# ON THE ACTIVATION MECHANISM OF L-ASPARTATE β-DECARBOXYLASE FROM PSEUDOMONAS DACUNHAE BY α-KETOGLUTARATE*

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Received May 10, 1968

L-Aspartate β-decarboxylase [L-aspartate 4-carboxy-lyase (E.C. 4.1.1.12)] has been isolated in apparently homogeneous form from Alcaligenes faecalis (Novogrodsky et al., 1963 ; Novogrodsky and Meister, 1964 ; Wilson and Meister, 1966) and in crystalline form from Achromobacter d-15 (Wilson, 1963 ; Wilson and Kornberg, 1963) and from Pseudomonas dacunhae (Chibata et al., 1967).

These enzymes are markedly activated by a small amount of α-keto acids. Novogrodsky et al. elucidated the activation mechanism by α-keto acids as follows : this enzyme has both L-aspartate decarboxylase activity and transaminase activity of broad specificity, and the transaminase activity contributed to the conversion of inactive PAMP-enzyme, which formed during the course of decarboxylation, to active PALP-enzyme. This mechanism, however, cannot explain the phenomenon that α-keto acids more activate the enzyme than PALP does.

This communication describes the activation mechanism of crystalline L-aspartate β-decarboxylase from Pseudomonas dacunhae by α-ketoglutarate.

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* This work was presented at the 19th Symposium on Enzyme Chemistry, Kanazawa, Japan. April 9, 1968 (Kakimoto et al.).

Crystalline aspartate β-decarboxylase was prepared as previously described by us (Chibata et al., 1967). Decarboxylase activity was assayed manometrically at 30°. The evolution of $CO_2$ was measured for initial 5 min. after the addition of L-aspartate. The main compartment of Warburg flasks contained sodium acetate buffer (pH 5.3), 400 μmoles; bovine serum albumin, 2 mg; sodium α-ketoglutarate, 5 μmoles; and the enzyme, 15 or 128.6 μg. Sodium L-aspartate buffered by 200 μmoles of the same buffer was added from the side arm after equilibration for 10 min. Total volume was 3.2 ml. Enzyme activity was expressed as μl $CO_2$ released per hr. per mg of protein under these conditions.

To investigate the activation mechanism of this enzyme by α-ketoglutarate, we examined whether our enzyme has transaminase activity or not. Using the following assay system, the formation of L-glutamic acid was examined by glutamic dehydrogenase method (Bergmeyer, 1963).

Assay system :

| | | |
|---|---|---|
| sodium acetate buffer (pH 5.3) | 600 | μmoles |
| L-amino acid | 200 | μmoles |
| α-ketoglutarate | 5 | μmoles |
| PALP | 4 | μmoles |
| bovine serum albumin | 2 | mg |
| enzyme | 1.02 | mg |

Total volume was made to 3.2 ml. After preincubation for 10 min. at 30°, the enzyme and the other constituents were mixed and incubated for 1 hr.

The formation of L-glutamate was detected only when L-aspartate was used as L-amino acid. Although, the transaminase activity was very low and almost negligible comparing to the decarboxylase activity. That is, the former was only 3 μmoles/hr/mg protein, corresponding approximately 1/1,300 of the latter. The ratio of the activities during the purification and heat denaturation indicates that these two activities were catalyzed by the single enzyme.

Novogrodsky et al. reported that when the enzyme from Alcaligenes was preincubated with a variety of amino acids, the conversion of the PALP-enzyme to PAMP-enzyme occurred and the enzyme was inactivated. In contrast to this, our enzyme from Pseudomonas was not inactivated by incubation with a number of amino acids.

