Molecular Characterization of Glutamine Synthetase from the Nitrogen-Fixing Phototrophic Bacterium *Rhodopseudomonas palustris* [1]

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The phototrophic bacterium *Rhodopseudomonas palustris* assimilated ammonium via glutamine synthetase and glutamate synthase. Diazotrophic and ammonium-grown cells had high levels of both enzymes, whereas enzymes of alternative assimilatory pathways were absent or had only low activities. Glutamine synthetase was purified to electrophoretic homogeneity within three steps by dye-ligand and ion exchange chromatography. Electron microscopy revealed a dodecameric molecular entity which was in accordance with parameters derived from electrophoretic techniques. The molecular weight of the enzyme monomer was 55800; that of the dodecamer 670000. The amino acid composition of *R. palustris* glutamine synthetase was determined and compared by a statistical method with other known enzyme compositions from prokaryotic and eukaryotic origins.

Introduction

Glutamine synthetase [EC 6.3.1.2, L-glutamate: ammonia ligase (ADP-forming)] catalyzes the primary reaction of ammonium assimilation in diazotrophic *Rhodospirillaceae* [1, 2]. Ammonia in turn represses the synthesis of nitrogenase with a possible involvement of glutamine synthetase [3, 4]. Additional to changes in the enzyme level, nitrogenase activity itself is controlled *in vivo* via a reversible short-term regulatory mechanism in response to added ammonium [5, 6]. Glutamine synthetase was also linked to this type of regulation, with a detailed mechanistic interpretation still outstanding [6, 7].

Glutamine synthetase, despite the recent interest in the peculiarities of nitrogen fixation in phototrophic bacteria, has not yet been obtained in a homogeneous form from any representative of this group to allow a study of its molecular and regulatory properties. The enzyme was partially purified from *Rhodopseudomonas capsulata* and preliminary evidence suggested a control mechanism via covalent modification [8]. We report here on the molecular properties of glutamine synthetase from *Rhodopseudomonas palustris* which was purified to homogeneity by dye-ligand and ion exchange chromatography. Evidence for its regulation via adenylylation/deadenylylation, feedback inhibition and kinetic control will be reported in a subsequent contribution.

[1] Part of this work was presented at the 3rd Internatl. Symp. on Photosynthetic Prokaryotes (Oxford, August 29, 1979) and the Internatl. Symp. N_2 fixation, Phytochem. Soc. of Europe (Brighton, September 18, 1979).

Abbreviations: SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.

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Materials and Methods

Organism and growth conditions

Rhodopseudomonas palustris (ATCC 17001) was grown photoheterotrophically at an illuminance of 3000 lux and a temperature of 28 ± 2 °C in the medium of Pfennig [5] with 15 mM NH_4Cl or with N_2 as source of nitrogen.

Cell-free extract

Cells after a growth period of 65 to 70 h were treated with 50 mg N-cetyl-N,N,N-trimethylammonium bromide per liter culture medium [9]. After incubation for 2.5 min in the light the cells were

harvested by centrifugation for 10 min at 13 700 × *g*. The cells were washed four times with 1 l of 10 mM Tris-HCl buffer, pH 7.1, containing 1 mM $MnCl_2$. Reference in the following to "buffer" specifies this Mn-containing buffer. The cell paste obtained from 8 l medium was resuspended with 20 ml of 50 mM buffer and stored at − 24 °C. For the preparation of a crude extract the thawed cell suspension was passed three times at 20000 psi through a French pressure cell. The homogenate was centrifuged at 10 °C for 20 min at 31 000 × *g*. The resulting supernatant was used either immediately for the purification of glutamine synthetase or stored at −24 °C.

Purification of glutamine synthetase

The crude extract obtained from 4 l of culture was adsorbed on a 1.5 × 11 cm column of Blue Sepharose Cl-6 B, which was equilibrated with buffer. The column was washed with approx. 120 ml of buffer until absorbance measurements at 280 nm were close to zero. Glutamine synthetase was eluted from the column with buffer which was 2 mM in ADP. The enzyme was directly adsorbed on top of a DEAE-cellulose column (DE-52, Whatman, 1 × 11 cm). The column had been equilibrated with buffer and was washed with at least 70 ml buffer after application of the enzyme. Glutamine synthetase was chromatographed with a linear buffer gradient from 10 to 500 mM Tris. The total gradient volume was 150 ml; the flow rate was 50 ml · h^{-1}. Fractions containing the enzyme were pooled and dialyzed overnight at 4 °C against 3 l of buffer. The dialyzed solution of glutamine synthetase was subjected to a second dye-ligand chromatography as described above. When necessary the enzyme was dialyzed once more against buffer to remove ADP. At all stages of purification, the enzyme could be stored at − 24 °C for more than a month with very little loss of activity.

