A Deoxyribonuclease Which Requires Nucleoside Triphosphate from Micrococcus lysodeikticus

III. EXCHANGE REACTION CATALYZED BY THE ENZYME PREPARATION

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SUMMARY

The ADP-ATP exchange reaction catalyzed by the highly purified Micrococcus lysodeikticus DNase has been investigated in detail. The exchange activity is eluted together with the DNase activity from triethylaminoethylcellulose and the ratio between both activities is relatively constant in the peak fractions. The exchange reaction between [8-

\[ ^{14} \text{C} \] \text{ADP} \] and ATP has an absolute requirement for ATP and divalent cations. The enzyme is not dependent on the presence of a DNA substrate. The enzyme does not catalyze a measurable exchange between ATP and orthophosphate. The optimum pH of the reaction is 8.5 in glycine-NaOH buffer.

\[ \text{Mn}^{2+} \] is the most effective metal ion but can be replaced, to some extent, by \[ \text{Mg}^{2+}, \text{Co}^{2+}, \text{or Ni}^{2+} \]. Of the nucleoside triphosphates tested, ATP is most effective in the presence of \[ \text{Mn}^{2+} \], while in the presence of \[ \text{Mg}^{2+} \] instead of \[ \text{Mn}^{2+} \], ATP and dATP are most effective at almost the same level. In contrast to the ADP saturation curve which shows typical Michaelis-Menten kinetics, the ATP saturation curve is sigmoidal, suggesting multiple binding of ATP on the enzyme. These results suggest that the enzyme and ATP react to form the enzyme-phosphate intermediate as the first step of the over-all reaction of DNA hydrolysis.

EXPERIMENTAL PROCEDURE

**Materials**—M. lysodeikticus DNase was purified and assayed as reported previously (1), and the concentrated hydroxylapatite fraction was used in all the experiments to be described unless otherwise noted. Unlabeled nucleotides were purchased from Sigma or Calbiochem. \[ \text{[8-}\] \text{C} \text{ADP} \] was obtained from Schwarz BioResearch. \[ \text{[\text{14}C]ATP} \] was prepared by the method of Weiss.

**Assay of Exchange Activity**—This assay measures the conversion of \[ \text{[8-} \] \text{C} \text{ADP} \] to \[ \text{[\text{14}C]ATP} \]. In the routine assay of the activity, the incubation mixture (0.09 ml) contained 6 \text{pmoles of gly}

\[ \text{cine-NaOH buffer, pH 8.5; 0.3 pmole of MnCl}_2; 0.75 \text{ pmole of 2-

\[ \text{mercaptoethanol}; 6 \text{ mmoles of [\text{14}C]ADP; 40 mmoles of ATP; and 20 to 30 \mu g of enzyme. Dilutions of enzyme were made as described previously (1, 2).**}

**RESULTS**

**Requirements for Reaction**—The requirements for the exchange reaction between \[ \text{[8-} \] \text{C} \text{ADP} \] and nonradioactive ATP are shown in Table I. The reaction has an absolute dependence on the presence of \[ \text{Mn}^{2+} \] and MnCl

\[ 2 \text{ is the most effective metal ion but can be replaced, to some extent, by Mg}^{2+}, \text{Co}^{2+}, \text{or Ni}^{2+}. \] Of the nucleoside triphosphates tested, ATP is most effective in the presence of \[ \text{Mn}^{2+} \], while in the presence of \[ \text{Mg}^{2+} \] instead of \[ \text{Mn}^{2+} \], ATP and dATP are most effective at almost the same level.
TABLE I

Requirements for exchange reaction

The complete reaction mixture was the same as described under "Experimental Procedure."

| Components                  | [³⁵S]ATP formed (nmol) |
|-----------------------------|------------------------|
| Complete system             | 1.72                   |
| Minus MnCl₂                 | <0.03                  |
| Minus ATP                   | <0.03                  |
| Minus enzyme                | <0.03                  |

TABLE II

Effect of divalent cations on exchange reaction

The assay was carried out as described under "Experimental Procedure" with the exception that MnCl₂ or other divalent cations noted were added at various concentrations. The value obtained with 1.0 × 10⁻⁴ M of MnCl₂ was set at 100%.

| Metal ions added | Relative exchange activity (nmol) |
|------------------|----------------------------------|
|                  | 0.5 × 10⁻⁴ M | 1.0 × 10⁻⁴ M | 4.0 × 10⁻⁴ M | 6.0 × 10⁻⁴ M | 10.0 × 10⁻⁴ M | %   |
| MnCl₂            | 45          | 100          | 91           | 30           | 35           | 30  |
| MgCl₂            | 22          | 32           | 36           | 36           | 35           | 35  |
| CoCl₂            | 7           | 9            | 11           | 10           | 10           | 10  |

Enzyme (µg) | Time in minutes | ADP-ATP Exchange (nmol) |
|------------|-----------------|-------------------------|
| 5          | 0               | 0.05                    |
| 10         | 5               | 0.13                    |
| 20         | 10              | 0.57                    |
| 30         | 15              | 1.25                    |
| 50         | 20              | 2.5                      |

**Fig. 2. A**, effect of enzyme concentration on ADP-ATP exchange reaction. The assay conditions were those as described under "Experimental Procedure" with the exception that the enzyme concentration was varied up to 30 µg of protein as indicated. B, time course of the exchange reaction. The assay conditions were those as described under "Experimental Procedure" with 20 µg of the enzyme, and the reaction mixtures were incubated for the periods as indicated.

