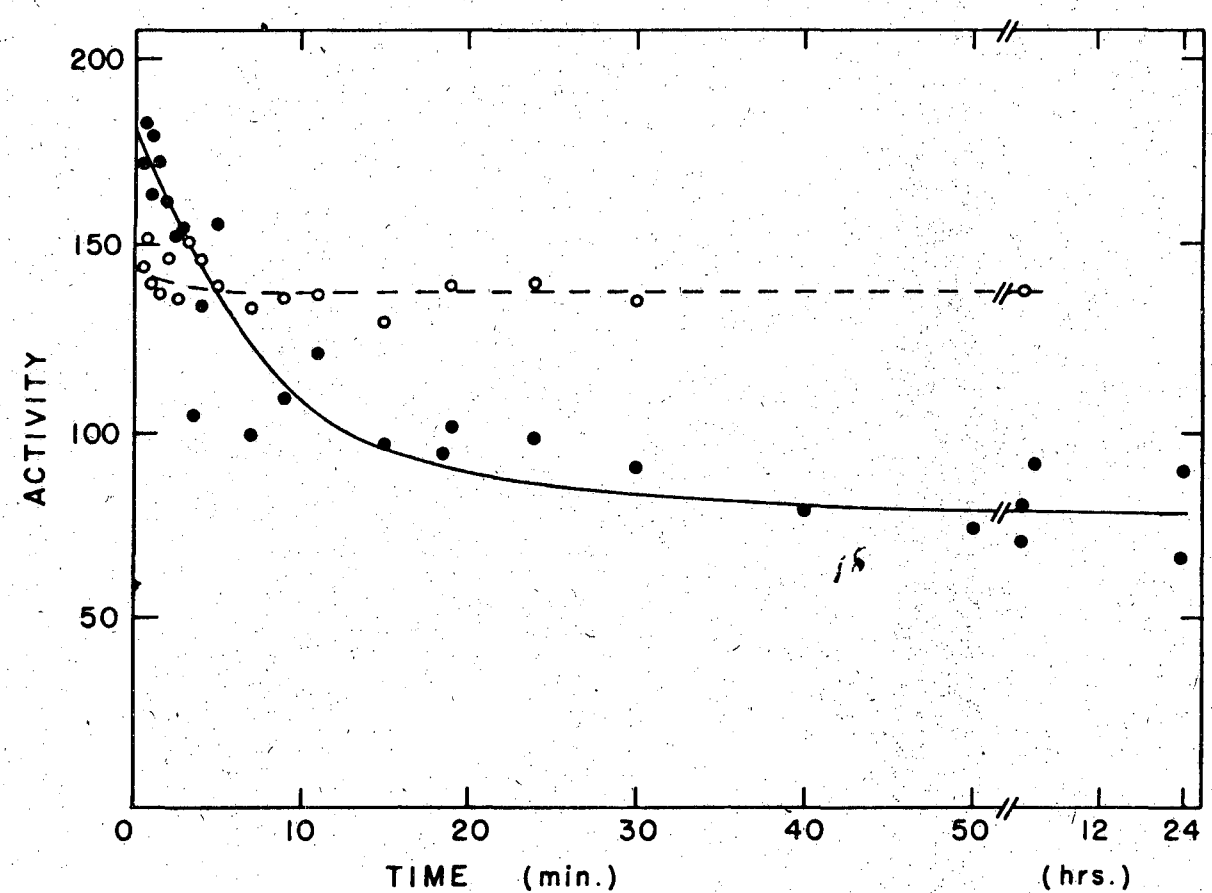

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Figure 2

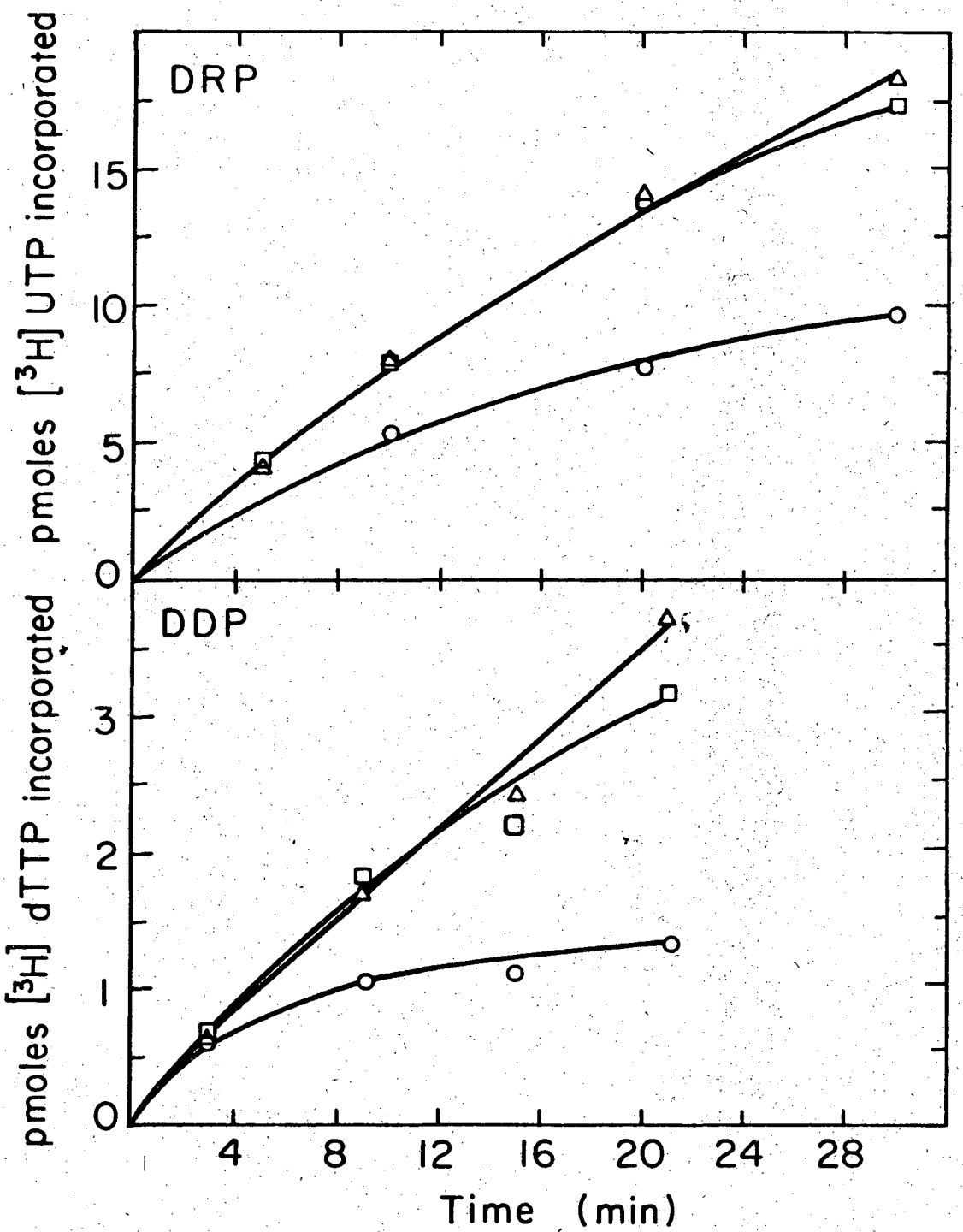

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Figure 3

