ORNITHINE DECARBOXYLASE FROM ESCHERICHIA COLI: STIMULATION OF THE ENZYME ACTIVITY BY NUCLEOTIDES

E. Hölttä, J. Jänne and J. Pispa

Department of Medical Chemistry, University of Helsinki, SF-00170 Helsinki 17, Finland

Received May 1, 1972

Summary. Ornithine decarboxylase (L-ornithine carboxy-lyase, EC 4.1.1.17) has been purified about 100-fold from cells of Escherichia coli F. The partially purified enzyme required pyridoxal phosphate and exhibited optimal activity at pH 8.5. The apparent K_m value for L-ornithine was 2 mM. At every stage of purification ornithine decarboxylase activity was enhanced by nucleotides, especially by nucleotide triphosphates, guanosine and deoxyguanosine triphosphate showing the highest affinity for the enzyme. In the presence of saturating concentrations of guanosine triphosphate the K_m value for L-ornithine was lowered to about one tenth of that in the absence of the nucleotide. The apparent K_m for guanosine triphosphate as the activator of ornithine decarboxylase was below 0.001 mM.

In E. coli, growing in neutral minimal medium, at least two pathways of putrescine biosynthesis are known (1,2): (a) direct decarboxylation of ornithine by ornithine decarboxylase and (b) decarboxylation of arginine by arginine decarboxylase (L-arginine carboxy-lyase, EC 4.1.1.19) and subsequent hydrolysis of the guanidino group of agmatine by agmatine ureohydrolase (EC 3.5.3.7) to yield putrescine. It also appears that E. coli possesses the genetic information for two different ornithine and arginine decarboxylases: one catabolic or inducible (3) and the other biosynthetic or constitutive (1,2) decarboxylase for both ornithine and arginine. The inducible and constitutive decarbosylases differ from each other in several properties as well as in conditions for formation (1). In contrast to the inducible decarboxylases (3,4,5) the constitutive decarboxylases have not been extensively characterized up to now.

In the present communication we have partially purified ornithine decarboxylase from cells of E. coli F growing in minimal medium. The enzyme had an alkaline pH optimum, required pyridoxal phosphate for optimal activity and was stimulated by various nucleotides. Guanosine and deoxyguanosine triphosphates were particularly effective activators in minute concentrations.

MATERIAL AND METHODS

Chemicals

DL-ornithine-1-^{14}C (specific radioactivity 37 mCi/mmole) was purchased from the Radiochemical Centre (Amersham) and treated before use as described earlier (6,7). ATP, ADP, dATP, dCTP and dGTP were purchased from Boehringer & Soehne, Mannheim, GDP from E.Merck AG, Darmstadt, AMP from Schwarz Laboratories, Mount Vernon, GTP, UTP and CTP from Calbiochem and the rest of nucleotides and related compounds used were obtained from Sigma. All the compounds were of highest or analytical grade of purity.

Assay of ornithine decarboxylase activity

The activity of ornithine decarboxylase was assayed by a modification of the method for the mammalian enzyme described earlier (6,7). The incubation mixture contained the following ingredients: 100 mM Tris-HCl buffer, pH 8.5 (at 20°), 5 mM dithiothreitol, 0.4 mM pyridoxal phosphate, 5 mM L-ornithine-1-^{14}C and the enzyme protein. The incubation were carried out at 37° for 30 min in glass centrifuge tubes as described earlier (7). The reaction was linear at least for 30 min and proportional to the amount of enzyme protein added.

The protein was measured by the method of Lowry *et al.* (8).

Partial purification of ornithine decarboxylase activity from E.coli

Cells of *E.coli* F were grown in a Tris maleate-buffered minimal medium containing 0.4% glucose at neutral pH (9) at 37° with vigorous aeration. The cells from middle log phase cultures (about 5 x 10^8 cells per ml) were chilled, centrifuged and resuspended in 10 mM Tris-HCl buffer, pH 7.5 (at 20°) containing 10 mM 2-mercaptoethanol and 0.1 mM EDTA (standard buffer). About 10 g of *E.coli* cells in 100 ml of the above buffer were disrupted in a Branson Sonifier using ethanol-ice bath for cooling. The sonication was performed at full power for 15 seconds at a time, for a total of 5 min. The sonicate was centrifuged at 20 000 x g_{max} for 20 min and the supernatant fraction was used for further purification. The sonic extract (Fraction 1) was fractionated with solid ammonium sulphate (Mann, enzyme grade) at 0°. The proteins precipitated between 0.4 and 0.6 saturation of ammonium sulphate were dissolved in 10 ml of the standard buffer and passed through a Sephadex G-25 column previously equilibrated with the standard buffer. The desalted ammonium sulphate fraction (Fraction 2) was applied to a DEAE cellulose column (Whatman, DE 52, 3 x 30 cm) which had been equilibrated with the standard buffer.