Enzyme assays

Transferase activity of glutamine synthetase

Glutamine synthetase was assayed during purification by the γ-glutamyl transferase reaction according to the procedure of Shapiro and Stadtman [10]. The reaction mixture contained in a total volume of 1 ml besides the enzyme, 62.5 µmol imidazole-HCl, pH 7.6; 0.375 µmol $MnCl_2$; 25 µmol potassium arsenate; 0.4 µmol sodium ADP; 25 µmol hydroxylamine; and 25 µmol L-glutamine. The pH of the reaction mixture was raised to 7.1 with 20% NaOH. The mixture was incubated at 35 °C for five to ten min, depending on the activity of the preparation. The reaction was stopped by the addition of 2 ml of stop mixture and the resulting γ-glutamylhydroxamate was measured at 500 nm in a spectrophotometer [10].

Biosynthetic activity of glutamine synthetase

This activity was measured in polycarbonate tubes by the liberation of inorganic phosphate. The assay described by Shapiro and Stadtman [10] was modified as follows: The complete mixture contained in a total volume of 0.5 ml, an enzyme aliquot, 50 µmol imidazole-HCl, pH 7.0; 4 µmol sodium ATP; 50 µmol L-glutamate; 25 µmol NH_4Cl; and 4 µmol $MnCl_2$ or 25 µmol $MgCl_2$. The reaction mixture was incubated at 35 °C for 10 to 30 min; afterwards inorganic phosphate was determined according to Taussky and Shorr [11].

Glutamate synthase (EC 1.4.1.14)

This enzyme was measured by following spectrophotometrically the 2-oxoglutarate-dependent oxidation of NAD(P)H at 340 nm. One ml of reaction mixture contained in addition to the enzyme, 50 µmol imidazole-HCl, pH 7.0; 2.5 µmol Na_2EDTA; 7.5 µmol 2-oxoglutarate; 0.25 µmol NADH or NADPH; and 10 µmol L-glutamine. The reaction was followed at 30 °C in a thermostated semi-micro cuvette of 1 cm light path [12]. All optical tests were started by the addition of substrate after first measuring unspecific NAD(P)H oxidation.

Glutamate dehydrogenase (EC 1.4.1.3) *alanine dehydrogenase* (EC 1.4.1.1), *and L-alanine:2-oxoglutarate aminotransferase* (EC 2.6.1.2)

The principle of enzyme activity measurements was identical to that for glutamate synthase. The reaction mixture for glutamate dehydrogenase contained the enzyme and the following components in a total volume of 1 ml: 50 µmol Tris-HCl, pH 8.0; 0.25 µmol NADH or NADPH; 50 µmol 2-oxoglutarate; 40 µmol NH_4Cl; and 10 µmol $MgCl_2$. The reaction was run at 30 °C. The reaction mixture for alanine dehydrogenase was identical to that described by Johansson and Gest [13]. Instead of 2-oxoglutarate, 5 µmol sodium pyruvate was added.

The reaction mixture for L-alanine:2-oxoglutarate aminotransferase contained in 1 ml besides the enzyme, 100 µmol phosphate buffer, pH 7.2; 0.18 µmol NADH; 5.5 units of lactate dehydrogenase; 5 µmol 2-oxoglutarate; and 15 µmol L-alanine. This reaction was run at 37 °C [14].

Polyacrylamide gel electrophoresis and determination of molecular weight

The purity of glutamine synthetase and the molecular weight of the enzyme were determined under denaturing conditions by the method of Weber and Osborn [15] in gels with 7.5 and 10% acrylamide. The following molecular weight standards were used: phosphorylase b (molecular weight, 94000), bovine serum albumin (67000), ovalbumin (43000), carbonic anhydrase (30000), soybean trypsin inhibitor (20100), and α-lactalbumin (14400). Approx. 10 µg of denatured glutamine synthetase was applied together with a mixture of 5 µg each of the denatured standard proteins to a gel tube of 10 cm length. A constant current of 8 mA per gel was applied.

