A POSSIBLE ROLE IN THE REGULATION OF PRIMARY AMINATION FOR A COMPLEX

OF GLUTAMINE: α-KETOGLUTARATE AMIDOTRANSFERASE AND

GLUTAMATE DEHYDROGENASE IN ESCHERICHIA COLI

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SUMMARY: Both glutamine: α-ketoglutarate amidotransferase and glutamate de-
hydrogenase activities are obtained from Escherichia coli grown in minimal
medium containing glucose and ammonia. These activities are not additive,
suggesting an interaction between them. Normally both of these activities
are equally inhibited by homoserine. In mutants resistant to homoserine
inhibition both activities are affected in parallel. Furthermore, the ratio
of the wild-type activities remains constant over a 30-fold range in purifi-
cation. This evidence suggests that these activities are associated with a
single complex. A possible role for this complex in the regulation of pri-
mary amination is discussed.

INTRODUCTION

The $NADP^+$-specific glutamate dehydrogenase (E.C. 1.4.1.4) reaction

carries out primary amination in Escherichia coli (1, 2), and is thus a key

enzyme in the nitrogen metabolism of this organism. Although regulation òf

the synthesis of this enzyme is well documented (1, 3), there is little evidence

for allosteric modification of its activity. Recent work in this laboratory

on the analysis of control patterns in the aspartate family of amino acids (4)

led to the prediction of an additional control mechanism in this set of

pathways, and subsequently, the existence of such a mechanism was confirmed

experimentally (5). Homoserine, the precursor of both threonine and methionine,

appears to inhibit $NADP^+$-specific glutamate dehydrogenase activity in vivo

and in vitro.

Since this is a central reaction in the highly branched set of

pathways for nitrogen metabolism, one might expect other allosteric modifiers

of glutamate dehydrogenase. In an examination of other nitrogenous products

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for their allosteric effect on glutamate dehydrogenase, we observed a gluta-

mine: α-ketoglutarate amidotransferase (oxidoreductase, $NADP^+$) activity in

extracts of E. coli B/r. This report describes some initial findings concern-

ing this activity.

METHODS

Enzyme Preparation

        Cultures of E. coli B/r were grown aerobically in glucose-minimal

medium (6) and harvested by centrifugation in either mid-log or stationary

phase. Crude extracts were prepared by ultrasonic disruption in distilled

water followed by centrifugation to remove whole cells and debris. Partial

purification of both glutamate dehydrogenase and the amidotransferase was

achieved by streptomycin sulfate treatment (1% w/v), ammonium sulfate frac-

tionation (40% to 55% saturation), and column chromatography on DEAE Sephadex

eluted by employing a concentration gradient of potassium chloride from 0.1 $\underline{M}$

to 0.6 $\underline{M}$.

Assays

        Glutamate dehydrogenase activity was measured according to the methods

of Halpern and Umbarger (1) and von Tigerstrom and Campbell (7). The

measurement of the amidotransferase activity in the forward direction was

identical to that of glutamate dehydrogenase except for the substition of

glutamine for ammonia. One unit of activity is equivalent to the formation

of 1 μmole of $NADP^+$ per minute. Protein was determined by the colorimetric

method of Lowry et al. (8).

RESULTS

        The "ammonia activity" and the "glutamine activity" in the crude

extract are of comparable magnitude (Table I). One explanation for this

observation is the presence of an active glutaminase (E.C. 3.5.1.2) in the

preparation, which could liberate ammonia, supplying substrate for glutamate

dehydrogenase. The glutaminase reaction is known to have a pH optimum of

TABLE I

"AMMONIUM" AND "GLUTAMINE" ACTIVITIES ASSAYED SEPARATELY
AND IN COMBINATION IN CRUDE EXTRACTS

Nitrogenous Substrate	Activity units/ml of Extract
NH_4^+ (40 mM)	0.228
Glu.N (40 mM)	0.184
NH_4^+ (40 mM) + Glu.N (40 mM)	0.220

8.0, which is identical to that used in our assay. We attempted to denature glutaminase by heat treatment at 55° for 30 minutes, knowing that glutamate dehydrogenase is relatively heat-stable (9). Unexpectedly, the "ammonia activity" disappeared and the "glutamine activity" remained upon heat treatment of fresh extracts. This result excludes the possibility that a glutaminase is responsible for the "glutamine activity" in Table I. The remaining activity, which appears to be a true glutamine: α-ketoglutarate amidotransferase, is essentially irreversible; no activity could be detected when NADPH and glutamate were used as substrates. We also observed that homoserine had no inhibitory effect on this heat-treated activity in the forward direction.

