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Short Communications

Purification and properties of glutamine synthetase I from *Rhizobium* sp. UMKL 20

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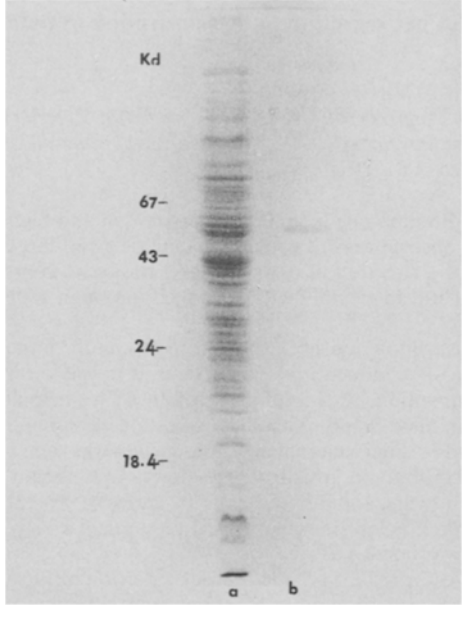
Summary. Glutamine synthetase I was purified from *Rhizobium* sp. UMKL 20 following polyethylene glycol precipitation. The enzyme had a subunit molecular weight of 58 kd. Apparent K_m values for ammonia and glutamate were 5.6 and 15.2 mM, respectively. Glutamine synthetase I activity was inhibited by several end products of glutamine metabolism. The purified enzyme was highly adenylylated ($E_n^- = 8.5$).
Key words. Rhizobium; glutamine synthetase I.

The enzyme glutamine synthetase (GS, EC 6.3.1.12) has been shown to play an important role in nitrogen assimilation in many microorganisms. Recent studies have focused on the mechanism of regulation of the enzyme and the molecular events leading to its expression. The GS from *E. coli* has been widely characterized by Stadtman and his co-workers[1].

Members of the family Rhizobiaceae have been shown to possess two forms of glutamine synthetase; GS I and GS II. GS I is heat stable and is similar to the GS from other microorganisms while GS II is heat labile (50°, 30 min)[2]. Besides its role in ammonia assimilation, GS I from *Rhizobium* is believed to play a role in the regulation of symbiotic nitrogen fixation by controlling the expression of nitrogenase[2,3]. The role of GS II is not known at present[3]. Recently it was shown that GS I may also be involved in salt tolerance in *Rhizobium*[4,5]. Under salt-stressed conditions, GS I activity increased several-fold[4]. In view of the important roles this enzyme may play in *Rhizobium*, I report here the purification and characterization of GS I from a salt-tolerant *Rhizobium*, UMKL 20[4].

Materials and methods. Rhizobium sp. UMKL 20 was obtained from the Department of Genetics and Cell Biology. Rhizobial cultures were maintained on agar slants of mannitol-yeast extract medium[4]. Liquid cultures were grown at 30° starting from a 2% inoculum (absorbance at 420 nm = 0.1). Total GS activity was assayed using the γ-glutamyl transferase assay described by Shapiro and Stadtman[6]. The biosynthetic assay of GS was carried out using ^{14}C-glutamate essentially as described by Prusiner and Milner[7]. The assay was modified with QAE-Sephadex (A25) replacing Dowex-1 (Cl^-) to improve the flow rate. All assays were carried out at pH 7.25, the isoactivity point of GS I from UMKL 20. GS I was purified using polyethylene glycol (PEG) precipitation essentially as described by Streicher and Tyler[8]. SDS-polyacrylamide slab gel electrophoresis was carried out using the procedure of Laemmli[9].

Results and discussion. Glutamine synthetase I was isolated and purified from *Rhizobium* sp. UMKL 20. Table 1 shows a summary of the purification procedure. The PEG precipitation of GSI occurs presumably because of a complex formed between GS I and DNA[8]. The procedure is a simple, fast and efficient method for the purification of GS I. Although the specific activity of the purified GS I is similar to other purified GSs[8,10,11] the yield was much lower. This is probably due to the difficulty encountered in solubilizing the acetone-precipitated pellet. The enzyme was judged to be homogeneous by SDS-polyacrylamide gel electrophoresis[9]. Only a single protein band was obtained, with a subunit mol.wt of 58 kd (fig.). This value is similar to the subunit molecular weight for the enzyme from other procaryotes[11]. Homogeneity was also apparent from immunodiffusion studies when antiserum against purified GS I was cross-reacted with crude extracts from strain UMKL 20. Only a single precipitin line was obtained[12] (data not shown). The purified enzyme was highly adenylylated, having an ade-



SDS-polyacrylamide gel electrophoresis of crude extracts (lane a) and purified glutamine synthetase I (lane b) from *Rhizobium* sp. UMKL20.

nylylation number (E_n^-) of approximately nine. In this respect GS I from strain UMKL 20 is similar to the GS from *Rhizobium japonicum* 61A96 and GS from other bacteria in being subjected to regulation by adenylylation and deadenylylation[1,3]. A similarly high degree of adenylylation has also been reported for the GS I from *Rhizobium japonicum* and is common for cultures grown under conditions where oxygen may be limiting[3].