The molecular weight of the native enzyme was determined by pore gradient electrophoresis with commercially available preformed gradient gels, 4 to 30% [16]. The molecular weight standards were thyroglobulin (669000), ferritin (440000), catalase (232000), lactate dehydrogenase (140000), and bovine serum albumin (67000). Electrophoresis was carried out with an Ultraphor preparative apparatus (Colora, Lorch, Bundesrepublik Deutschland) for 2500 volt-hours at 6 °C. The electrode reservoirs contained a total of 10 l of 90 mM Tris, 80 mM boric acid, and 2.5 mM Na_2EDTA. The pH was 8.4. After electrophoresis, when required, the position of the tracking dye was determined by a gel scanner. Staining and destaining followed published procedures [15]. The positions of the stained protein bands were again determined by scanning of the gel at 560 nm with a densitometer. Values of electrophoretic mobility were calculated relative to the tracking dye (for SDS electrophoresis) or relative to the total length of the gel (for pore gradient electrophoresis). The experimental data for the electrophoretic mobility versus log molecular weight were fitted by linear regression analysis.

The isoelectric point of glutamine synthetase was determined by preparative isoelectric focusing in dextran gel with a Multiphor electrophoresis apparatus (LKB, München) according to the instructions given by the manufacturer. The focusing conditions were as follows: ampholyte concentration, 2%; pH range, 4 to 6; temperature, 6 °C: power constant at 7 W for 14 h. The enzyme was identified with small gel aliquots by the transferase activity. The pH at the position of the enzyme was measured with an Ingold surface electrode, model 403-30-M3.

Electron microscopy

An enzyme preparation was used directly after elution from the second dye-ligand chromatography without removal of ADP. The protein concentration was 0.6 mg per ml. The preparation was diluted 10-fold with water and negatively stained with 2% aqueous uranyl acetate [17]. These negatively stained samples were observed in and micrographs were taken with a Philips EM 400 electron microscope. To determine the molecular symmetry of glutamine synthetase, image analysis was performed by the technique of Markham *et al.* [18].

Amino acid analysis

An electrophoretically homogeneous enzyme sample was extensively dialyzed against 10 mM phosphate buffer, pH 7.0, and brought to dryness by lyophilization. The dried sample was dissolved in quartz double-distilled water to give a protein concentration of 0.74 mg per ml. Samples were made 6 N in HCl and hydrolyzed under nitrogen for 20 and 72 h at 110 °C. Amino acids were analyzed by single long column technique with a Biocal 200 amino acid analyzer, equipped with a Spectra-Physics computing integrator.

Protein determination

Protein concentrations were estimated by the method of Lowry with bovine serum albumin as a standard.

Chemicals

Nucleotides, sodium dodecyl sulfate, L-glutamine, imidazole, 2-oxoglutarate, and L-alanine were purchased from Serva, Heidelberg. Sodium pyruvate, L-glutamate, and potassium arsenate were from Sigma, München; lactate dehydrogenase was from Boehringer, Mannheim. Blue Sepharose Cl-6B, mo-

Table I. Enzymes of ammonium assimilation in *Rhodopseudomonas palustris.*

Nitrogen source	Enzymes						
	GS[a]	GS[b]	GOGAT[c] NADH	GOGAT[c] NADPH	Ala-DH[d]	AOGAT[e]	GDH[f] NAD(P)H[g]
N_2	133	1880	51.4	0	3.1	0	< 0.4
NH_4^+	63	1280	46.4	0	2.0	2.8	1.9

Enzyme source was a cell-free extract from cells that were not treated with detergent, passed only twice through the French press, and centrifuged for 10 min at 10800 × *g*. All enzyme activities are given as nmol · min^{-1} · mg $protein^{-1}$.

