PROTECTION AND REACTIVATION OF CARBAMYL PHOSPHATE SYNTHETASE WITH SULFATE

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Purified preparations of carbamyl phosphate synthetase from mammalian liver have been reported to be reasonably stable in concentrated ammonium sulfate solutions but very unstable in dilute buffer solutions (Metzenberg, Hall, Marshall, and Cohen, 1957). In this investigation of purified carbamyl phosphate synthetase from Streptococcus lactis 8039, it was noted that ammonium sulfate not only protects the enzyme upon storage but also prevents the destructive effects of dialysis and of heat treatment; and furthermore, is effective in restoring to a large extent the carbamyl phosphate synthetase activity to preparations which have been inactivated by dialysis and heat treatment. The sulfate ion rather than the ammonium ion appears to be responsible for both the stabilization and the restoration of the carbamyl phosphate synthetase activity.

Purified preparations of carbamyl phosphate synthetase from sonic extracts of S. lactis were obtained by precipitation with ammonium sulfate (50 to 75 per cent of saturation); adsorption on a DEAE-cellulose column and elution with 0.08 M phosphate buffer, pH 8; and precipitation of the active fractions with ammonium sulfate (90 per cent of saturation) followed by dialysis against 0.04 M Tris[1] buffer, pH 8.5, for 2 hours at 4°.

[1]Abbreviations: Tris, tris(hydroxymethyl)aminomethane; ATP, adenosine triphosphate.

This purified preparation with a specific activity of 1200 (μmoles of product formed per mg. of protein per hour) was then diluted with 0.04 M Tris buffer, pH 8.5, to 1 mg. per ml. of protein. Carbamyl phosphate synthetase activity was determined before and after various treatments by incubating a rate limiting amount of enzyme (10 to 40 μg. of protein) with a reaction mixture containing ammonium carbamate[2], 250 μmoles; ATP, 10 μmoles; magnesium chloride, 10 μmoles; Tris buffer, 50 μmoles; DL-ornithine monohydrochloride, 20 μmoles; and ornithine transcarbamylase, 5 μg. protein, specific activity 77,000[3], in a total volume of 1 ml. at a pH of 8.8. After 15 minutes incubation at 38°, citrulline was determined colorimetrically (Archibald, 1944) on appropriate aliquots.

As indicated in Table I, there is an 80 per cent loss in enzymatic activity on dialysis for 18 hours at 4° against Tris buffer and better than an 80 per cent loss on heating at 60° for 2 minutes. This loss in activity is prevented by high concentrations of ammonium sulfate. The addition of 0.04 M magnesium chloride to the Tris buffer in lieu of ammonium sulfate does not prevent the destruction due to dialysis.

When carbamyl phosphate synthetase is inactivated as described in Table I, the enzymatic activity can be largely restored by allowing the inactivated preparation to stand in an ammonium sulfate solution, pH 8.5, for 1 hour at 4°. These results are presented in Table II. The degree to which the enzyme is reactivated is dependent upon the concentration of the ammonium sulfate solution to which the enzyme is exposed and not upon the amount of sulfate present during the enzymatic conversion of

[2]Preparation previously described (Gmelin, 1936).

[3]Purification previously described (Ravel, Grona, Humphreys, and Shive, 1959).

TABLE I

STABILITY OF PURIFIED PREPARATIONS OF CARBAMYL PHOSPHATE SYNTHETASE

Treatment	Per Cent of Control
None	100
Stored at -20° for 1 week	40
Stored at -20° for 1 month in 0.5 M ammonium sulfate	100
Dialyzed 18 hours at 4° against 0.04 M Tris, pH 8.5	20
Dialyzed 18 hours at 4° against 0.04 M Tris, 0.5 M ammonium sulfate, pH 8.5	98
Heated 2 minutes at 60°	14
Heated 2 minutes at 60° in 0.5 M ammonium sulfate	86
Heated 10 minutes at 60° in 0.5 M ammonium sulfate	77

carbamate to carbamyl phosphate. Concentrations of ammonium sulfate above 1 M are not appreciably more effective. Inactivated preparations can be stored for a period of several months at -20° without losing their ability to be reactivated. Both sodium and potassium sulfate are effective in restoring enzymatic activity whereas ammonium carbonate, ammonium chloride, potassium phosphate and the residue from the ignition of ammonium sulfate are without effect under the same conditions (pH 8.5 for 1 hour at 4°).

When carbamyl phosphate synthetase is inactivated by heating at 60° for 2 minutes, the activity can be partially restored by ammonium sulfate. If, however, the dilute (1 mg. per ml. of protein) enzyme solution is heated for longer periods of time, the activity cannot be restored by standing in an ammonium sulfate solution.

TABLE II

REACTIVATION OF CARBAMYL PHOSPHATE SYNTHETASE BY SULFATE

Treatment Following Inactivation	Per Cent of Control
I. Inactivated by dialysis*	
None	20
Ammonium sulfate, 0.25 M	33
Ammonium sulfate, 0.5 M	54
Ammonium sulfate, 1.0 M	63
Sodium sulfate, 0.5 M	51
Potassium sulfate, 0.35 M	40
Ammonium carbonate, 0.5 M	19
Ammonium chloride, 2 M	25
Potassium phosphate, 0.5 M	8
II. Inactivated by heat treatment*	
None	14
Ammonium sulfate, 1 M	46

*As in Table I.

These data indicate that the sulfate ion rather than the ammonium ion is responsible for both the stabilization and the restoration of the carbamyl phosphate synthetase activity. Although high concentrations of sulfate are required, factors other than ionic strength are involved since other salt solutions which do not contain sulfate but which are of equivalent ionic strengths are without effect; and the same concentration of sulfate is required for reactivation in the presence of high concentrations of other salts. High concentrations of sulfate might actually introduce a sulfate moiety into the enzyme molecule or perhaps exert a specific ionic effect in forming and

stabilizing a specific structure of the enzyme. A comparison of sulfate and the biological sulfate donor, 3-phosphoadenosine-5-phosphosulfate, would be of interest in the reactivation reaction. Also of interest would be the effect of sulfate on the extreme lability of mammalian carbamyl phosphate synthetase as well as on the enhanced thermal lability of frog liver carbamyl phosphate synthetase by substrates and cofactors (Caravaca and Grisolia, 1959).

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