The "glutamine activity," however, cannot be entirely independent of the "ammonia activity" because these are not additive in the presence of both substrates (Table I). In view of this, we believe the glutamine activity normally functions in close association with the "ammonia activity" even though we have been able to separate the "glutamine activity" by heat treatment. The two activities also vary in a coordinate fashion when cells are grown in nutrient broth, minimal medium containing succinate plus ten amino acids, or ammonia-limited minimal medium with glucose as carbon source. Additional evidence for this close association of the two activities is provided by glutamate dehydrogenase mutants that are resistant to inhibition by homo-

TABLE II

INHIBITION BY HOMOSERINE OF GLUTAMINE: α-KETOGLUTARATE AMIDOTRANSFERASE
AND GLUTAMATE DEHYDROGENASE ACTIVITIES FROM WILD-TYPE AND
INHIBITION RESISTANT MUTANTS

Enzyme Source	% inhibition	
	NH_4^+	Glu.N
wild-type	22	21
Hr 19	0	3
Hr 20	1	2

Assays performed using crude extracts with and without homoserine
(13 mM final concentration), as described in Methods.

TABLE III

PARTIAL PURIFICATION OF GLUTAMINE: α-KETOGLUTARATE AMIDOTRANSFERASE
AND GLUTAMATE DEHYDROGENASE

	Volume, ml	Total protein, mg	Total activity, units		Specific activity, units/μg protein	
			NH_4^+	Glu.N	NH_4^+	Glu.N
Crude extract	24.0	910	339	276	.372	.303
Streptomycin treatment	22.0	658	215	132	.328	.201
Ammonium sulfate fraction	12.4	221	134	121	.607	.547
DEAE-Sephadex:						
Major peak	4.4	8.3	99.0	81.5	11.9	9.84
Minor peak	4.6	23.1	1.8	11.5	.078	.497

serine (5). In the wild-type extract, the two activities are equally inhibited

by homoserine. The loss of this inhibition in the mutants is parallel for the

two activities. These results are summarized in Table II.

The partial purification of the enzymes is shown in Table III. The

two activities were co-purified throughout these procedures. In the final

step the "glutamine activity" was eluted in two separate peaks from a DEAE

Sephadex column. The major peak coincides with the sole peak of "ammonia

activity" in position and specific activity, while the minor peak has no

corresponding peak of "ammonia activity." Major and minor peaks with these

same properties were also obtained by centrifugation in sucrose gradients.

DISCUSSION

Glutamine: α-ketoglutarate amidotransferase was first observed in

Aerobacter aerogenes when this organism was grown under conditions of limiting

ammonia (10). The enzyme is normally repressed when ammonia is present in

excess. The level of glutamate dehydrogenase is repressed under conditions

where amidotransferase is induced, suggesting that the enzymes function in-

dependently. These observations may indicate that this amidotransferase

normally serves an auxiliary role and is of major importance only under those

conditions that cause it to be induced.

For most organisms of the Bacillus group glutamate dehydrogenase

appears to be absent. Other reactions are responsible for primary amination.

In some instances alanine dehydrogenase is believed to perform this function

(11), although Bacillus subtilis mutants lacking this activity appear to grow

normally (12). In the case of Bacillus megaterium primary amination is re-

ported to occur via glutamine: α-ketoglutarate amidotransferase which has

been recently discovered in this organism (13). Elmerich and Aubert(13) found

this activity in B. subtilis as well and suggest that the coupled enzymes

(glutamine synthetase-glutamine: α-ketoglutarate amidotransferase) might

represent the pathway of glutamate synthesis in bacteria which lack glutamate

dehydrogenase.