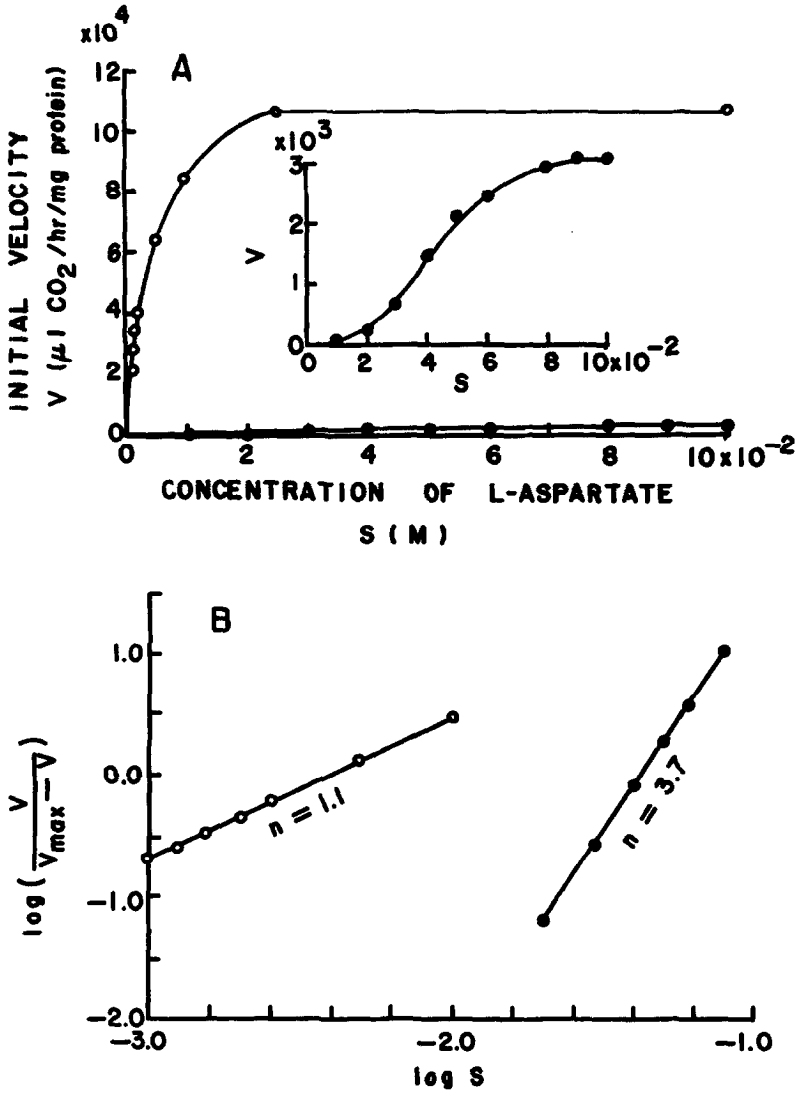


Fig. 1. The effect of aspartate concentration on initial velocity in the presence ( —○— ) and absence ( —●— ) of α-ketoglutarate. A : initial velocity with respect to aspartate concentration, B : Hill plots of data from A.

These observations suggest that α-ketoglutarate does not activate the Pseudomonas enzyme by means of transamination, but activates by other mechanism.

The effect of the substrate concentration on initial velocity is shown in Fig. 1A. In the absence of α-ketoglutarate, the enzyme follows sigmoid kinetics with respect to aspartate. In the presence of α-ketoglutarate, however, the enzyme gives normal hyperbolic kinetics. The $V_{max}$ for aspartate in the absence of α-ketoglutarate is 3,000, in the presence of α-ketoglutarate is 106,000. The apparent Km for aspartate is 100 mM in the absence of α-ketoglutarate and 5.5 mM in the presence of α-ketoglutarate. This fact may be due to that the binding of α-ketoglutarate to the enzyme increases the catalytic activity. The Hill plots (Brown and Hill, 1922-1923) are given in Fig. 1B. In the presence of α-ketoglutarate the slope(n) was 3.7, and it became 1.1 by addition of α-ketoglutarate.

These observations strongly suggest that this enzyme is a typical allosteric protein and α-ketoglutarate is an allosteric activator (Monod et al., 1965).

In some allosteric enzymes, reversible dissociation or association occurs, which may or may not be accompanied by a loss in catalytic activity or by marked changes in susceptibility to allosteric effects.

Ultracentrifugation studies revealed that α-ketoglutarate does not affect the sedimentation constants of this enzyme ($S_{20,w}$ = 19.1 S, C = 1.06).

Details of the studies on the activation mechanism of this enzyme will be published elsewhere.

ACKNOWLEDGEMENTS

We are indebted to Dr. K. Kakiuchi of Institute for Protein Research, Osaka University for performing the ultracentrifugation experiments. Thanks are also due to Mr. T. Takayanagi, and Dr. K. Fujii for their encouragements in this work and Miss T. Nakagawa for technical assistance.

REFERENCES

Bergmeyer, Hans-Urich, Methods of Enzymatic Analysis, Academic Press, 1963, P. 384.

Brown, W.E.L., and Hill, A.V., Proc. Roy. Soc. (London), Ser. B. 94, 297 (1922-1923).

Chibata, I., Kakimoto, T., Kato, J., Shibatani, T., and Nishimura, N., Biochem. Biophys. Res. Commun., 26, 662 (1967).

Kakimoto, T., Kato, J., Shibatani, T., Nishimura, N., and Chibata, I., Proceedings of the 19th Symposium of Enzyme Chemistry, 1968, P. 207.

Monod, J., Wyman, J., and Changeux, J.P., J. Mol. Biol., 12, 88 (1965).

Novogrodsky, A., Nishimura, J.S., and Meister, A., J. Biol. Chem., 238, 1903 PC (1963).

Novogrodsky, A., and Meister, A., J. Biol. Chem., 239, 879 (1964).

Wilson, E.M., Biochim. Biophys. Acta, 67, 345 (1963).

Wilson, E.M., and Kornberg, H.L., Biochem. J., 88, 578 (1963).

Wilson, E.M., and Meister, A., Biochemistry, 5, 1166 (1966).