The column was washed with 150 ml of the buffer used for equilibration and connected to a linear NaCl gradient from 0.1 to 0.4 M in the same buffer (total gradient volume was 1000 ml). Ornithine decarboxylase activity was eluted at about 850 ml after the connection of the gradient. The ten most active fractions were pooled (Fraction 3, 103.5 ml), concentrated to 6.5 ml in a pressurized ultrafiltration cell (Amicon corp.) and applied to a Sephadex G-200 column (2.5 x 30 cm) previously equilibrated against the standard buffer. Ornithine decarboxylase activity emerged from the column at 1.45 times the void volume. Four most active fractions were pooled (Fraction 4), concentrated and subjected to second gel filtration on the same Sephadex G-200 column. Six most active fractions were pooled as designated as Fraction 5.

Table 1

Partial purification of ornithine decarboxylase activity from E.coli F

Fraction	Total activity μmoles of CO_2/ 30 min	Specific activity μmoles of CO_2/ 30 min per mg protein	Purification -fold
1. Sonic extract	510	0.367	
2. Ammonium sulphate fraction (0.4-0.6 saturation)	329	0.467	1.3
3. DEAE cellulose	22.5	0.963	2.6
4. Sephadex G-200 I	46.0	13.64	37
5. Sephadex G-200 II	27.4	36.83	100

Sonic extract was prepared from about 10 g of E.coli cells and processed as described in the text. The marked decrease in the total activity after DEAE cellulose column and the recovery of the activity after Sephadex G-200 column was due to salt (present in the pooled DEAE fraction) inhibition of the enzyme (see also text).

RESULTS AND DISCUSSION

Table 1 summarizes the purification procedure used for E.coli ornithine decarboxylase. The overall purification was about 100-fold with a yield of about 5%. The crude sonic extract catalyzed a stoichiometric release of radioactive carbon dioxide from ornithine-1-^{14}C and formation of putrescine-^{14}C from ornithine-5-^{14}C. This stoichiometry was kept through the whole purification procedure. The partially purified ornithine decarboxyl-

ase (Fraction 5) was stabile for at least three weeks when stored at 0°. After ammonium sulphate fractionation the enzyme showed a relative requirement for exogenous pyridoxal phosphate since the omission of pyridoxal phosphate from the incubation mixture resulted in a decrease of about 80% in the enzyme activity. This finding differs from the absolute pyridoxal phosphate requirement reported by Morris and Pardee (2) for E.coli K-12 ornithine decarboxylase. The addition of isonicotinic acid hydrazine (5 mM), semicarbazide (5 mM) or canaline (2 mM) to the incubation mixture in the absence of exogenous pyridoxal phosphate inhibited the enzyme by 90-100% further suggesting the participation of pyridoxal phosphate in the catalytic activity. The refined enzyme showed a sharp pH optimum at about 8.5 in Tris-HCl at 20°

Table 2

Effect of nucleotides on ornithine decarboxylase activity

	Addition	mM	Ornithine decarboxylase activity	Change (%)
Experiment 1	None	-	20.30	-
	ATP	1.0	57.58	+183
	ADP	1.0	28.22	+ 39
	AMP	1.0	25.27	+ 24
	cyclic AMP	1.0	23.87	+ 18
	Adenosine	1.0	25.08	+ 24
	UTP	1.0	56.29	+177
	UDP	1.0	46.03	+127
	UMP	1.0	25.34	+ 25
	CTP	1.0	37.23	+ 83
	GTP	1.0	93.01	+358
	GMP	1.0	80.11	+294
Experiment 2	None	-	23.64	-
	dATP	1.0	19.42	- 18
	dCTP	1.0	26.62	+ 12
	dGTP	1.0	85.13	+260
	ATP	1.0	55.87	+136
	CTP	1.0	34.20	+ 44
	GTP	1.0	88.35	+273
Experiment 3	None	-	21.12	-
	Guanine	1.0	20.06	- 6
	Guanosine	1.0	20.42	- 4
	Deoxyguanosine	1.0	27.35	+ 29
	GTP	1.0	74.55	+252

Ornithine decarboxylase activity was assayed in the absence or presence of nucleotides using Fraction 4 preparation (5.4 μg) as the source of the enzyme. Standard incubation conditions were used except that the concentration of L-ornithine was reduced to 1 mM. The enzyme activity is expressed as nmoles of CO_2 released per 30 min.

and practically no activity at pH values of 4 to 5 indicating that the enzyme was rather constitutive than inducible (1,2). Fraction 4 enzyme preparation formed a single symmetric peak of activity in isoelectric focusing at pH 4.0 to 4.5. Putrescine acted as a weak product inhibitor. Spermidine and spermine at a concentration of 5 mM in the presence of 1 mM L-ornithine caused 60 and 80% inhibition, respectively. This is in good agreement with the data recently reported by Morris et al. (10).

