Purification of Glutamine Synthetase from *Rhodospirillum rubrum* by Affinity Chromatography *

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Glutamine synthetase (E.C. 6.3.1.2, L-glutamate: ammonia ligase (ADP-forming)), GS, is a key enzyme in the nitrogen metabolism of prokaryotes.[1] It has been suggested that GS is also involved in the metabolic regulation of nitrogenase activity in photosynthetic bacteria.[2-5] There are, however, no reports on the extensive purification of GS from photosynthetic bacteria. Johansson and Gest[6] using a partially purified preparation showed that GS from *Rhodopseudomonas capsulata* is regulated by an adenylylation/deadenylylation mechanism similar to that in *Escherichia coli*.

Nitrogenase activity in *Rhodospirillum rubrum* is regulated on a metabolic level as demonstrated by the "switch-off" effect obtained with ammonia, asparagine or glutamine.[7] In order to further investigate the possible role of GS in this regulation mechanism we have purified GS from *R. rubrum* using a very rapid and simple procedure involving cetyltrimethylammonium bromide (CTAB) treatment and affinity chromatography.

Experimental. R. rubrum strain S1 was grown anaerobically in the light in the medium described before[7] with N_2 or ammonium sulfate as nitrogen source. The cells were harvested at the end of the exponential phase. Immediately prior to harvesting, CTAB was added to the culture ($A_{660} = 1.6-3.2$) to give a final concentration of 0.025–0.05 mg/ml. The harvested cells were washed three times with 50 mM imidazole-HCl pH 7.4 containing 0.5 mM $MnCl_2$. The washed cells were resuspended in the same buffer (3:1 w/v) and broken in a Ribi Cell Fractionator at 140 MPa. The crude extract obtained was centrifuged at 45 000 *g* for 60 min and the clear supernatant used for further purification.

In a standard purification 30–40 ml of supernatant (2–5 mg protein/ml) was made 4 mM with respect to Mn^{2+} and pumped on to a 5 ml ADP–hexane–agarose column, which was equilibrated with 50 mM imidazole-HCl pH 7.0 containing 4 mM $MnCl_2$. This buffer was used in the following. The column was washed with buffer, followed by 0.5 M KCl in buffer, in both cases until no more protein was eluted as indicated by A_{280} measurements. After washing with 2–3 bed volumes of buffer to remove KCl, GS was eluted with 4 mM ADP in buffer. GS activity was determined as the γ-glutamylhydroxamate transferase activity according to Stadtman *et al.*[8] with imidazole buffer at pH 7.38. Protein was estimated as described by Lowry *et al.*[9]

Results and discussion. In this purification procedure the CTAB-treatment is of great importance since membranes as well as proteins that interfere with the elution of GS from the affinity column, are removed in this step. CTAB has previously[6] been used to stabilize the adenylylation state of GS in *Rps. capsulata* and recently in a purification procedure for GS from *Rps. palustris*.[10]

The use of ADP–agarose for the affinity chromatography was based on the fact that the *E. coli* enzyme has a specific binding site for nucleotides.[11] The *R. rubrum* enzyme apparently binds specifically to the ADP–agarose since we found that (1) the binding is Mn^{2+}-dependent, (2) no activity could be eluted with 0.5 M KCl, (3) GS was eluted with Mn–ADP and the elution was dependent on the ADP-concentration.

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Table 1. Purification table for glutamine synthetase isolated from nitrogen fixing *R. rubrum*.

Fraction	Volume (ml)	Protein conc. (mg/ml)	Activity[a] (u/ml)	Total activity (u)	Specific activity (u/mg)	Purification factor	Recovery (%)
Crude extract	75	18.6	65	4875	3.5	1	100
Supernatant, after 45 000 *g* 60 min.	40	5.8	70	2800	12.1	3.5	57
ADP-agarose chromatography	15	1.6	200	3000	125	35.7	62

[a] GS-activity measured as γ-glutamylhydroxamate transferase activity. 1 unit = 1 μmol γ-glutamylhydroxamate/min.

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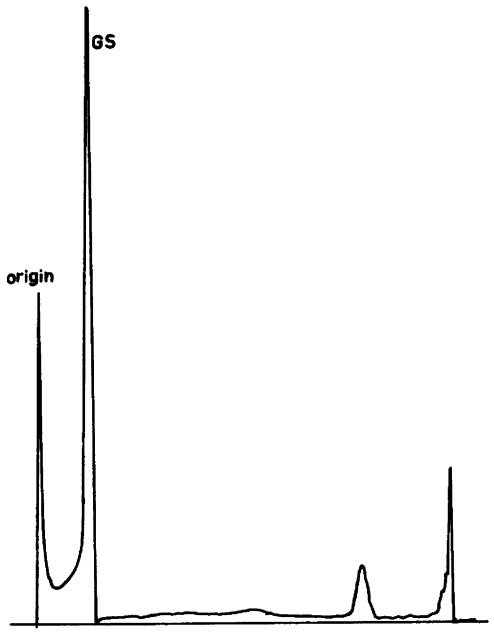

Fig. 1. Polyacrylamide gel electrophoresis of purified GS (100 μg). The gels were stained with Coomassie Brilliant Blue R-250 and scanned at 540 nm.

The use of both the CTAB-treatment and the ADP – agarose chromatography results in a purified GS with very high specific activity and with good recovery (Table 1). With this procedure purified GS can be obtained in one day, starting from the cell culture.

The GS preparations showed only one major band on polyacrylamide gel electrophoresis under non-denaturing conditions (Fig. 1) and only one band could be detected when SDS polyacrylamide gel electrophoresis was used. Preliminary results from molecular weight determinations using gel filtration (Sepharose-6B) indicate a value between 550 000 – 580 000 daltons. This is similar to values reported for GS from non-photosynthetic bacteria.[1] The molecular weight of the subunit, as estimated on SDS polyacrylamide gel electrophoresis, is around 50 000.

We are now using this purification procedure to obtain GS from *R. rubrum* grown with different nitrogen sources in order to study the involvement of GS in the regulation of nitrogen fixation and to further investigate whether GS from *R. rubrum* is regulated by an adenylylation/deadenylylation mechanism.

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