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Effects of Cations on Amino Acid Decarboxylases

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(Received 8 October 1957)

Metzler, Ikawa & Snell (1954) have proposed a general mechanism for reactions catalysed by pyridoxal in which there is a requirement for a multivalent metal ion; a metal chelate of the Schiff base formed from pyridoxal phosphate and the amino acid is postulated as an intermediate. Most of their evidence in support of this theory was obtained from non-enzymic reactions of amino acids catalysed by pyridoxal which required one of the metal ions Cu^{2+} , Fe^{3+} or Al^{3+} for activity. Guirard & Snell (1954) found that the histidine decarboxylase of *Lactobacillus* 30a required pyridoxal phosphate and Fe^{3+} or Al^{3+} ions for activity, other cations being relatively ineffective or inhibitory. A requirement for Fe^{3+} ions was also found by McCullough, Pilgian & Daniel (1957) for an enzyme in *Escherichia coli* O111:B4, which decarboxylated aminobenzoates in the presence of pyridoxal phosphate. Copper ions are known to inhibit many bacterial amino acid decarboxylases (Taylor & Gale, 1945).

It was therefore thought to be of interest to test whether an activation by multivalent cations is a characteristic property of other amino acid decarboxylases. The experiments described in this paper show that many bacterial decarboxylases are activated by bivalent cations.

EXPERIMENTAL

The strains of *Escherichia coli* and *Clostridium welchii*, their growth, collection and washing and the preparation of cell-free extracts were as previously described (Eggleson, 1957). Dialysis of cell-free extracts was carried out against water at 2° for 18–24 hr.

Amino acid decarboxylase activities of bacterial preparations were examined in a Warburg apparatus at 25°. In most experiments the cups contained 0.2 ml. of *m*-sodium acetate buffer, pH 4.5, 20 μg . of pyridoxal phosphate, 0.6–10 mg. dry wt. of enzyme; metal salt solution (where added) was in the main compartment, and 0.2 ml. of 0.25 *M*-L-amino acid was in the side bulb. The final volume in each cup was 2 ml. The histidine decarboxylase of *E. coli* F was found to have a higher pH optimum than the other bacterial enzymes; these experiments were therefore carried out at pH 5.5.

Extracts were made of vegetable marrow or orange fruit, by macerating the tissue in a Waring Blender and straining the pulp through muslin, followed by dialysis against ice-water overnight. Experiments with the glutamic acid decarboxylase in citrus fruit (Axelrod, Jang & Lawrence, 1955) and in marrow were carried out in a total volume of 4 ml., at least half of which consisted of enzyme solution. The cups also contained 0.4 ml. of *m*-acetate buffer, pH 5.5, 20 μg . of pyridoxal phosphate and 0.2 ml. of 0.25 *M*-L-glutamate.

Brains from rats were ground up in a mortar with sand and 2 vol. of water and then dialysed for about 5 hr. against ice-water. Each Warburg cup contained 3 ml. of enzyme, 0.2 ml. of *m*-sodium phosphate buffer, pH 6.8, 20 μg . of pyridoxal phosphate and 0.2 ml. of 0.25 *M*-L-glutamate in a total volume of 4 ml. The gas space was filled with N_2 , a freshly scraped stick of yellow phosphorus placed in the centre well and the incubation carried out at 35°.

The cations used were in the form of solutions of: KCl , Li_2SO_4 , NH_4Cl , AgNO_3 , MgCl_2 , MnCl_2 , CoCl_2 , CaCl_2 , BaCl_2 , NiSO_4 , FeSO_4 , ZnCl_2 , CuSO_4 , SnCl_2 , HgCl_2 , FeCl_3 , AlCl_3 (SnCl_2 and FeCl_3 were dissolved in 0.01 *N*-HCl; FeSO_4 was dissolved in water immediately before use). Unless otherwise stated, the final concentration of the salt added to the Warburg cups was *mm*. Each cation effect was examined at least twice with each of the enzymes tested.