At every stage of the purification the enzyme activity was stimulated by various nucleotides at subsaturating concentrations of L-ornithine. In the presence of 1 mM ornithine the crude extract was stimulated about two-fold by the addition of 1 mM GTP; under similar conditions GTP produced a stimulation of 4 to 5-fold when Fractions 4 to 5 were used as the source of the enzyme. Accordingly, the overall purification of ornithine decarboxylase was about 200-fold when the assays were made in the presence of nucleotides.

Table 2 lists the effect of various nucleotides on ornithine decarboxylase activity. At a concentration of 1 mM in the presence of subsaturating concentrations of L-ornithine (1 mM) it seemed that the ribonucleotide tri-

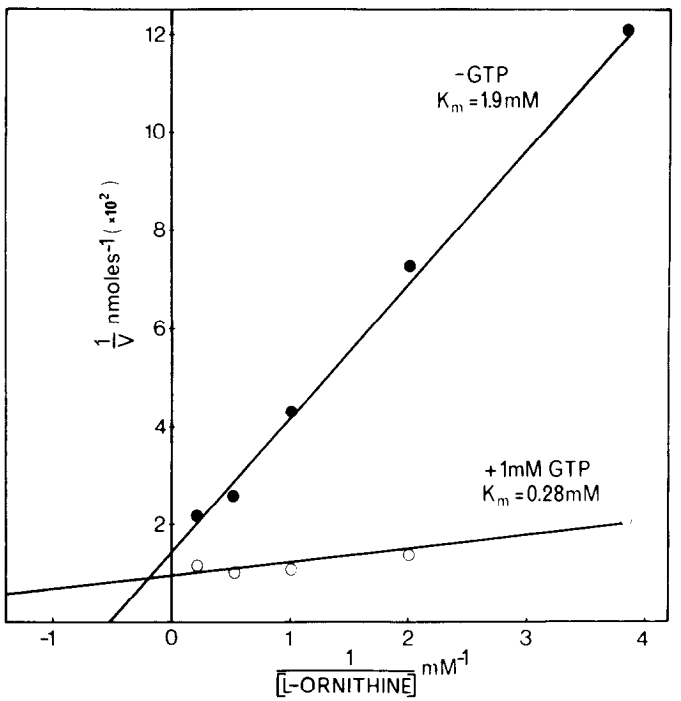

Fig. 1. Effect of ornithine concentration on ornithine decarboxylase activity in the presence or absence of 1 mM GTP. Ornithine decarboxylase activity was assayed under standard incubation conditions using 5.2 µg of Fraction 4 preparation as enzyme. The double reciprocal lines were plotted by computer by the least squares method.

phosphates, dGTP and some other ribonucleotide mono- and diphosphates were best activators for the enzyme. Maximal stimulation was achieved with GTP which, as did dGTP, showed the far highest 'affinity' for ornithine decarboxylase.

Fig. 1 shows the effect of substrate concentration on ornithine decarboxylase activity. In the absence of GTP the apparent K_m value for L-ornithine was about 2 mM. In the presence of GTP (1 mM) the apparent K_m value was lowered to about one tenth of that in the absence of the nucleotide.