[a] Mn^{2+}-dependent biosynthetic activity of glutamine synthetase.
[b] Transferase activity of glutamine synthetase.
[c] Glutamate synthase.
[d] Alanine dehydrogenase.
[e] L-Alanine: 2-oxoglutarate aminotransferase.
[f] Glutamate dehydrogenase.
[g] Indicated activities detected with either NADH or NADPH.

lecular weight standards, and polyacrylamide gradient gels were from Pharmacia, Freiburg. N-Cetyl-N,N,N-trimethylammonium bromide and other chemicals were from Merck, Darmstadt; ampholytes were purchased from LKB, München.

Results and Discussion

Pathway of ammonium assimilation in R. palustris

R. palustris grows in the light with ammonium or N_2 as source of nitrogen [19]. Under both growth conditions glutamine synthetase and an NADH-specific glutamate synthase were found in cell-free extracts (Table I). Whereas the level of glutamate synthase was nearly independent of the nitrogen source, both, the activity and the level of glutamine synthetase showed a marked increase in the diazotrophic culture. Because of the high levels of the two enzymes it is likely that they form the dominant route for ammonium assimilation in *R. palustris.* Enzymes for alternative pathways were either absent or showed low activities to make a major role in ammonium incorporation unlikely. An NADPH-specific alanine dehydrogenase was present in cells grown either with NH_4^+ or N_2. Glutamate dehydrogenase and L-alanine: 2-oxoglutarate aminotransferase were only found with low activity levels in ammonium-grown cultures but not under diazotrophic conditions. When the organism was supplied with 10 mM glutamate, glutamine synthetase activity was found comparable to that of ammonia-grown cells, indicating that this enzyme was constitutive.

Glutamine synthetase and glutamate synthase activities were demonstrated in nitrogen-fixing *Rhodospirillum rubrum* [2, 20], *Rhodomicrobium vannielii, Rhodopseudomonas sphaeroides* [2], *R. capsulata* [13], and in *Rhodopseudomonas acidophila* [21]. None of these enzymes was studied in highly purified form and no molecular data are available so far.

The nucleotide specificity of glutamate synthase exhibits an interesting species-dependency among the *Rhodospirillaceae.* NADPH-dependent enzymes were found in *R. rubrum* [1, 2, 20], *R. capsulata* [13] and *R. sphaeroides* [2]. NADH-dependent enzymes were found in *R. vannielii* [2], *R. acidophila* [21], and in *R. palustris* (this paper). Contrary to our finding, which was corroborated several times with different enzyme batches, Brown and Herbert [2] described from an antarctic isolate of *R. palustris,* grown on nitrate, an NADPH-specific glutamate synthase. This poses the interesting question whether there are differences in nucleotide specificity in this organism even among different strains, or whether there exists a nutrional dependency with respect to the source of nitrogen.

Purification of Glutamine Synthetase

The purification procedure described in Materials and Methods provides an efficient and facile way to obtain homogeneous glutamine synthetase from *R. palustris* with three purification steps only. Detergent treatment of the intact cells, in addition to a possible stabilizing effect of the adenylylation state of the enzyme, aggregated most of the membranous material and unwanted protein of broken cells. A low-speed centrifugation yielded therefore already a crude extract whose specific activity for glutamine synthetase was routinely higher than three units per mg protein.

We have shown previously the binding of nitrate reductase to blue dextran [22]. Since then the binding of the dye Cibacron Blue 3GA to nucleotide-dependent enzymes was shown to mimic the binding of substrate [23]. Table II shows the nucleotide specificity of *R. palustris* glutamine synthetase for the biosynthetic and transferase activity. GTP, ITP and UTP could slightly substitute for ATP in the Mn^{2+}-dependent biosynthetic activity. The transferase activity required ADP, replacable to a slight extent by GDP, IDP and UDP; nucleotide triphosphates slightly inhibited this activity. The nucleotide requirement of glutamine synthetase thus made dye-ligand chromatography a promising technique for rapid purification. During the preparation of this work Tabita and coworkers have used Blue Sepharose for purification of the enzyme from *Azotobacter vinelandii* and have also shown that it binds the glutamine synthetase from *R. sphaeroides* [24].

Table II. Nucleotide specificity of *R. palustris* glutamine synthetase.

