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Short Communication

Characterization of Some Bacterial Glutamate Synthase

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細菌 Glutamate Synthase의 特性

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Since its existence was first demonstrated by Meers et al., (1970)[1] glutamate synthase has been found in many bacteria and plants.[2,3] It is generally accepted that the glutamine synthetase-glutamate synthase pathway is a major route of entry of ammonia into glutamate in bacteria, which operates in the presence of the low concentrations of ammonia. The regulation mechanism of glutamine synthetase activity and synthesis in *Escherichia coli* has been studied in detail by Holzer, Stadtman and colleagues.[4] Comparative studies have been made on the enzymes of many other organisms and on their control systems.[4] On the other hand, even though glutamate synthase has been purified from several bacteria,[2,4] little is known about this enzyme, especially its catalytic and molecular properties, for understanding the function and control system of the glutamine synthetase-glutamate synthase pathway. In the preceding papers describing glutamate synthase of *Brevibacterium flavum*, we found that the properties of the enzyme such as molecular weight, reaction mechanism and so on were different from those of *E. coli* glutamate synthase.[5,6] Yelton and Yoch reported that molecular weight of glutamate synthase of *Rhodospirillum rubrum* was different when it was prepared in the presence and in the absence of sodium chloride or potassium chloride.[7] This indicates that it is necessary to compare the properties of the enzymes prepared by different procedures and sources, because *B. flavum* glutamate synthase was purified without using such slats or ammonium sulfate but the enzyme of *E. coli* was reported to have prepared with them.[8]

In this study, comparative studies on the molecular structure and reaction mechanism of the enzymes of *B. flavum*, *Gluconobacter suboxydans* and *E. coli*, which had been prepared by the two different procedures were carried out.

Glutamate synthase was purified from the organisms in the presence and absence of 0.1 M potassium chloride. Procedure A: The enzymes of *G. suboxydans* and *E. coli* were purified according to the method described in the previous paper consisting of ammonium phosphate fractionation, DEAE-cellulose column chromatography and Sepharose 6B gelfiltration.[5] Procedure B: The buffer containing 2 mM α-ketoglutarate, 0.2 mM EDTA and 0.1 M potassium chloride was employed in this procedure. Ammonium sulfate was also used for precipitation of the enzyme. Other conditions were the same as those in procedure A. The *B. flavum* enzyme was prepared by the method described previously.[5] Fig. 1 shows the profiles of gel filtration of the enzymes prepared by the procedure A and B. The molecular weight of glutamate synthase of *E. coli* was shown to be increased when it was prepared in the presence of potassium chloride (II-B), but the molecular weight of the *G. suboxydans* was invariable (I-B). The molecular weight of *B. flavum* enzyme incubated with potassium chloride was increased, and the enzyme was simultaneously inactivated (III-B). This may be due to the same effect caused by ammonium sulfate to the enzyme inactivation shown in the previous paper.[5] It might be suggested that the disagreement in the reaction mechanism

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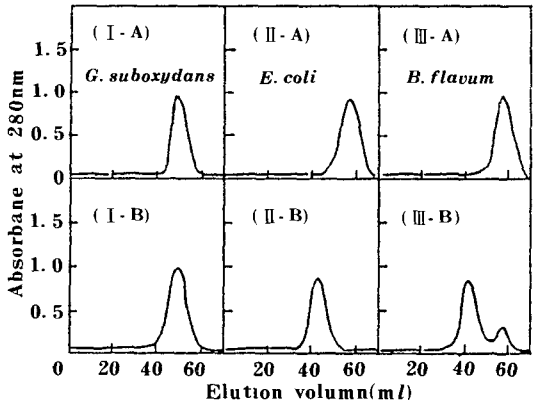

Fig. 1. Effect of potassium chloride on the molecular weight of glutamate synthase.

(Ⅰ) The *G. suboxydans* enzyme was prepared by the procedure A and B. (Ⅱ) The *E. coli* enzyme was prepared by the procedure A and B. (Ⅲ-A) The *B. flavum* enzyme was prepared by the method described in previous paper[5]. (Ⅲ-B) The *B. flavum* enzyme was preincubated with 0.1M potassium chloride in 0.02M potassium phosphate buffer (pH 6.5) at 4℃ overnight. The gel filtration was carried out on a Sepharose 6B column (1.2× 120cm) with (A) 0.02M potassiumphosphate buffer (pH 6.5) or (B) the buffer containing 0.1 M potassium chloride.

between the glutamate synthase of *E. coli* and that of *B. flavum* was due to the variation in the enzyme molecule caused by potassium chloride. Fig. 2 shows double reciprocal plots of the reactions by the *G. suboxydans* enzyme prepared by the procedure A. The parallel lines were the same as those observed with the enzyme of *B. flavum*, and indicating a hexa

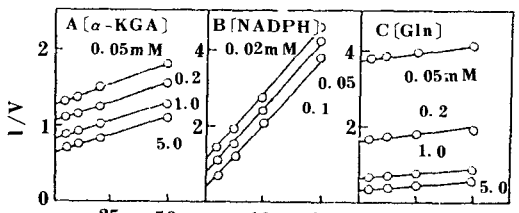

Fig. 2. Initial velocity patterns for the overall reaction of glutamate synthase prepared from *G. suboxydans*.

