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Properties and Kinetics of Glutamate Dehydrogenase of *Corynebacterium glutamicum*

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Corynebacterium glutamicum 의 Glutamate Dehydrogenase 의 효소학적 성질과 Kinetics

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A 150-fold purified preparation of NADPH-specific glutamate dehydrogenase of *Corynebacterium glutamicum* (1) was used for the determination of kinetic parameters of the substrates, NADPH, NH_4Cl, and α-ketoglutarate in the direction of glutamate synthesis. The kinetic constants determined from this study suggest a biosynthetic role for the enzyme. Based on the analysis of the result derived from initial velocity, the reaction mechanism was postulated to be ordered addition with NADPH as a first substrate to bind in the forward direction. Of the several metabolites tested for a possible function in the regulation of glutamate dehydrogenase activity, only malate and citrate were appeared to have an appreciable influence on the enzyme. Potassium chloride showed to be the most effective for the enzyme activity.

Most bacteria and fungi have either an NAD(H)- or and NADP(H)-specific glutamate dehydrogenase (2). It seems likely that the presence and regulation of anabolic or catabolic glutamate dehydrogenase activities might be of considerable importance. Members of the genus *Bacillus* are in general thought to be lack glutamate dehydrogenase activity (3, 4) but NADP specific activity has been found in four species (4, 5). Shiio and Ozaki (2) described the partial purification, general properties, and reaction mechanism of glutamate dehydrogenase from a glutamate producing *Brevibacterium flavum*. The kinetics and regulation of glutamate dehydrogenase purified from *Salmonella typhimurium* has been reported (6).

Previously we reported the purification of NADPH-dependent glutamate dehydrogenase of *Corynebacterium glutamicum* (1). This communication describes the results of an investigation for the kinetics and properties of the purified glutamate dehydrogenase.

Materials and Methods

Bacterial strain

Corynebacterium glutamicum ATCC13058 culture was prepared and glutamate dehydrogenase was purified as described previously (1). The previously purified enzyme was used for kineties studies.

Chemicals

NADPH and α-ketoglutarate were purchased from Sigma Chemical Co.

Enzyme assays

Glutamate dehydrogenase was assayed in the forward direction. Enzyme activity was determined by

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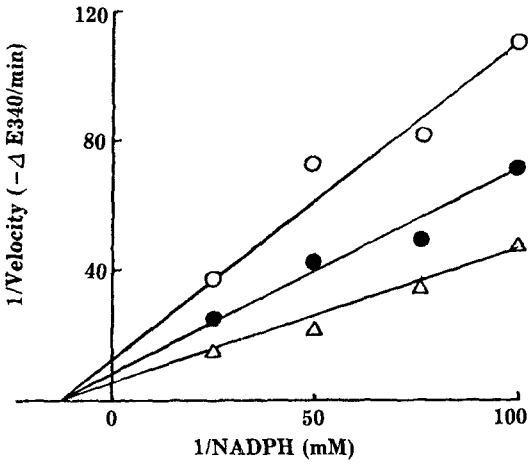

Fig. 1. Double reciprocal plots of the reaction velocity against NADPH at the 100 mM ammonium chloride.
The concentrations of α-ketoglutarate were 1 mM (○), 2 mM (●), and 10 mM (△).

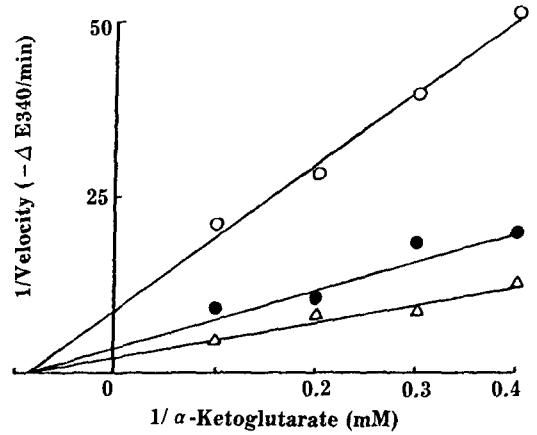

Fig. 2. Double reciprocal plots of the reaction velocity against α-ketoglutarate at the 0.1 mM.
The concentrations of ammonium chloride were 5 mM (○), 20 mM (●), and 40 mM (△).

measuring the oxidation of NADPH spectrophotometrically at 340 nm (7). For kinetics experiments the substrate concentrations were varied as indicated in the test. The initial velocity was determined by the increase in absorbance at 340 nm.

Results and Discussion

A detailed kinetic analysis was undertaken to determine the machanism of the multi-substrate reaction catalized by glutamate dehydrogenase of *C. glutamicum*. Initial velocity patterns and product inhibition studies were conducted with the purified glutamate dehydrogenase reaction considered in terms of the reductive amination. Kinetic constants were determined for the substrates in the forward direction. The results of this analysis are presented in the followings.

The reductive amination reaction

The substrates of glutamate dehydrogenase in the reductive amination reaction are α-ketoglutarate, ammonia, and NADPH. The binding affinities and Michaelis constants of these substances were determined by the analysis modified from that of Frieden (8). All the reaction velocity results were obtained at the same conditions described previously (1). Initial velocity patterns were obtained by using each of the substrates as the variable substrate in combination with another as the changing fixed substrate.

1) NADPH

Fig. 1 shows a double-reciprocal plot of reaction velocities against varying NADPH concentrations. Straight lines intersecting the horizontal axis indicate that these are reversible conections among NADPH and α-ketoglutarate, and NADPH and ammonia. The K_m of NADPH was 0.08 mM

2) α-Ketoglutarate

Fig. 2 shows double reciprocal plots of the reaction velocity against α-ketoglutarate in the presence of a constant level of NADPH (0.1 mM) and changing fixed ammonium chloride. The K_m value was 11.57 mM, and this value was independent of the ammonium concentration.