Nucleotide	Biosynthetic activity Mn^{2+}-dependent (%)	Transferase activity (%)
None	0	25.6
ATP	100	18.1
ADP	3	100
AMP	0	25.4
GTP	37	19.2
UTP	19.2	19.4
CTP	1.7	12.7
ITP	11.6	23
GDP	0	47.4
UDP	0	31.1
CDP	0	29.4
IDP	1.5	37.0

The nucleotide concentration was 8 mM in the biosynthetic assay and 0.4 mM in the transferase assay. Control activity with ATP in the biosynthetic assay was 2.6 μmol · min^{-1} · mg $protein^{-1}$; the activity with ADP in the transferase assay was 63.7 μmol · min^{-1} · mg $protein^{-1}$.

R. palustris glutamine synthetase bound strongly to the dye matrix. It could be eluted from it with high yield (> 60%) by ADP. Fig. 1 shows the elution profile of the first dye-ligand chromatographic step used in the purification procedure. This step usually gave an eight to ten-fold purification. Because of the acidic nature of the enzyme with an isoelectric point of 5.5, anion exchange chromatography was used as further purification step. After the second affinity chromatography, specific activities in the transferase assay above 100 μmol min^{-1} · mg $protein^{-1}$ were obtained. This is more than five-fold higher than the activity reported for a partially purified enzyme

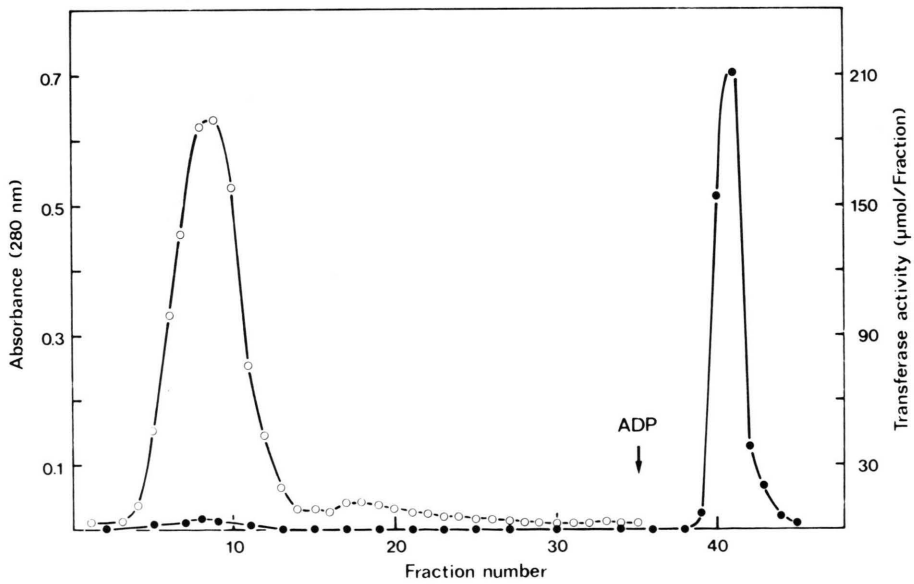

Fig. 1. Elution profile of glutamine synthetase from the first dye-ligand chromatography. A crude extract was applied to a column as described in Materials and Methods. Glutamine synthetase was followed by its transferase activity (●). For measurement of the protein at 280 nm (○), samples were diluted 20-fold with buffer. Glutamine synthetase was nearly quantitatively adsorbed on the column and eluted as extremely sharp peak with 2 mM ADP.

