BBA Report

BBA 61234

Exclusion of cyanate as intermediate in ornithine transcarbamoylase action

J CARRERAS, A CHABAS and S GRISOLIA

Department of Biochemistry and Molecular Biology University of Kansas Medical Center, Kansas City, Kan 66103 (U S A)

(Received October 11th, 1971)

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

$$\text{Carbamoyl phosphate} + \text{L-ornithine} \rightleftharpoons \text{L-citrulline} + P_i + H^+$$

The equilibrium favors markedly citrulline synthesis[1] The enzyme has been extensively purified from many sources and it has been crystallized[2] However, the mechanism is poorly understood

Reichard[3], and Reichard and Hanshoff[4] 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 phosphate[5] Stark[6] 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 possiblity that enzymic transcarbamoylation also proceeded through cyanate, as suggested by Stark[6], 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 citrulline[9] and it was found free from $^{14}CNO^-$ 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 inhibitors[11] provides additional evidence that cyanate is not an intermediate

This work was supported by a Grant-in-Aid from the U S Public Health Service AM 13119 and AM 01855 Dr J Carreras would like to acknowledge support of a National Institute of Health International Postdoctoral Fellowship

REFERENCES

1 P P Cohen and M Marshall, in P D Boyer, H Lardy and K Myrback, *The Enzymes*, Vol 6, Academic Press, New York, 1962, p 327
2 S H Bishop and S Grisolia, *Biochim Biophys Acta*, 139 (1967) 344
3 P Reichard, *Acta Chem Scand* , 11 (1957) 523
4 P Reichard and G Hanshoff, *Acta Chem Scand* , 10 (1956) 548
5 M E Jones, in S P Colowick and N O Kaplan, *Methods in Enzymology*, Vol 5, Academic Press, New York, 1962, p 903
6 G R Stark, *Biochemistry*, 4 (1965) 1030
7 E A Werner, *The Chemistry of Urea*, Longmans, Green and Co , New York, 1923
8 L Spector, M E Jones and F Lipmann, in S P Colowick and N O Kaplan, *Methods in Enzymology*, Vol 3, Academic Press, New York, 1957, p 653
9 D Hunninghake and S Grisolia, *Anal Biochem* , 16 (1966) 200
10 J Caravaca and S Grisolia, *J Biol Chem* , 235 (1960) 684
11 M Nakamura and M E Jones, in H Tabor and C W Tabor, *Methods in Enzymology*, Vol 17A, Academic Press, New York, 1970, p 286