3) Ammonium ion

Fig. 3 shows double reciprocal plots of the reaction velocity against ammonium chloride concentration at 20 mM α-ketoglutarate and changing fixed NADPH concentration.

Effect of various salts on glutamate dehydrogenase activity

Various inorganic salts were used for the effect on enzyme activity were examined. Potassium chloride was the most effective, and K_2SO_4 was more effective than NaCl in lower concentration of 10 mM. The maximum activation was found at

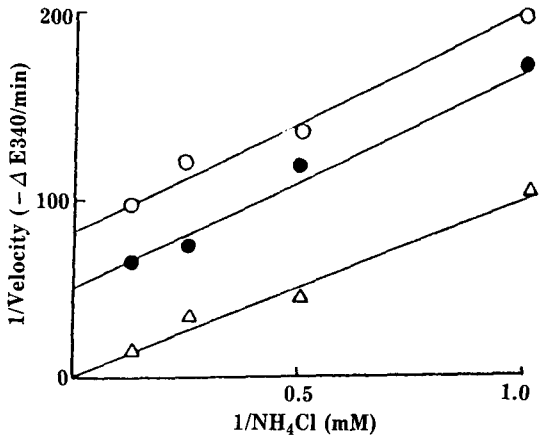

Fig. 3. Double reciprocal plots of the reaction velocity against ammonium chloride at the 20 mM α-ketoglutarate.
The concentrations of NADPH were 0.02 mM (○), 0.04 mM (●), and 0.01 mM (△).

Table 1. Effect of various salts on glutamate dehydrogenase activity.

Addition	Concentration (mM)	Relative activity (%)
None		100
KCl	100	145
	10	130
	1	119
NaCl	100	96
	10	120
	1	110
KNO_3	100	96
	10	135
	1	113
K_2SO_4	100	33
	10	144
	1	125

100 mM KCl as shown in Table 1.

Modulation of glutamate dehydrogenase by effectors

A glutamate dehydrogenase from diverse sources is known to be regulated by a variety of metabolites. Therefore it is of interest to study the interaction of regulatory ligands with glutamate dehydrogenase of *C. glutamicum*. These compounds include TCA cycle related intermediates, such as pyruvate, citrate, L-malate, and succinate. These intermediates were used at the concentration of 1 mM. The glutamate dehydrogenase reaction rate was measured in the presence or absence of these metabolites, and the effect of them was expressed as a relative activity. Of the intermediates tested, L-malate and citrate which showed relative activity of 66% and 68% respectively were relativly good inhibition. Pyruvate and succinate showed relative activity of 78% and 88% respectively (Table 2.)

Table 2. Effects of organic acids on glutamate dehydrogenase activity.

Addition (1 mM)	Relative activity (%)
None	100
Sodium pyruvate	78
Malate	66
Citrate	68
Sodium succinate	88

Table 3. Effects of amino acids on glutamate dehydrogenase activity.

Addition (1 mM)	Relative activity (%)
None	100
Glutamate	84
Tryptophan	113
Arginine	94
Histidine	92
Glutamine	99
Proline	130
Alanine	118
Phenylalanine	107
Leucine	103

Effects of amino acids on glutamate dehydrogenase activity

Effects of L-amino acids on the forward reaction was investigated. The result is shown in Table 3. None of the amino acids used was found to be significantly effective on forward reaction of glutamate dehydrogenase. However, among amino acids used, proline and alanine somewhat stimulated the forward reaction.

The kinetic parameters determined for the bin-

ding of substrates to glutamate dehydrogenase of *C. glutamicum* showed that the values derived from the result are differ significantly from those reported for other organisms. The K_m for NADPH (0.08 mM) is suggestive of a tightly bound enzyme-cofactor complex as the initial step in the forward direction. The K_m value for NADPH of this bacterial enzyme is different from that determined for the *Bacillus licheniformis* (0.12 mM) (5) and *B. flavum* (0.027 mM) (2). The second substrate required in the amination reaction is α-ketoglutarate (K_m value of 11.57 mM), compared with K_m value of 5.72 mM for *B. flavum* (2).

The data of kinetic analysis of glutamate dehydrogenase from *B. flavum* (2) also showed that the enzyme reaction can be described by the ordered Ter Bi mechanism, in which NADPH, α-ketoglutarate and NH_4Cl combine with the enzyme, and glutamate and NADP are released from the enzyme in this order.

Coulton and Kapoor (10) reported that glutamate has been appeared to be associated with relatively high K_m value in comparison to the value for α-ketoglutarate in the forward direction. One may conclude from this that *in vivo* reaction is essentially undirectional in favor of glutamate biosynthesis.

The results of the kinetic analyses of glutamate dehydrogenase of *C. glutamicum* may be summarized by postulating for the addition of sustrates; NADPH binds initially to the enzyme, α-ketoglutarate is the second substrate, and ammonium ion is considered to be the last and the second substrate to bind. This result is agreed with the binding order, but different from the K_m values of several substrates for glutamate dehydrogenase of *B. flavum* (2).

요 약

*Corynebacterium glutamicum*의 NADPH-specific glutamate dehydrogenase를 이용하여 NADPH, NH_4Cl, α-ketoglutarate의 기질에 대한 kinetics를 고찰하였다. 이들의 kinetic constants를 측정함으로서 정반응에로의 효소반응 기작은 첫번째 효소와 반응하는 기질이 NADPH임을 확인할 수 있었다. Glutamate dehydrogenase 활성의 조절을 위한 metabolites의 효과를 고찰하여 본 결과 malate와 citrate만이 효소에 억제 효과를 나타내었으며, potassium chloride는 효소활성에 가장 많은 영향을 주었다.

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