Apparent K_m values of GS I calculated from linear double reciprocal plots for ammonia and glutamate were 5.6 and 15.2 mM, respectively (data not shown). No inhibition of the enzyme by ammonia was detected in the presence of up to 25 mM ammonium chloride. However, in whole cells, a decrease in the biosynthetic activity of GS I was reported in *Rhizobium* cultures subjected to ammonia shock[3]. The effects of various end products of glutamine metabolism on the activity of GS I were also studied to determine whether the enzyme is subjected to regulation by the effectors. Table 2 shows that pyrophosphate at 5 mM had a very strong inhibitory effect on GS I. The other effectors were only moderately effective in affecting the activity of GS I. Such inhibitory patterns are similar to the GS from *E. coli* and many bacteria[1] which are subjected to regulation by feedback inhibition. These results showed that GS I from *Rhizobium* sp. UMKL 20 has similar characteristics to the GS from *E. coli* and many other microorganisms. Such characteristics would indicate that it is possible to use techniques established for the *E. coli* enzyme in detailed studies of the GS from *Rhizobium*.

Table 1. Purification of glutamine synthetase I from *Rhizobium* sp. UMKL 20

Protein fraction	Specific activity[a]	Yield (%)
Crude extract	0.47	–
Polyethylene glycol precipitation	2.20	53.5
Acetone precipitation	18.10	15.0

[a] The specific activity, expressed in units per mg protein, refers to the transferase activity at pH 7.25 (see Methods section).

Table 2. Effectors of glutamine synthetase I from *Rhizobium* sp UMKL 20

Coumpound (5 mM)	% activity
None	100
Glutamine	77.5
Glycine	68.2
α-Ketoglutarate	68.1
Aspartate	66.7
AMP	53.8
ADP	27.9
Pyrophosphate	< 5

All compounds were added at a final concentration of 5 mM to the standard reaction mixture for the biosynthetic assay as described in the Methods section. Mg : ATP was maintained at 1 : 1.

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Reversal of net secretion to net absorption of potassium in rat large intestine by dietary potassium depletion

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Summary. Feeding rats a diet low in potassium and high in sodium for 2 weeks led to a reversal of net potassium secretion to net potassium absorption in ligated segments of distal large intestine (colon descendens and rectum) under in-vivo conditions. This change in the direction of net potassium transport is probably important for the maintenance of potassium homeostasis.
Key words. Rat intestine; diet, low potassium, high sodium; potassium homeostasis; potassium transport.

Sodium absorption and potassium secretion by the large intestine appear to be influenced by diet. Thus, it is well known that sodium absorption by the colon is enhanced in animals on a low sodium diet[2-5] and potassium secretion is stimulated in animals fed a high potassium diet[6]. It was the aim of the present short study to investigate the effect of potassium depletion on potassium, sodium and chloride transport by rat distal large intestine (colon descendens and rectum). An in vivo sac technique was employed[7].

Methods. Adult male Sprague-Dawley rats (initial mean b.wt 233 g), which were housed in a colony room illuminated from 07.00 to 19.00 h, were fed for about 2 weeks either a normal potassium (= NK) or a low potassium (= LK) diet. To obtain a low potassium diet the K_2HPO_4 of the mineral mixture used for the diets was replaced by Na_2HPO_4. Thus, only traces of potassium (0.04%) were present in the LK-diet. The potassium content of the NK-diet was 0.45%. The sodium content of the diets was 0.72% (LK-diet) and 0.45% (NK-diet). Both diets contained the same basic components (13% casein supplemented with 1%, D,L-methionine; 76.7% corn starch; 3.3% soybean oil; 2% vitamin mixture; 5% mineral mixture). The composition of the mineral mixtures used for both diets is shown in table 1.

Food and water were offered to the rats all the time. The weight gain of the LK-rats was significantly lower than that of the NK-rats (table 2) reflecting a small depression of food in-