Biosynthetic and structural relationships of Compound X and carbamyl phosphate*

The similar behavior of carbamyl phosphate and Compound X* has been shown with mammalian and bacterial preparations[5]. In spite of the evidence[3,4] indicating Compound X to be a discrete chemical entity, JONES *et al.*[1] appear to believe Compound X and carbamyl phosphate to be the same compound. In addition to the evidence already presented it is shown in this paper (Table I) that during spontaneous decomposition carbamyl phosphate is broken down to a carbamate and inorganic phosphate, and that after the release of inorganic phosphate the carbamate is split to CO_2 and NH_3.

TABLE I

SPONTANEOUS DECOMPOSITION OF CARBAMYL PHOSPHATE

Incubation time	*Citrulline synthesis*	*$PO_4^{=}$ release*	*Ammonia release*	*CO_2 release*
min	μM	μM	μM	μM
0	8.00	0.0	0.0	0.0
30	5.00	3.20	2.00	1.98
60	2.74	5.40	3.30	3.30
90	1.70	6.40	4.26	4.30
120	1.14	7.10	4.94	5.00

CP lithium salt[1] was dissolved, adjusted to pH 5.0 with cold acetic acid and made 0.16 M. This solution was diluted to 0.004 M with 0.05 M acetate buffer pH 5.0 before using. Incubation at 38°. Samples were analyzed for CP as previously described for Compound X[3] since in this test Compound X and carbamyl phosphate react in the same manner[5]. Inorganic phosphate was measured by the method of LOWRY AND LOPEZ[6], carbon dioxide was measured manometrically, and ammonia using a final volume of 10 ml and 3 ml of Nessler's reagent[7].

TABLE II

CARBAMYL PHOSPHATE SYNTHESIS IN BACTERIAL EXTRACTS

Time in minutes	0	10	20
μM CP Found	0	2.3	2.9

Final substrate concentrations in micromoles per 2 ml: $MgSO_4$, 15; ATP, 3; $(NH_4)_2CO_3$, 80; 3-D-potassium phosphoglycerate, 50; tris (hydroxymethyl) aminomethane buffer pH 8.5, 80; bacterial extract, 0.66 mg protein per tube, of a supernatant fluid (20,000 × g for 20 minutes) of an ultrasonic-treated streptococcus strain[5]; 2 mg of muscle preparation[3] per tube, (this supplementation is necessary for maximum activity, since the bacterial preparation shows little 3-D-phosphoglyceric mutase and enolase[8]). Incubation at 30°. Under these conditions this system when supplemented with ornithine showed 10.8 and 20.5 micromoles of citrulline in 10 and 20 minutes respectively. Citrulline synthesis is not affected in this system by the addition of AG up to 10 micromoles per ml. CP accumulation was measured essentially as previously described for Compound X, except that the pH was 6.5 during the enzymic test[3]. Attempts to remove co-factors by acetone fractionation or passing the enzyme through Dowex 1 and 2 columns resulted in partial loss of activity which was not reactivated by the addition of heated crude preparations or by the addition of Dowex eluates.

As previously shown[4], Compound X under equal conditions liberates first inorganic phosphate which is followed by a slow release of CO_2 and by a very delayed split of NH_3.

In spite of the similarity between the carbamyl kinase of bacteria and of mammals[5], it is clear that the mechanisms of biosynthesis of reactive carbamyl groups are not entirely equal since the mammalian system requires AG or related compounds, whereas the bacterial extracts used in this

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work do not show any stimulation upon the addition of AG or related compounds. It appears then logical to assume by analogy that the bacterial enzyme might possess an active center with a structure similar to acetyl glutamate or related compounds. If this is the case two possibilities are open, either that carbamyl phosphate remains attached to the enzyme until reaction with ornithine or other acceptor, or that carbamyl phosphate is split off from the enzyme site before further reaction. As shown in Table II the second possibility seems to be true.

Still to be investigated is whether or not Compound X is split to free carbamyl phosphate before reacting with ornithine or other acceptor and whether this splitting is enzymic or non-enzymic[3].

A further subject for investigation will be to determine the possible existence of a "carbamyl or acetyl active center" in the "native" mammalian enzyme which may be masked or denatured during the isolation of the enzyme[3].

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* The following abbreviations are used throughout this paper: AG, acetyl glutamate; CP, carbamyl phosphate[1]; Compound X, the active carbamyl phosphate intermediate formed from AG or related compounds[2]; ATP, adenosinetriphosphate. All analytical methods and procedures have been described in preceding papers[3,4].

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α-L-Formamidinoglutaric acid as a formylating agent*

The isolation of α-L-formamidinoglutaric acid (FAG)[1,2,3,4] from incubation mixtures of histidine or urocanic acid with mammalian liver preparations suggested that FAG might be an intermediate in the pathway of the metabolism of histidine as a donor of the one carbon unit. Since in these enzymic digests FAG accumulated and no further degradation was observed, it was assumed that a suitable formyl acceptor was absent in these extracts[1], although the possibility could not be excluded that activation of histidine as formyl donor followed a pathway other than that through urocanic acid and FAG[5].

We wish to report two lines of evidence which strongly suggest that FAG or a compound in equilibrium with it is an obligatory intermediate in reactions in which histidine acts as a formylating agent. When FAG (5 μM) was incubated with folic acid (0.25 μM) and 0.5 ml of a soluble enzyme extract from rat liver (supernatant of 1:1 liver homogenate with 0.05 M phosphate buffer, pH 7.2, centrifuged at 140,000 × g at 0–4° for 2 hours) in a total volume of 0.8 ml, a sky-blue-fluorescing compound was formed which behaved identically with synthetic 10-formylfolic acid[6] upon paper chromatography with six solvents and ionophoresis. Under these conditions total conversion of folic acid to "formyl-folic acid" was obtained within an incubation time of 3 hours at 37° in the dark. The enzyme extract was inactivated by treatment with Dowex-2-Cl or prolonged dialysis and could be reactivated to a varying degree by the addition of 0.2 ml of boiled (2 min, 100°) enzyme extract. The efficacy of the boiled enzyme was abolished by treatment with Dowex-2-Cl or charcoal. The activity of the inactivated enzyme could also be restored by folic acid which had been reduced either with Adams' catalyst in 0.1 N NaOH[7] or with $NaBH_4$. The enzymic formation of "formylfolic

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