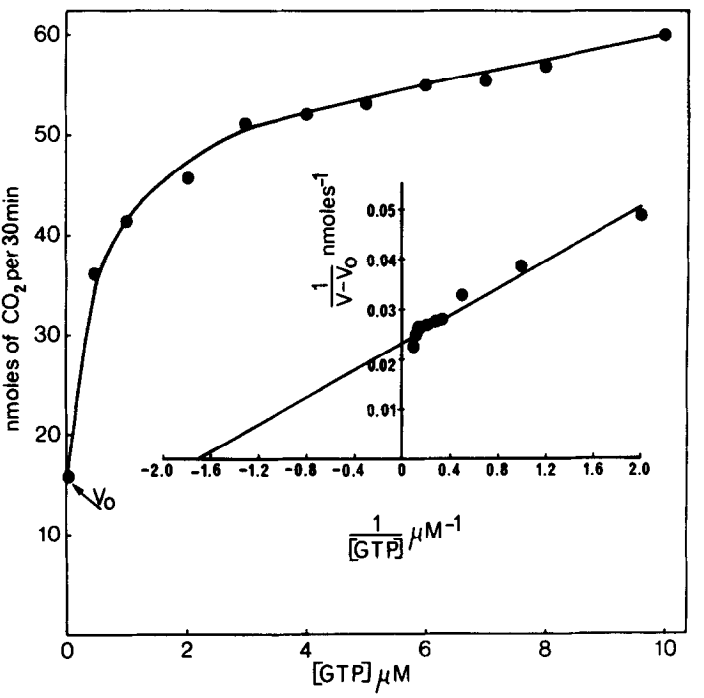

Fig. 2. Effect of GTP concentration on ornithine decarboxylase activity. Ornithine decarboxylase activity was assayed under standard incubation conditions except that the concentration of L-ornithine was reduced to 1 mM. Fraction 5 preparation (1.9 μg) was used as the source of the enzyme. V_o, the activity in the absence of GTP, V, the activity in the presence of GTP.

Fig. 2 shows the effect of GTP concentration on ornithine decarboxylase activity in the presence of 1 mM L-ornithine. As shown, the apparent K_m value for GTP as the activator of the enzyme (Fraction 5) was extremely low, below 1 μM. On the molar basis GTP and also dGTP appeared to be the far most active nucleotide studied. The apparent K_m as effector for ATP, UTP, GMP and GDP was in the range of 0.1 to 0.4 mM. Cyclic GMP was slightly inhibitory for ornithine decarboxylase. In several experiments

where the concentration of GTP was varied at subsaturating levels of substrate, the double reciprocal lines for the measurement of the effector K_m for GTP were consistently slightly concave downward thus indicating a possibility of negative co-operativity (11).

The mechanism of the action of GTP and other nucleotides on ornithine decarboxylase activity of E.coli is still unknown. Interestingly, the activity of ornithine decarboxylase seems to be sensitive to high ionic strength but was considerably protected by the presence of 1 mM GTP. This might suggest the interaction of GTP with the possible subunits of the enzyme. The physiological significance, if any, of the nucleotide stimulation of ornithine decarboxylase is not clear. The minute amounts of GTP needed for maximal stimulation of ornithine decarboxylase might indicate that this or some other nucleotide could be an integral part of the enzyme molecule and absolutely necessary for the catalytic activity. This is contrast to the mammalian ornithine decarboxylase which seems to be relatively insensitive to any low molecular weight effect studied so far (7, 12).

ACKNOWLEDGEMENTS

This work has been supported by grants from the National Research Council for Medical Sciences, Finland and from the Sigrid Jusèlius Foundation. The skilful technical assistance of Mrs. Riitta Leppänen, Mrs. Sirkka Kanerva and Mrs. Alli Viljanen is gratefully acknowledged.

REFERENCES

1. Morris, D.R., and Pardee, A.B., Biochem.Biophys.Res.Commun., 20, 697 (1965).
2. Morris, D.R., and Pardee, A.B., J. Biol. Chem., 241, 3129 (1966).
3. Gale, E.F., Biochem. J., 34, 392 (1940).
4. Blethen, S.L., Boeker, E.A., and Snell, E.E., J. Biol. Chem., 243, 1671 (1968).
5. Boeker, E.A., and Snell, E.E., J. Biol. Chem., 243, 1687 (1968).
6. Raina, A., and Jänne, J., Acta Chem. Scand., 22, 2375 (1968).
7. Jänne, J., and Williams-Ashman, G.H., J. Biol. Chem., 246, 1725 (1971).
8. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J., J. Biol. Chem., 193, 265 (1951).
9. Oleson, A.E., Pispa, J.P., and Buchanan, J.M., Proc.Natl.Acad.Sci., U.S., 63, 473 (1969).
10. Morris, D.R., Wu, W.H., Applebaum, D., and Koffron, K.L., Ann. N.Y. Acad. Sci., 171, 968 (1970).
11. Koshland, D.E., Jr., in "The Enzymes", Vol. I, Boyer, P.D. (ed.), Academic Press, New York, p. 341 (1970).
12. Friedman, S.J., Halpern, K.V., and Canellakis, E.S., Biochim. Biophys. Acta, 261, 181 (1972).