The Nonenzymatic Activation of Acetate by Adenosine Triphosphate-Bivalent Metal Chelates

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A nonenzymatic transfer of a phosphoryl group from the polyphosphate chain of adenosine triphosphate was first described in connection with the reaction shown in Equation 1. The reaction, which occurs under mild conditions and at low concentrations of reactants, is absolutely dependent on the presence of bivalent metal ions, and is stimulated by univalent metal ions. The most effective bivalent metal ion was found to be Ca++. When Mn++ ion was used to satisfy the bivalent metal ion requirement, the most effective univalent metal ion was found to be K+. The reaction was formulated as a nucleophilic attack of orthophosphate on a chelate of ATP and bivalent metal. Chelation increases the electrophilic character of the phosphorus atom of ATP, and thus facilitates the nucleophilic attack by orthophosphate. The bivalent metal ion also screens the negative charges of ATP from those of the attacking molecule. Alkali metal ions stimulate the reaction by neutralizing the residual negative charges of the active chelate (1-3). The influence of metal ions on the acceptor molecule (orthophosphate) has not so far been studied.

Carboxylic acids such as acetate, glycine, and β-alanine can act as acceptors in place of orthophosphate. When the reaction is run in the presence of hydroxylamine, the product can be trapped as a hydroxamate (Equation 2). Nonenzymatic phosphorylation reactions which are catalyzed by bivalent metal ions may have been the prototype reactions for the biochemical evolution of those enzymes which utilize the free energy of hydrolysis of polyphosphates to drive chemical transformations. The natural trapping agents of the aminoacyl phosphates could have been amino acids instead of hydroxylamine. The reaction products would then have been peptides instead of hydroxamates. Such reactions may have provided the rudiments of an autocatalytic duplicating system which resulted in the formation of polypeptides from amino acids (4). This paper presents a study of the nonenzymatic transfer reaction from ATP to acetate. The most effective bivalent metal ion in this reaction is Be++, and the optimal pH is about 5.2.

EXPERIMENTAL PROCEDURE

Reagents—Various salts of ATP were purchased from the Sigma Chemical Company. Other reagents were of reagent grade, except RbCl and CsCl which were a purified grade obtained from E. H. Sargent and Company, Chicago, Illinois.

Methods—Acethydroxamic acid was measured colorimetrically as the ferric ion complex (5). The sample to be assayed was diluted to a volume of 1.5 ml with water. The ferric ion solution (1.5 ml) was then added, and the optical density of the resulting solution was read at 540 nm with a spectrophotometer cell with a 1-cm light path. The ferric ion solution consisted of 3.0% ferric chloride in 1.2 N HCl. A calibration curve was prepared with authentic acethydroxamate (kindly supplied by Dr. W. Jencks).

RESULTS

Requirements for Nonenzymatic Activation of Acetate—The experiment shown in Table I demonstrates that ATP, acetate, and bivalent metal ions are required for the reaction. Omission of one of these substances results in a marked decrease or in the disappearance of acethydroxamate formation. The reaction rate is linear with time (Fig. 1). A small amount of hydroxamate is obtained in the absence of bivalent metal ion at zero time (about 0.04 μmole). This may be due to an impurity present in one of the reagents. An additional small amount of hydroxamate is formed on incubating the reaction mixture in the absence of bivalent metal ions (about 0.38 μmole per hour). This is much less than the amount of hydroxamate formed in the presence of bivalent metal ions (0.48 μmole per hour).

pH Optimum—The reaction has a pH optimum at about pH 5.2 (Fig. 2). When Be++ ions were used, it was found impractical to determine the reaction rate at pH values higher than 5.5, because of the formation of precipitates.

Bivalent Metal Ion Specificity—A comparison of the effectiveness of different bivalent metal ions in the reaction is shown in Table II. Be++ ions are the most effective, followed by Ni++ and Co++. Ca++ ions, which are the most effective ions in Reaction 2, are among the least effective when acetate is the acceptor (Reaction 2). A satisfactory comparison of the activity of Cu++ ions with the activities of other bivalent metal ions was not obtained. The results with cupric ions were erratic, and the apparent amount of hydroxamate formed was not proportional to the time of incubation, presumably because of the reduction of Cu++ to Cu+ by hydroxylamine.
TABLE I
Formation of acetohydroxamate

The complete reaction mixture contained (in μmoles): Tris salt of ATP, 50; Tris acetate-acetic acid buffer, 800; hydroxylamine hydrochloride, 400; and Be(NO$_3$)$_2$, 30. Final volume 1.0 ml, pH 5.2, 38°, 1 hour.

<table>
<thead>
<tr>
<th></th>
<th>Acetohydroxamate formed (μmole)</th>
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<tbody>
<tr>
<td>Complete reaction mixture</td>
<td>0.48</td>
</tr>
<tr>
<td>Zero time</td>
<td>0.02</td>
</tr>
<tr>
<td>Be$^{++}$ omitted</td>
<td>0.08</td>
</tr>
<tr>
<td>Acetate omitted</td>
<td>0.06</td>
</tr>
<tr>
<td>ATP omitted</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Fig. 1. Time course of acetohydroxamate formation. The reaction mixture contained (in μmoles per ml): Tris salt of ATP, 50; Tris acetate-acetic acid buffer, 800; hydroxylamine hydrochloride, 400; and Be(NO$_3$)$_2$, 30. pH 5.2, 38°. Samples were withdrawn and analyzed at the times indicated. ○, complete reaction mixture; X, Be(NO$_3$)$_2$ omitted.