**TABLE III**

Effect of various nucleoside triphosphates on exchange reaction

The assay was performed as described under "Experimental Procedure" with the exception that ATP was replaced by 40 nmol of nucleotides as indicated in the presence of MnCl₂ (1.0 × 10⁻⁴ M) or MgCl₂ (5.0 × 10⁻⁴ M).

| Nucleotides | [³⁵S]ATP formed (nmol) |
|-------------|------------------------|
| ATP         | 2.80                   |
| GTP         | 0.42                   |
| CTP         | 0.11                   |
| UTP         | 0.15                   |
| dATP        | 0.78                   |
| dGTP        | 0.49                   |
| dCTP        | 0.05                   |
| dTTP        | 0.13                   |

Effect of pH on Exchange Reaction—Maximum activity was observed at pH 8.5 in glycine-NaOH buffer, while with Tris-HCl buffer at pH 8.3, approximately 85% of the maximum activity was obtained (Fig. 1). At pH 9.4 in glycine-NaOH buffer, the optimum pH for ATP-dependent DNase activity, only 60% of maximum exchange activity was observed.

Effect of Divalent Cations on Exchange Reaction—The most effective metal ion was Mn²⁺ at 1 × 10⁻⁴ M and at higher concentrations considerable inhibition was observed (Table II). Mn²⁺ could be replaced, to some extent, by Mg²⁺, Co²⁺, or Ni²⁺. Thus the cation requirement for the exchange reaction is different from that for ATP-dependent DNase reaction, in which Mg²⁺ is more effective than Mn²⁺.

Effect of Enzyme Concentration on Exchange Reaction—The exchange rate was directly proportional to the amount of enzyme added, up to 30 µg of protein, when a 10-min incubation period was used (Fig. 2A). The enzyme (20 µg) catalyzed the exchange reaction, showing linear response up to about 15 min (Fig. 2B).
Effect of ATP concentrations on ADP-ATP exchange. 

A, the assay was carried out as described under "Experimental Procedure" with the exception that the ATP concentration was varied as shown. B, experimental conditions were as described in A with the exception that MnCl₂ was replaced by 5.0 × 10⁻⁴ M MgCl₂. Inset shows a Lineweaver-Burk plot.

Effect of ADP concentrations on exchange reaction. 

A, the exchange assay was carried out as described under "Experimental Procedure" with the exception that the ADP concentration was varied as indicated. B, the experimental conditions were as described in A with the exception that Mn²⁺ was replaced by 5.0 × 10⁻³ M Mg²⁺ and incubation time was 30 min. Inset shows a Lineweaver-Burk plot.

Chromatography of ATP-dependent DNase and ADP-ATP exchange activities on TEAE-cellulose. The Sephadex G-200 fraction was adsorbed and eluted from TEAE-cellulose as described previously (1). Fractions were collected and assayed for the DNase and ADP-ATP exchange activities as described under "Experimental Procedure."

ATP exchange by altering the Vₘₐₓ for ATP. In both reactions, the ATP saturation curve was sigmoidal, and, therefore, the Lineweaver-Burk plot gave a concave curve.

Effect of ADP Concentrations on Exchange Reaction—In contrast to the ATP saturation curve, the relationship between the concentration of ADP and the velocity of ADP-ATP exchange reaction showed typical Michaelis-Menten kinetics, with the Kₘ value of 3.3 × 10⁻⁶ M or 4.0 × 10⁻⁶ M in the presence of Mn²⁺ or Mg²⁺, respectively (Fig. 4, A and B).

Chromatography of Exchange and DNA Hydrolysis Activities—The ADP-ATP exchange activity and ATP-dependent DNase activity behave similarly during the routine purification procedures. When the Sephadex G-200 fraction was subjected to the second TEAE-cellulose column chromatography, the first peak of ADP-ATP exchange activity eluted from the column coincided with the peak of DNase activity, and the ratio of exchange activity was relatively constant in the most active six fractions (Fig. 5), whereas the second peak of ADP-ATP exchange activity which could be separated from the first peak was devoid of DNase activity.