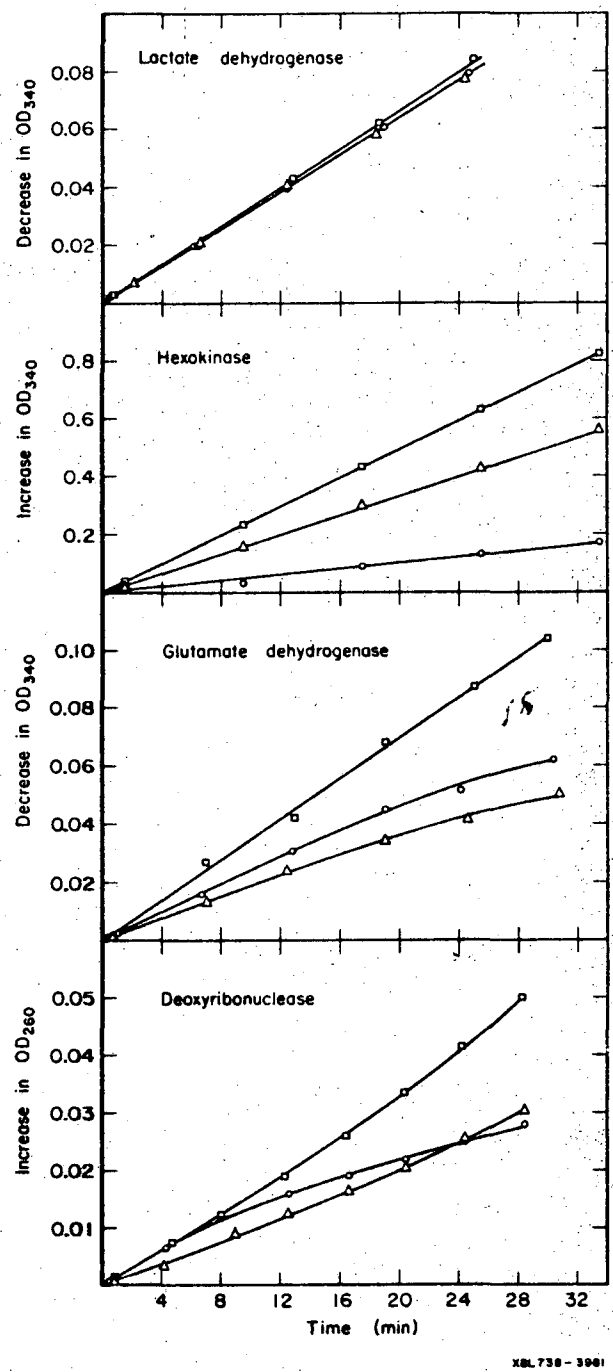

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Figure 4

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