RESULTS

Bacterial enzymes. The results shown in Table 1 indicate that some bivalent cations accelerate the decarboxylations of L-lysine, L-arginine, L-glutamate and L-ornithine. The largest activations (40–50 %) and the most extensive (by seven cations) were found with the L-lysine decarboxylase of *E. coli* 8571. In this enzyme system, Mg^{2+} , Mn^{2+} , Co^{2+} , Ca^{2+} , Ba^{2+} , Ni^{2+} and Fe^{2+} ions were about equally effective, Al^{3+} ions about half as active and Zn^{2+} , Sn^{2+} and Fe^{3+} ions were without effect. The cations Cu^{2+} , Hg^{2+} and Ag^{+} were very strongly inhibitory, as already noted by Taylor & Gale (1945). Other strains and other decarboxylases were most frequently activated by Mg^{2+} , Mn^{2+} and Co^{2+} ions. There were considerable differences between the eight enzymes, e.g. an activation by Ca^{2+} or Fe^{2+} ions was found with only three, and by Ni^{2+} ions with only four, of these enzymes. Zinc ions had no effect where tested and in the same systems Sn^{2+} ions were inhibitory. The L-histidine decarboxylase of *E. coli* F, however, was not activated by any of the nine metal ions tested; six of these were inhibitory. The univalent cations K^{+} , Li^{+} and NH_4^{+} had little or no effect on the decarboxylations of lysine, arginine or ornithine. With seven of the eight enzymes, Fe^{3+} and Al^{3+} ions were inhibitory to varying degrees.

These activations by bivalent cations were obtained at the optimum pH of the enzymes. No activation was found at the neutral point, even when the enzymes were stimulated by the addition of inorganic phosphate (Eggleson, 1957).

Table 2 shows that the addition of Mg^{2+} ions had a greater effect in the presence of pyridoxal phosphate than in its absence. An exception to this was seen with the histidine decarboxylase, where only the coenzyme activated.

Although dialysis reduced the activity of the decarboxylases the activating effect of adding Mg^{2+} ions alone (without pyridoxal phosphate) was found to be the same as before dialysis (see Table 3).

The effect of a chelating agent, ethylenediamine-tetra-acetate (EDTA) is shown in Table 4. Its inhibitory effect on lysine and glutamate decarboxylations was only small and could be reversed by the addition of extra Mg^{2+} ions.

Plant-tissue and brain glutamic decarboxylases. Dialysed extracts of vegetable marrow and orange fruit which decarboxylated L-glutamic acid were not activated by any of the cations tested. The orange enzyme was inhibited by Al^{3+} ions, and both the marrow and orange enzymes were inhibited by Fe^{2+} and Fe^{3+} ions. The bivalent cations Mg^{2+} , Mn^{2+} , Co^{2+} , Ca^{2+} , Ba^{2+} and Ni^{2+} had no effect. The dialysed brain enzyme was inhibited by Fe^{2+} , Co^{2+} and Ni^{2+} ions. Other cations had no

Table 1. Effect of cations on bacterial amino acid decarboxylases

Organism	Amino acid	Cation added											Control (μ l. of CO ₂)			
		K ⁺	Li ⁺	NH ₄ ⁺	Ag ⁺	Mg ²⁺	Mn ²⁺	Co ²⁺	Ca ²⁺	Ba ²⁺	Ni ²⁺	Fe ²⁺		Fe ³⁺	Al ³⁺	
<i>E. coli</i> N.C.I.B. 8571	L-Lysine	0	0	+12	-97	+46	+47	+40	+43	+46	+39	+39	-96	-6	-21	237
<i>E. coli</i> F	L-Arginine	0	0	0	—	+18	+6	0	0	0	-10	-7	0	-38	-73	318
<i>E. coli</i> F	L-Histidine	—	—	—	—	0	0	-17	0	-7	-21	-16	—	—	-63	122
<i>E. coli</i> 4	L-Arginine	—	—	—	—	+20	+11	+14	0	0	0	-8	—	—	-54	195
<i>E. coli</i> 4	L-Lysine	—	—	—	—	+28	+12	+18	0	+5	+8	0	—	—	-67	232
<i>E. coli</i> 4	L-Glutamate	—	—	—	—	+20	0	+13	0	+10	+11	-8	—	—	-20	206
<i>E. coli</i> 4	L-Ornithine	0	0	0	—	+21	+19	+22	+23	+6	+10	+9	-77	0	-50	91
<i>C. welchii</i> S.R. 12	L-Glutamate	—	—	—	—	+14	+12	+19	+11	+9	0	—	—	—	-18	280

The experimental conditions are described in the text. The concentration of each cation was mM. With the first three organisms dialysed cell-free extracts were used; otherwise whole cells were used. Results are average percentage changes (greater than 5%) in the rates of evolution of CO_2 in the first 30 min. of incubation at 25°. The last column shows the CO_2 output in the control, where no cation was added.

Table 2. *Effect of magnesium ions on decarboxylases in the absence and in the presence of pyridoxal phosphate*

Dialysed cell-free extracts of *E. coli*, strain 8571 with L-lysine, strain F with L-arginine and L-histidine, were used. Pyridoxal phosphate (20 μ g.) and mm-MgCl₂ were added; other details are as in the text. Results show μ l. of CO₂ liberated/60 min. at 25°.