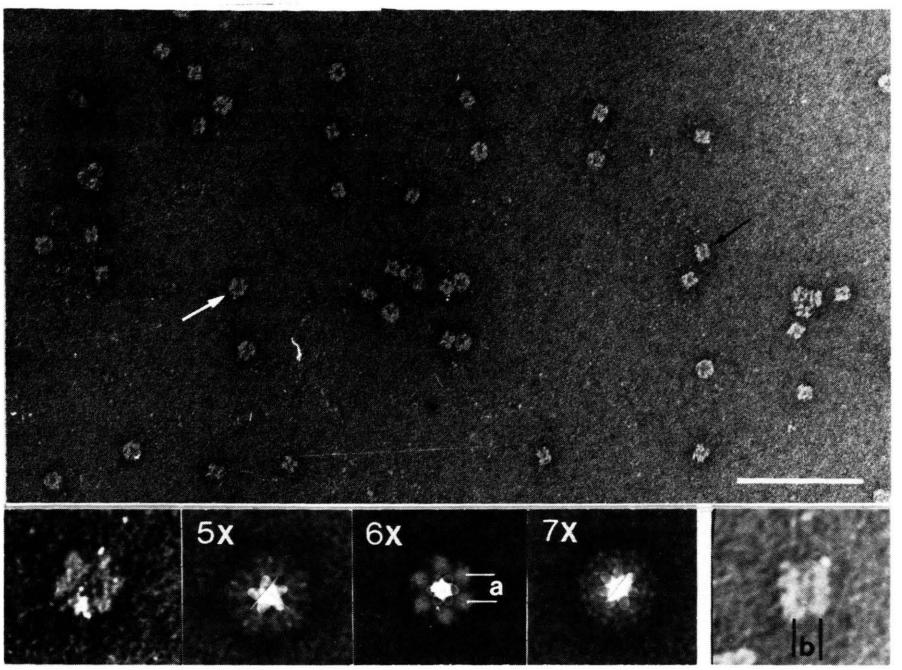

Fig. 2. Electron micrograph of purified and negatively stained glutamine synthetase from *Rhodopseudomonas palustris*. The white bar indicates 0.1 μm. The black arrow points to a side-on molecule. The white arrow indicates the face-on molecule which was analyzed by the Markham rotation technique in the lower row (magnification 610000 ×). On six-fold rotation the hexameric symmetry became clearly visible; five-fold and seven-fold rotations served as controls. The subunit distance (a) within the plane of the hexameric subunit assembly was 5.4 nm; the distance between two planes (b) was 5.7 nm.

Table III. Purification of glutamine synthetase of *Rhodopseudomonas palustris*[a].

Fraction	Total protein (mg)	Total units[b]	Specific activity[c]	Yield (%)
Crude extract	193	665	3.4	100
1st Dye-ligand chromatography	13	365	28.1	54.9
DEAE-cellulose chromatography	7.5	364	48.5	54.7
2nd Dye-ligand chromatography	1.7	182	107.1	27.4

[a] Purification from 4 l of ammonium culture with approx. 12 g of cells.
[b] One unit corresponds to 1 μmol γ-glutamylhydroxamate formed per min in the Mn^{2+}-dependent transferase assay.
[c] Units per mg protein.

from *R. capsulata* [8], and is comparable to activities for homogeneous glutamine synthetase from *Escherichia coli* [25], or *A. vinelandii* [24, 26]. The enzyme activities and yields at different stages of purification are summarized in Table III.

Electron microscopy

The electron microscopic appearance of a negatively stained enzyme preparation is shown in Fig. 2. The most frequently found structures were ring-like molecules, sometimes interspersed, with more rectangular, bilayered structures. These structures are face-on and side-on views of the enzyme as they were shown first with glutamine synthetase from *E. coli* [17]. The molecular dimensions of the *R. palustris* enzyme are compiled in Table IV. The diameter of the molecule and the subunit distances are slightly higher than equivalent dimensions of the *E. coli* enzyme in accordance with a slightly higher molecular weight (see below).

Molecular weight

Purified glutamine synthetase gave a single band in SDS polyacrylamide gel electrophoresis with 7.5 or 10% gels (Fig. 3). With known standard proteins, the molecular weight of this band was calculated to be 55800. The same preparation gave in pore

gradient electrophoresis (Fig. 4) molecular weight limits of 57900 (minimal value) and 582000 (maximal value). Besides these two forms of the enzyme, representing the monomeric and dodecameric form of the enzyme, protein bands at intermediate molecular weights of 123000, 209000, and 288000 were found. These are most likely dimeric, tetrameric, and hexameric subunit assemblies. No odd numbered complexes were found, indicating that subunit assembly proceeds in a distinct pattern of monomer-monomer, dimer-dimer, and hexamer-hexamer aggregation. The molecular weight of the dodecamer deduced from SDS polyacrylamide gel electrophoresis was 669600; calculated from the minimal value in pore gradient electrophoresis it was 694800. The directly measured molecular weight of the dodecamer and intermediate molecular weights were smaller than the multiples of the monomer. Since these oligomeric molecules deviate from globular shape they will migrate within the gel until the pore size impedes further migration due to the smallest dimension of the molecule [27]. Attempts to determine the molecular weight from sedimentation equilibrium failed, because the enzyme behaved as a polydisperse system. Apparently the subunit binding is not tight, since the non-denaturing pore gradient electrophoresis also yielded enzyme fragments.