Effect of Alkali Metal Ions—Alkali metal ions at a concentration of 0.1 M have little or no effect on the rate of the reaction (Table III). This is in contrast to the influence of these ions on Reaction 1. Either alkali metal ions exert no influence on the acetate-activating reaction, or the negative findings are the result of the high ionic strength of the reaction mixture which may obliterate stimulating effects of 0.1 M alkali metal ions.

Influence of Varying the ATP/Be$^{++}$ Ratio—Fig. 3 shows the influence of varying the concentration of Be$^{++}$ on the rate of the reaction. A maximal rate is attained only in the presence of precipitates. Fig. 4 shows the influence of varying the concentration of ATP on the rate of the reaction. A maximal rate is attained when the ATP/Be$^{++}$ ratio is made about 1. There is no further increase in the rate of the reaction when the ATP/Be$^{++}$ ratio is made less than 1.

Effect of Acetate Concentration—The rate of the reaction is proportional to the acetate concentration from 0 to 0.8 M (Fig. 5).

DISCUSSION

The nonenzymatic activation of acetate described in this paper probably involves the initial formation of an acyl phosphate.
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FIG. 3. The effect of beryllium ion concentration on transphosphorylation. The reaction mixture contained (in μmoles): Tris salt of ATP, 50; Tris acetate-acetic acid buffer, 800; hydroxylamine hydrochloride, 400; and Be(NO₃)₂ as indicated. Final volume 1.0 ml, pH 5.2, 38°, 1 hour. O, no precipitate; X, light precipitate; Δ, precipitate formed during incubation.

FIG. 4. The effect of ATP concentration on transphosphorylation. The reaction mixture contained (in μmoles): Tris acetate-acetic acid buffer, 800; hydroxylamine hydrochloride, 400; and Tris salt of ATP as indicated. Final volume 1.0 ml, pH 5.2, 38°, 1 hour. The Be(NO₃)₂ concentration was (O) 12.5, and (X) 25 μmoles per ml.

FIG. 5. The effect of acetate concentration on transphosphorylation. The reaction mixture contained (in μmoles): Tris salt of ATP, 50; hydroxylamine hydrochloride, 400; Be(NO₃)₂, 30; and Tris acetate-acetic acid buffer as indicated. Final volume 1.0 ml, pH 5.2, 40°, 1 hour.

which, in the presence of hydroxylamine, reacts further to yield acethydroxamate. The nature of the acyl phosphate formed in the nonenzymatic reaction has not yet been investigated. The initial product might be acetyl phosphate, or acetyl-AMP, or both. The nonenzymatic activation of acetate shows an absolute, or almost absolute, dependence on bivalent metal ions (Table I). An ATP/Be⁺⁺ ratio of 1.66 was used for most of the experiments reported here. This ratio was chosen from practical convenience, since it avoids the formation of precipitates at ATP concentrations of 0.05 M. The curve of the pH optimum (Fig. 2) indicates that acetate, and not undissociated acetic acid, is the reactive species. The rate of hydroxamate formation is not rate-limiting at the pH values shown in Fig. 2. The results shown in Figs. 2, 3, and 4 suggest that the active form of ATP in the reaction is the chelate (ATP-Be)⁻. The single negative charge of this chelate may be neutralized by ion pair formation or chelation with additional cations (2, 3). The results are in harmony with a reaction mechanism which involves a nucleophilic attack by the acetate anion on the ATP-metal chelate, a mechanism which is similar to that proposed for Reaction 1 (2).

When acetate acts as phosphate acceptor, Be⁺⁺ ions are the most effective (Table II), and the optimal pH is about 5.2. When orthophosphate acts as phosphate acceptor, Ca⁺⁺ as well as Sr⁺⁺ and Ba⁺⁺ ions are the most effective (9), and the optimal pH is 9.0. These striking differences in specificity suggest that binding between the bivalent metal ion and the acceptor molecule must be taken into account in attempts to explain the mechanism of the nonenzymatic reactions. Mg⁺⁺ ions are among the least effective in the nonenzymatic reaction, yet the ATP-Mg chelate is a highly efficient phosphorylating agent in the enzymatic reaction. The enzyme may achieve this modification of properties by binding the ATP Mg chelate through the vacant coordination valencies of the metal.

A nonenzymatic activation of acetate by adenosine triphosphate has been described. Hydroxylamine was used to trap the activated acetate as acethydroxamate. The reaction requires bivalent metal ions and has a pH optimum of about 5.2. Of the bivalent metal ions tested, Be⁺⁺ proved to be the most effective. The reaction rate is optimal at ratios of adenosine triphosphate to bivalent metal of 1.0 or less.

SUMMARY

REFERENCES
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