

BBA Report

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Exclusion of cyanate as intermediate in ornithine transcarbamoylase action

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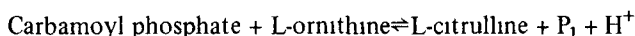
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SUMMARY

The failure of cyanate to dilute [^{14}C] carbamoyl phosphate during citrulline formation with ornithine transcarbamoylase (carbamoyl phosphate L-ornithine carbamoyltransferase, EC 2.1.3.3) excludes the participation of cyanate in the reaction which seems to proceed by a single displacement mechanism

Ornithine transcarbamoylase (carbamoyl phosphate L-ornithine carbamoyltransferase, EC 2.1.3.3) catalyzes the reaction



The equilibrium favors markedly citrulline synthesis¹. The enzyme has been extensively purified from many sources and it has been crystallized². However, the mechanism is poorly understood.

Reichard³, and Reichard and Hanshoff⁴ found lack of exchange between ^{32}P and carbamoyl phosphate in the absence of ornithine and lack of exchange between [^{14}C] ornithine and citrulline when phosphate was omitted. Therefore, it was concluded that the reaction probably involved a single-displacement mechanism. For such a mechanism the transcarbamoylase must activate the unreactive carbamoyl carbon of carbamoyl phosphate by increasing its electrophilic character. Additional evidence against the formation of a carbamoyl-enzyme is the lack of enzymic arsenolysis of carbamoyl phosphate⁵. Stark⁶ suggested, as another possibility, the enzymic dissociation of carbamoyl phosphate when ornithine is also bound to the enzyme with cyanic acid production which is then the true carbamoylating agent. This mechanism would be also compatible with the exchange experiments^{3,4}.

Since we found that carbamoylation of proteins with carbamoyl phosphate appears to proceed *via* cyanate, the possibility that enzymic transcarbamoylation also proceeded through cyanate, as suggested by Stark⁶, was tested. [^{14}C] Carbamoyl

phosphate was incubated with ornithine and ornithine transcarbamoylase in the presence of a large pool of cyanate. As illustrated in the table, cyanate did not inhibit the conversion of carbamoyl phosphate to citrulline. Moreover, there was no dilution of the ^{14}C . Similar results were obtained in duplicate experiment.

TABLE I

FAILURE OF CYANATE TO DILUTE [^{14}C] CARBAMOYL PHOSPHATE DURING CITRULLINE FORMATION WITH ORNITHINE TRANSCARBAMOYLASE

Each tube contained in 2 ml 50 mM Tris-HCl (pH 7.0) and [^{14}C] carbamoyl phosphate (specific activity, 3280 counts/min per μmole), ornithine, KCNO and ornithine transcarbamoylase as indicated. After 10 min at 30° , the reaction was stopped by addition of 1 ml of concentrated HClO_4 . After 30 min at room temperature dry ice was added and air bubbled through. Citrulline and ^{14}C were measured. Controls with cyanate alone were included to correct for any cyanate transformation.⁷ [^{14}C] Carbamoyl phosphate was prepared, essentially as described by Spector *et al.*⁸, from K^{14}CNO purchased from Amersham/Searle Corp. Purity was tested by conversion to citrulline⁹ and it was found free from $^{14}\text{CNO}^-$ as tested by paper chromatography (*n*-butanol-acetic acid-water, 60:20:20, v/v/v). L-Ornithine was from Sigma Chemical Co. KCNO was from Fisher Chemical Co. Ornithine transcarbamoylase was partially purified from frog liver.¹⁰

Additions			Ornithine transcarbamoylase (units) ¹¹	Citrulline formed (μmoles)	Specific activity (counts/min per μmole)
Carbamoyl phosphate (μmoles)	KCNO (μmoles)	Ornithine (μmoles)			
1	—	—	—	0.0	—
—	100	—	—	0.0	—
1	—	10	—	0.02	—
1	100	10	—	0.0	—
1	—	10	20	1.0	3080
1	100	10	20	0.96	3160

From these experiments the participation of cyanate in the ornithine transcarbamoylase reaction seems excluded. Moreover, the fact that neither cyanate nor azide are inhibitors¹¹ provides additional evidence that cyanate is not an intermediate.

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