Table IV. Molecular properties of glutamine synthetase from *Rhodopseudomonas palustris.*

Molecular weight from pore gradient PAGE		Proposed subunit assembly
Highest value	582000	α_{12}
Lowest value	57900	α (monomer)
Intermediate values	123000	α_2
	209000	α_4
	288000	α_6
Molecular weight from detergent PAGE	55800	α (monomer)
Type of monomers	One	
Quarternary structure from electrophoretic analysis and electron microscopy	Dodecamer	
Molecular dimensions	(mean ± S.D.)	Sample size
Diameter (*d*)	15.3 ± 0.13 nm	29
Height (*h*)	11.1 ± 0.47 nm	12
Subunit distances[a] (*a*)	5.1 ± 0.08 nm	
(*cf.* Fig. 4) (*b*)	5.5 ± 0.33 nm	

[a] Assuming an ideal hexameric structure these values were calculated as $a = d/3$, and $b = h/2$,

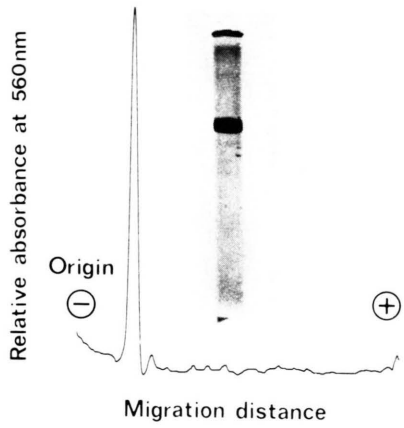

Fig. 3. SDS polyacrylamide gel electrophoresis and densitometer trace of purified glutamine synthetase. The densitometer trace is derived from a 10% gel to which 10 µg of enzyme was applied. The gel was scanned at 560 nm after electrophoresis, staining and destaining. The gel picture represents 12 µg of enzyme on a 7.5% gel. The origin is at the upper end of the gel.

The molecular properties of *R. palustris* glutamine synthetase are summarized in Table IV. In conclusion, electron microscopic evidence and molecular weight determination by electrophoretic techniques coincide with respect to the quarternary structure of the enzyme. Glutamine synthetase from *R. palustris* thus resembles the enzyme from other prokaryotic sources like *E. coli* [25] or *A. vinelandii* [26, 28]. With these two organisms it has in common its regulation via adenylylation and deadenylylation and other kinetic properties (Alef and Zumft, unpublished).

Amino acid composition

The results of the amino acid analysis are shown in Table V. There is a slight prevalence of acidic residues in accordance with the isoelectric point of 5.5. When comparing the amino acid composition to that of glutamine synthetase from *E. coli* [25] or *A. vinelandii* [28] a striking similarity becomes evident. These glutamine synthetases have not only an identical function but also an identical regulatory mechanism. With respect to the *E. coli* enzyme, there are about 50 more amino acids in agreement with the slightly higher molecular weight of *R. palustris* glutamine synthetase. The largest differences were found in the content of threonine, serine, and lysine.

