Glutamate-1-semialdehyde aminotransferase from *Sulfolobus solfataricus**

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Glutamate-1-semialdehyde aminotransferase (GSA-AT) from the extremely thermophilic bacterium *Sulfolobus solfataricus* has been purified to homogeneity and characterized. GSA-AT is the last enzyme in the C₅ pathway for the conversion of glutamate into the tetrapyrrole precursor δ -aminolaevulinic acid (ALA) in plants, algae and several bacteria. The active form of GSA-AT from *S. solfataricus* seems to be a homodimer with a molecular mass of 87 kDa. The absorption spectrum of the purified aminotransferase is indicative of the presence of pyridoxamine

5'-phosphate (PMP) cofactor, and the catalytic activity of the enzyme is further stimulated by addition of PMP. 3-Amino-2,3-dihydrobenzoic acid is an inhibitor of the aminotransferase activity. The N-terminal amino acid sequence of GSA-AT from *S. solfataricus* was found to share significant similarity with the eukaryotic and eubacterial enzymes. Evidence is provided that ALA synthesis in *S. solfataricus* follows the C₅ pathway characteristic of plants, algae, cyanobacteria and many other bacteria.

INTRODUCTION

δ -Aminolaevulinic acid (ALA) is the universal precursor for the biosynthesis of chlorophyll, haem, and other tetrapyrroles. In animals, yeast and a limited number of bacteria ALA is formed by the condensation of succinyl coenzyme A with glycine, catalysed by the enzyme ALA synthase [1,2]. In contrast, plants, algae, cyanobacteria and many other bacteria synthesize ALA from glutamate by a C₅ pathway in which the carbon skeleton of glutamate is converted into ALA by a series of reactions including the activation of the γ -carboxyl carbon of glutamate by ligation to tRNA, reduction of the activated carbon to an aldehyde, yielding glutamate 1-semialdehyde (GSA) and, as a final step, transamination of GSA to ALA [3–5].

GSA aminotransferase (GSA-AT) catalyses the transamination of GSA to ALA. GSA-AT was first purified and studied in barley [6,7]. Moreover the complete nucleotide sequence of the gene encoding GSA-AT has been determined in *Synechococcus* [8], barley [9] and *Escherichia coli* [8]. Comparative studies of the primary structure of these GSA-ATs indicate an overall identity of 41 % [10]. These enzymes consist of two identical subunits with a molecular mass of approx. 46 kDa, whereas the GSA-AT from *Chlamydomonas* is a monomer with the same molecular mass as that found for the subunits of barley and *Synechococcus* enzyme [11].

As regards the cofactor requirement, (1) the barley enzyme is insensitive to the addition of pyridoxal 5'-phosphate (PLP) or pyridoxamine 5'-phosphate (PMP) cofactor [7,12], (2) the *Synechococcus* GSA-AT activity is stimulated by the addition of PMP [7,13], and (3) the *Chlamydomonas* enzyme activity is increased only by the addition of PLP and not by PMP [11].

GSA-ATs studied so far are very sensitive to the inhibitor 3-amino-2,3-dihydrobenzoic acid (gabaculine) [12,14], and it has been assumed that the inhibition of tetrapyrrole synthesis by gabaculine is due to irreversible inactivation of these enzymes [15,16].

Sulfolobus solfataricus belongs to a genus of aerobic thermophilic sulphur-dependent bacteria that have been classified within a subgroup of Archaea named crenarchaeotes [17]; or, alternatively, it has been suggested that these bacteria comprise a

distinct prokaryotic group designated eocytes [18,19]. On the basis of a phylogenetic comparison of the genes encoding rRNA, eocytes have been placed closer to the nuclear-cytoplasmic descendent line of eukaryotes than to eubacteria or other archaeobacteria [20]. Because aplastidic eukaryotes synthesize ALA by a pathway differing from that used by the vast majority of prokaryotes [21], it was of interest to determine the ALA synthetic route in eocytes in the light of the proposed phylogenetic affiliation of eocytes to eukaryotes.

Matters and Beale [22] have already reported some preliminary data on GSA-AT, determined on a crude extract of *S. solfataricus*. This paper reports the purification and characterization of GSA-AT from *S. solfataricus*.

MATERIALS AND METHODS

Micro-organism and culture conditions

S. solfataricus strain MT-4 was isolated from an acidic hot spring in Agnano, Naples, Italy. The organism was grown at 87 °C in a 90 litre fermenter with low mechanical agitation and an aeration flux of 30 ml/min per litre of broth [23]. The standard culture medium was the Deutsche Sammlung von Mikroorganismen 182 medium with glucose as the sole carbon source. The pH of the culture media was adjusted to 3.5 with 0.1 M H₂SO₄.

Cell growth was quantified turbidimetrically at 540 nm. Cells were harvested in the early stationary phase of growth by continuous-flow centrifugation on an Alfa Laval model LAB 102 B-20 centrifuge. The pellet was then collected by centrifugation at 9000 g for 30 min at 4 °C.