DISCUSSION

A unique feature of DNA degradation by the action of M. lysozymaticus DNase is the absolute participation of nucleoside triphosphate in the hydrolysis reaction. Details of the mechanism of nucleotide requirement for the reaction are presently undefined. The DNA-dependent cleavage of ATP to ADP and inorganic orthophosphate, as reported in the preceding paper (2), shows that the nucleotide does not act as an allosteric effector. A similar type of activation of enzyme by ATP has been described for succinyl-CoA synthetase (13), γ-glutamylcysteine synthetase (14), glutathione synthetase (15), ATP-citrate lyase (16, 17), or pyruvate carboxylase (18). These enzymes catalyze an exchange of phosphate between ATP and ADP, and in this type of activation ATP interacts with the enzyme, and enzyme-phosphate complex is formed as an activated intermediate (16, 17).
In this paper, evidence was presented that the highly purified preparation of *M. lysodeikticus* DNase can also catalyze an exchange of ADP with ATP in the absence of DNA. The properties of the exchange were shown to be similar to those of ATP-dependent DNA hydrolysis reaction catalyzed by the same preparation, but several differences between the two activities were observed. The DNase preparation used in these experiments is a highly purified one, but not homogeneous as judged by polyacrylamide gel electrophoresis. However, no detectable radioactive ATP is formed from [W]ADP by the final enzyme preparation in the absence of ATP (Table I). This indicates that the preparation is virtually free of adenylate kinase which, if present, could in principle catalyze apparent ADP-,4TP exchange. Adenylate kinase activity contaminating crude extracts of *M. lysodeikticus* is almost completely removed by the procedures of TEA-cellulose chromatography and Sephadex G-200 filtration during the purification. Furthermore, the exchange activity was eluted together with the DNase from TEA-cellulose and the ratio between both activities is relatively constant in the peak fractions. Therefore, the data presented in this paper provide the evidence that the exchange reaction and the ATP-requiring DNase reaction are catalyzed by a single enzyme protein. It may imply that the exchange reaction represents a portion of over-all reaction of the DNA hydrolysis. Thus, the over-all reaction may involve the following reactions.

\[
\text{Enzyme} + \text{ATP} \rightarrow \text{enzyme-P}_i + \text{ADP} \quad (1)
\]

\[
\text{Enzyme-P}_i + \text{DNA} \rightarrow \text{oligonucleotide} + \text{enzyme} + \text{P}_i \quad (2)
\]

It is possible, therefore, to rationalize that the difference in some properties of the exchange reaction and of the over-all reaction could be due to the influence of the property of Reaction 2 to that of the over-all reaction.

Several attempts have been made to isolate the predicted enzyme-phosphate complex directly by incubating the enzyme and Mn\(^{2+}\) or Mg\(^{2+}\) with [\gamma\,-P\,]ATP and separating the radioactive complex from free [\gamma\,-P\,]ATP by the Sephadex gel filtration, but so far all have been unsuccessful. It appears, therefore, that the binding of ATP to the enzyme is freely reversible.

Another interesting feature of the exchange reaction is that the ATP saturation curve is sigmoidal, and the Lineweaver-Burk plot gives a concave curve suggesting multiple binding of ATP on the enzyme. These data, together with the previous finding that the ratio of the amount of ATP hydrolyzed versus the number of cleaved phosphodiester bonds during the DNA degradation by *M. lysodeikticus* DNase is calculated to be approximately 3:1 instead of 1:1 (2), seem to shed light on the role of nucleotide in the reaction mechanism. More precise kinetic studies on this line are now in progress in our laboratory, in order to settle the question.

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REFERENCES

1. Anai, M., HIRAHASHI, T., and Takagi, Y., *J. Biol. Chem.*, 245, 767 (1970).
2. Anai, M., HIRAHASHI, T., YAMANAKA, M., and Takagi, Y., *J. Biol. Chem.*, 245, 775 (1970).
3. Anai, M., Sekagaku, 39, 196 (1967).
4. Voitis, G. F., and Buttin, G., *Biochim. Biophys. Acta*, 224, 29 (1970).
5. Voitis, G. F., and Buttin, G., *Biochim. Biophys. Acta*, 224, 42 (1970).
6. Buttin, G., and Wright, M. R., *Cold Spring Harbor Symp. Quant. Biol.*, 33, 259 (1968).
7. Osahi, M., *Proc. Natl. Acad. Sci. U. S. A.*, 64, 1296 (1969).
8. Winder, F. G., and Cougclan, M. P., *Biochem. J.*, 111, 679 (1969).
9. Winder, F. G., and Lavin, M., *Biochem. J.*, 115, 6 (1969).
10. Bardour, E. D., and Clark, A. J., *Proc. Natl. Acad. Sci. U. S. A.*, 65, 955 (1970).
11. Goldmark, P. J., and Linn, S., *Proc. Natl. Acad. Sci. U. S. A.*, 67, 434 (1970).
12. Weiss, B., Live, T. R., and Richardson, C. C., *J. Biol. Chem.*, 243, 4530 (1968).
13. Kaufman, S., *J. Biol. Chem.*, 216, 153 (1955).
14. Webster, G. C., and Varner, J. E., *Arch. Biochem. Biophys.*, 52, 22 (1954).
15. Snoke, J. E., and Bloch, K., *J. Biol. Chem.*, 213, 825 (1955).
16. Inoue, H., Suzuki, F., Tanioka, H., and Takeda, Y., *Biochem. Biophys Res Comm.*, 59, 609 (1967).
17. Inoue, H., Suzuki, F., Tanioka, H., and Takeda, Y., *J. Biochem.*, 63, 89 (1968).
18. Schutten, M. C., and Utter, M. F., *J. Biol. Chem.*, 240, 3714 (1965).
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