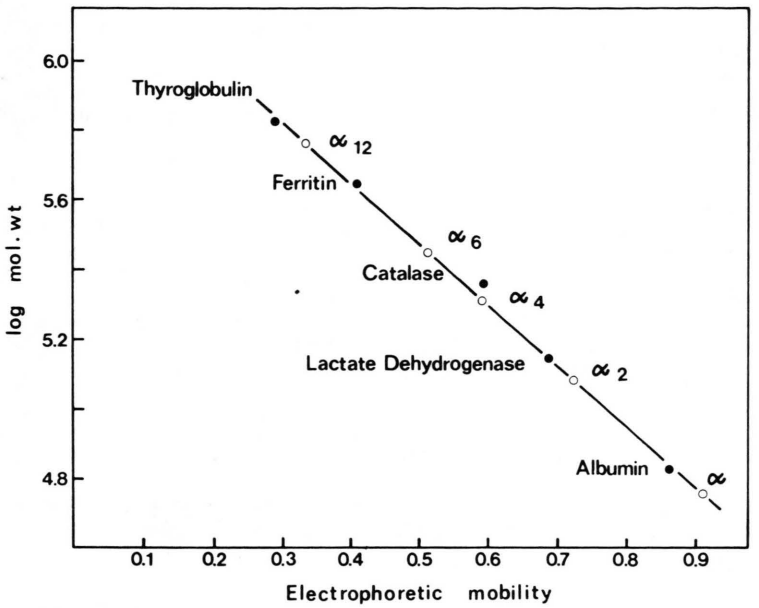

Fig. 4. Molecular weight determination of native glutamine synthetase by pore gradient electrophoresis. Experimental details are described in Materials and Methods. The molecular weights of the monomer (α) and the various subunit assemblies are given in Table IV.

Table V. Amino acid composition of *R. palustris* glutamine synthetase[a].

Amino acid	Residue/subunit[b]	Nearest integer
Asp	55.5 ± 2.4	56
Thr	29.0 ± 1.0	29
Ser[c]	37.3 ± 1.7	37
Glu	51.9 ± 2.2	52
Pro	33.6 ± 1.2	34
Gly	42.3 ± 2.8	42
Ala	38.6 ± 0.5	39
Cys[d]	9.0 ± 1.3	9
Val[e]	27.7 ± 0.4	28
Met[d,f]	16.4 ± 1.2	16
Ile	27.9 ± 1.8	28
Leu[e]	32.7 ± 0.2	33
Tyr[f]	10.0 ± 0.1	10
Phe	24.4 ± 1.4	24
Lys	37.8 ± 2.0	38
His	16.2 ± 1.0	16
Arg	21.3 ± 1.3	21
Trp	nd[g]	
Total	511.6	512

[a] Unless otherwise indicated, the averages of triplicate hydrolyses for 20 and 72 h in 6N HCl at 110 °C are listed.
[b] Moles amino acid per 55 8000 molecular weight subunit ± S.D. of all determinations.
[c] Determined by extrapolation to zero time of hydrolysis.
[d] Includes oxidized forms.
[e] Determined as the 72 h value.
[f] Determined as the 20 h value.
[g] Not determined.

A comparison of the amino acid compositions of prokaryotic and plant glutamine synthetases by statistical means is shown in Table VI, together with the most fundamental molecular properties of these enzymes. The composition divergence (D), which is a measure of the overall dissimilarity of the composition of two proteins, was used by Harris and Teller [29] to compare a large number of proteins with known sequence. Values of the compositional divergence $D < 7 \cdot 10^{-2}$ would indicate a non-fortuitous similarity of composition. The data of Table VI compare the composition of *R. palustris* glutamine synthetase to the known compositions of four bacterial glutamine synthetases, a cyanobacterium, a fungus, and two higher plants. They all show values below $7 \cdot 10^{-2}$ suggesting a considerable sequence homology irrespective of the source of the enzymes. Although the quarternary structure and regulatory properties of glutamine synthetases from plant sources are different, the composition divergence is still small and suggests a possible development from a common ancestor. A similar comparison of glutamine synthetases was extended by Kleinschmidt and Kleiner [29] also to animal sources; these authors proposed even an evolutionary relationship of glutamine synthetases to ATP-hydrolyzing proteins in general.

Table VI. Compositional divergence (D) of *R. palustris* glutamine synthetase from the same enzyme of other prokaryotic and plant sources.

Origin of glutamine synthetases	Value of D × 10^2 versus *R. palustris*	mol. wt	Subunit	
			No.	*mol. wt*
E. coli [25]	5.6	600000	12	50000
A. vinelandii [28]	4.0	650000	12	53000
Bacillus subtilis [30]	5.7	600000	12	50000
B. stearothermophilus [31]	4.8	620000	12	51000
Anabaena cylindrica [32]	4.9	591000	12	49000
Neurospora crassa [33]	5.3	360000	8	45000
Glycine soja [34]	5.4	376000	8	47300
Pisum sativum [35]	6.1	360000	8	45000

The composition divergence (D) was calculated from the amino acid composition given in the cited references according to the method of Harris and Teller [29] which weights all residues equally. Tryptophanresidues were omited.

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