	Pyridoxal phosphate absent			Pyridoxal phosphate present		
	No additions	Mg ²⁺ ions	Increase due to Mg ²⁺ ions	Pyridoxal phosphate	Pyridoxal phosphate and Mg ²⁺ ions	Increase due to Mg ²⁺ ions
L-Lysine	163	215	+ 52	353	447	+ 94
L-Arginine	220	226	+ 6	316	372	+ 56
L-Histidine	87	86	- 1	126	121	- 5

Table 3. *Effect of dialysis on the activation of L-lysine decarboxylase by magnesium ions without added pyridoxal phosphate*

Cell-free extract of *E. coli* 8571; other details are as in the text, except that pyridoxal phosphate was omitted. Results are μ l. of CO₂ liberated/30 min. at 25°.

	Additions		Increase due to Mg ²⁺ ions
	None	Mg ²⁺ ions (mm)	
Enzyme not dialysed	322	427	+ 105
Enzyme dialysed	237	341	+ 104

effect, except for Mn²⁺ and Ca²⁺, which gave activations of about 10% in some experiments but not in others, possibly owing to variations in the removal of diffusible material. The addition of EDTA (0.0025M) had no effect on either the plant-tissue or brain enzymes.

DISCUSSION

The experiments reported here show that activation by bivalent cations is widespread among bacterial amino acid decarboxylases which require pyridoxal phosphate. An exception is the histidine decarboxylase of *E. coli* F, which was stimulated by pyridoxal phosphate, but not by any of the cations tested. The plant-tissue and brain glutamic acid decarboxylases were not stimulated by bivalent cations, which may have been due to a sufficient amount of enzyme-bound cation, not removed by dialysis.

The effect of the cations in the bacterial systems

can be accounted for by the hypothesis of Metzler *et al.* (1954). This hypothesis is given some support by the data of Table 2, which shows that the rates of decarboxylation of lysine and arginine by *E. coli* were highest when Mg²⁺ ions and the coenzyme were both present.

Other enzymes requiring pyridoxal phosphate have recently been shown to be activated by Mg²⁺ ions, e.g. heart-muscle glutamic-oxaloacetic transaminase (Happold & Turner, 1957), kynureninase of *Neurospora* (Jakoby & Bonner, 1953) and cystathionase of *E. coli* (Wijesundera & Woods, 1953). On the other hand, an activation by the univalent ions K⁺, NH₄⁺ and Rb⁺ has been reported for the pyridoxal phosphate-requiring tryptophanase of *E. coli* (Happold & Struyvenberg, 1954). Blakley (1955) found stimulation of serine synthesis by pyridoxal phosphate in fractionated rabbit-liver preparations but no activation by cations or inhibition by EDTA, and concluded that this enzyme system may not require any metal ion.

It appears, then, that in some enzyme systems pyridoxal phosphate acts in conjunction with either multi- or univalent cations, whereas in others its action may be independent of cations.

SUMMARY

1. The activity of seven amino acid decarboxylases in *Escherichia coli* and *Clostridium welchii* acting upon L-lysine, L-arginine, L-ornithine and L-glutamate was increased (up to about 45%) by some or all of the bivalent cations Mg²⁺, Mn²⁺, Co²⁺, Ca²⁺, Ba²⁺, Ni²⁺ and Fe²⁺. The activation with Mg²⁺ ions was greater in the presence than in

Table 4. *Effect of ethylenediaminetetra-acetate on amino acid decarboxylases in dialysed bacterial extracts*

Dialysed cell-free extracts of *E. coli*, strain 8571 with L-lysine, strain F with L-glutamate, were used; other details are as in the text. Results are μ l. of CO₂ liberated in 30 min. (with L-lysine), in 90 min. (with L-glutamate), at 25°.

	Additions			
	None	Mg ²⁺ ions (mm)	EDTA (mm)	EDTA (mm) + Mg ²⁺ ions (2 mm)
L-Lysine	125	202	99	208
L-Glutamate	307	350	285	340

the absence of pyridoxal phosphate. A histidine decarboxylase of *Escherichia coli* F was stimulated by pyridoxal phosphate but not by any of the nine metal ions tested.

2. Ethylenediaminetetra-acetate had only a small inhibitory effect, which was reversed by addition of Mg^{2+} ions.

3. With the same group of enzymes, K^+ , Li^+ and NH_4^+ ions had little or no effect and, except with lysine decarboxylase of *Escherichia coli* 8571, the trivalent ions Fe^{3+} and Al^{3+} were inhibitory.

4. The L-glutamic acid decarboxylases of vegetable marrow, orange fruit or rat brain were not stimulated by cations nor inhibited by ethylenediaminetetra-acetic acid.

5. The results are discussed in relation to the hypothesis of Metzler *et al.* (1954).

This work was aided by a grant from the Rockefeller Foundation to this Department.

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