(A) NADPH was varied at fixed concentrations of α-KGA as indicated. The concentration of glutamine was 20mM. (B) Glutamine was varied at fixed concentrations of NADPH. The concentration of α-KGA was 20mM. (C) α-KGA was varied at fixed concentrations of glutamine. The concentration of NADPH was 0.3mM. Initial velocity (V) was expressed with decrease of 1.0 in the optical density at 340nm (1cm light path) per min.

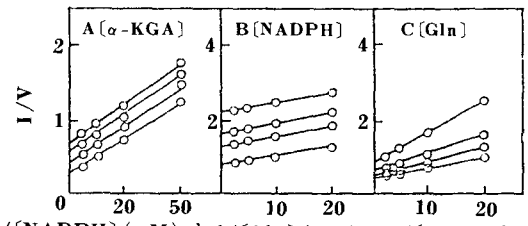

Fig. 3. Initial velocity patterns for the overall reaction of glutamate synthase from *E. coli*.

The concentrations of the substrates and other conditions were the same as in Fig. 2.

-uni ping pong mechanism.[6] The same result was obtained with the enzyme prepared by the procedure B(data not shown). The initial velocity patterns with the *E. coli* enzyme prepared by the procedure A were parallel between NADPH and either α-KGA or glutamine while the pattern between α-KGA and glutamine was intersecting (Fig. 3). These result agreed with those reported by Rendina and Orme-Johson.[9] Their experiments with the *E. coli* enzyme indicated a uni uni bi bi ping pong mechanism (Fig. 4). The result for the enzyme prepared by the procedure B. was the same that of the enzyme prepared by the procedure A (data not shown)

Glutamate synthase of *E. coli* and *G. suboxydans* exhibited one peak of activity on the gel filtration with Sepharose 6B in the presence or absence of 0.1 M KCl. In the preasence of KCl, however, the molecular weight of *E. coli* glutamate synthase was larger than that in the absence of KCl. When KCl was added to the enzyme solution of *B. flavum* and the buffer for gel filtration, two peaks were appeared. Therefore, the lower molecular weight form of *B. flavum* and *E. coli* glutamtes synthase seems to associate into

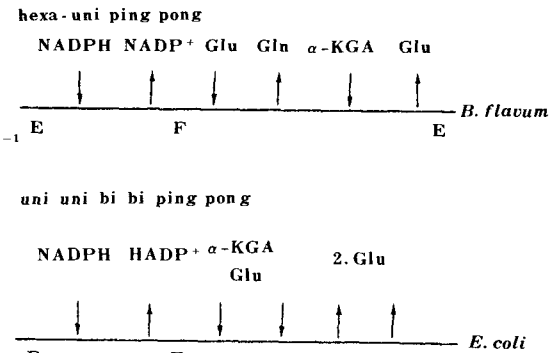

Fig. 4. Schematic representation of the steady-state kinetic mechanisms for the reaction catalyzed by glutamate synthase.

higher molecular weight form in the presence of KCl. On the othe hand, the results of kinetic analyses (Fig. 2 and 3) shows that the glutamate synthase reaction mechanism is dependent on the enzyme sources but not the variety of the molecule.

References

1. J.L. Meers, D.W. Tempest and C.M. Brown: *J. Gen. Microbiol.*, **64,** 187 (1970).
2. B. Tyler: *Ann. Rev. Biochem.*, **47,** 1127 (1978).
3. B.J. Miflin, P.J. Lea and R.M. Wallsgroe: glutamine; Metabolism, Enzymology, and Regulation, (Mora J. and R. Palacios ed.) p. 213, Academic Press, New York (1980).
4. A. Ginsburg and E.R. Stadtman: The Enzymes of Glutamine Metabolism, (Prusiner S. and E.R. Stadtman-ed.) p. 9, Academic Press, New York (1973).
5. H.C. Sung, T. Tachiki, H. Kumagai and T. Tochikura: *J. Ferment. Technol.*, **62,** 371 (1984).
6. H.C. Sung, T. Tachiki, H. Kumagai and T. Tochikura; *J. Ferment, Technol.*, **63,** 5 (1985).
7. M.M. Yelton and D.C. Yoch: *J. Gen. Microbiol.*, **123,** 335 (1981).
8. H.C. Sung. T. Tachiki, H. Kumagai and T. Tochikura: *J. Ferment. Technol.*, **62,** 569 (1984).
9. A.R. Rendina and W.H. Orme-Johson: *Biochemistry,* **17,** 5388 (1978).