E. coli appears to be quite different from these other organisms with

respect to glutamine: α-ketoglutarate amidotransferase in that this activity

is normally present even in an ammonia-rich environment. Furthermore, the

association of this activity with glutamate dehydrogenase and the coordinate regulation of their synthesis appear to be unique among the organisms studied to date. However, it may be more general, since we have also detected glutamine: α-ketoglutarate amidotransferase and glutamate dehydrogenase activities in extracts of E. coli K12, Salmonella typhimurium, and Acineto-bacter calco-aceticus when these organisms were grown in a minimal medium containing glucose and ammonia. The bifunctional activity of the complex could arise from bifunctional subunits, or from a collection of subunits with separate activities, or simply from the association of subunits. In any event, glutamate dehydrogenase activity is the more labile of the two. Evo-lutionary differences might accentuate this property and explain the apparent absence of this activity in some organisms. The observation that a mutant of B. subtilis acquires glutamate dehydrogenase activity (14) is interesting in this regard.

Halpern and Umbarger (1) showed that glutamate dehydrogenase activity in E. coli grown in a glucose minimal medium was sufficient to account for primary amination whereas the level of aspartate ammonia-lyase (E.C. 4.3.1.1) was too low to contribute significantly. These and other results implied a primarily synthetic role for glutamate dehydrogenase and a primarily degrada-tive role for aspartate ammonia-lyase. Vender and Rickenberg (2) found a mutant of E. coli lacking glutamate dehydrogenase could still grow and fix ammonia. They measured aspartate ammonia-lyase activity under conditions of growth on glycerol and implied that this activity was responsible for primary amination. A re-evaluation of this question in light of the glutamine: α-ketoglutarate amidotransferase activity seemed in order. Dr. Rickenberg kindly supplied us with his W1317^G mutant and we obtained neither glutamate dehydrogenase nor glutamine: α-ketoglutarate amidotransferase activity from cells grown in a glucose-minimal medium. This observation shows that the amidotransferase does not contribute to primary amination in this mutant

strain; it also provides further evidence for the formation of a complex with these two activities.

The association of glutamate dehydrogenase and glutamine: α-ketoglutarate amidotransferase activities could conceivably have an important regulatory role in the branched pathways of nitrogen metabolism. According to this hypothesis, high ammonia levels in the environment would, by competition with glutamine, inhibit the less efficient production of glutamate from glutamine. Under low ammonia conditions, the less efficient mechanism of ammonia assimilation would be progressively employed. Thus, the association of these two activities may perform the same function, but at the metabolic level, as does the inverse regulation of the synthesis of these activities in Aerobacter. We are currently performing experiments to test these conjectures.

ACKNOWLEDGMENTS

REFERENCES

1. Y. S. Halpern, and H. E. Umbarger, J. Bacteriol., 80, 285 (1960).
2. J. Vender, and H. V. Rickenberg, Biochim. Biophys. Acta, 90, 218 (1964).
3. F. Varricchio, Biochim. Biophys. Acta, 177, 560 (1969).
4. M. A. Savageau, (submitted for publication).
5. A. M. Kotre, S. J. Sullivan, and M. A. Savageau, (submitted for publication).
6. B. D. Davis, and E. A. Mingioli, J. Bacteriol., 60, 17 (1950).
7. M. vonTigerstrom, and J. J. R. Campbell, Can. J. Microbiol., 12, 1005 (1966).
8. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem., 193, 265 (1951).
9. C. Frieden, J. Biol. Chem., 240, 2028 (1965).
10. D. W. Tempest, J. L. Meers, and C. M. Brown, Biochem. J., 117, 405 (1970).
11. S. C. Shen, M. M. Hong, and A. E. Braunstein, Biochim.Biophys. Acta, 36, 290 (1959).
12. E. Freese, S. W. Park, and M. Cashel, Proc. Nat. Acad. Sci. U.S.A., 51, 1164 (1964).
13. C. Elmerich and J.-P. Aubert, Biochem. Biophys. Res. Commun., 42, 371 (1971).
14. J. M. Wiame, and A. Piérard, Nature, 176, 1073 (1955).