GSA-AT assay

GSA-AT activity was measured by using chemically synthesized GSA [24]. The assay mixture contained, in a total volume of 0.5 ml of 0.1 M Mops buffer, pH 6.5, 50 mM laevulinic acid (LA), 10 mM dithiothreitol (DTT) and 0.02 mM PMP, with 0.025 mM GSA as substrate. Reactions were incubated for 7 min at 60 °C and stopped by adding 10 μ l of 70 % (v/v) perchloric

Abbreviations used: ALA, δ -aminolaevulinic acid; DTT, dithiothreitol; gabaculine, 3-amino-2,3-dihydrobenzoic acid; GSA, glutamate 1-semialdehyde; GSA-AT, GSA aminotransferase; LA, laevulinic acid; PLP, pyridoxal 5'-phosphate; PMP, pyridoxamine 5'-phosphate; PTH, phenylthiohydantoin.

* Dedicated to Professor A. Ballio on the occasion of his 75th birthday.

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acid. The precipitated proteins were removed by centrifugation at 14000 *g* for 30 min at 4 °C. Supernatant (0.4 ml) was transferred to a 1.5 ml tube and the pH was adjusted to 7.2 with 0.1 ml of 0.5 M Na₃PO₄ and 8 µl of 10 M NaOH. Ethyl acetoacetate (0.05 ml) was added and the mixture was placed in a boiling-water bath for 10 min. The tube was then cooled to room temperature and an equal volume (0.558 ml) of modified Ehrlich's reagent was added. After 30 min at room temperature the mixture was centrifuged at 14000 *g* for 40 min at 4 °C. The absorption spectrum of the supernatant was recorded from 450 to 600 nm and the absorbance at 553 nm was determined to quantify the ALA pyrrole, with the use of a molar absorption coefficient of $7.2 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$ [25]. Control reactions without enzyme or substrate were performed and the background value was subtracted.

To determine the effect of gabaculine on the activity of GSA-AT, the enzyme was incubated at 60 °C for 2 min in a mixture containing 0.05 M LA, after which gabaculine at different concentrations was added. Samples were first incubated at room temperature for 20 min and then the GSA was added to start the enzymic reaction as described before. To investigate the cofactor requirement of GSA-AT, the enzyme was incubated in the presence of different concentrations of PMP cofactor (from 0 to 100 µM) and assayed by following the procedure described.

GSA-AT purification

Preparation of crude cell extract

Wet cells (66 g) were suspended in 55 ml of 40 mM Tris/HCl buffer, pH 8. Approximately one-third volume of sand was added and the suspension was ground in a mortar. The mixture was further homogenized in the stainless steel chamber of a Sorvall Omni-Mixer for 3 min at half speed and for 1 min at full speed. The homogenate was centrifuged at 14000 *g* (Beckman JS-21, rotor JA-20) for 30 min at 4 °C. The supernatant was further ultracentrifuged at 35000 *g* for 30 min at 4 °C, extensively dialysed against 50 mM Tris/HCl buffer, pH 7.0, and then stored at -80 °C before further use.

Superdex 200 gel-filtration chromatography

The gel-filtration column used in the initial purification of GSA-AT was connected to an FPLC System (Pharmacia). The column (Superdex 200 16/60) was equilibrated with 50 mM Tris/HCl buffer, pH 7.0, containing 0.1 M NaCl, at a flow rate of 1.5 ml/min. Calibration was performed with the following molecular mass standards: β -amylase (200 kDa), β -galactosidase (116 kDa), BSA (66 kDa), ovalbumin (44 kDa), carbonic anhydrase (29 kDa) and cytochrome *c* (12.4 kDa). The soluble protein extract from *S. solfataricus* was dialysed against the gel-filtration running buffer and then loaded on the Superdex column. The active fractions were pooled and concentrated by ultra-filtration on an AMICON PM 10 filter.

FPLC Mono Q ion-exchange chromatography

Anion-exchange chromatography was performed on the Pharmacia FPLC system in a sodium phosphate running buffer, pH 7.0. Active protein fractions from gel filtration were applied to a pre-equilibrated Mono Q HR5/5 column at a flow rate of 1 ml/min, and the GSA-AT enzyme was eluted with a linear gradient of NaCl from 0.05 to 0.4 M in 1 h. Fractions with GSA-AT activity were collected and dialysed against 50 mM Tris/HCl buffer, pH 7.0, before further analysis.

Protein determination

The amount of protein in each sample was determined with the Bio-Rad Protein Assay [26], with BSA as standard.

Molecular mass determination

The molecular mass of native GSA-AT was determined with a SMART System on a Superose 12 gel filtration column (PC3.2/30; Pharmacia, Uppsala, Sweden). The column was eluted with 50 mM sodium/Mops buffer, pH 7.5, containing 0.15 M NaCl, and the eluate was monitored at 280 and 220 nm (flow rate 0.1 ml/min). The column was calibrated with the same molecular mass standards as in the gel-filtration step on the Superdex 200 column.

Electrophoresis

Polyacrylamide (12%, w/v) gel slab electrophoresis in 0.1% SDS (SDS/PAGE) was performed as described by Laemmli [27]. For molecular mass determination, the gel was calibrated with phosphorylase *b* (97.4 kDa), ovalbumin (44 kDa), carbonic anhydrase (29 kDa), trypsin inhibitor (20.1 kDa) and α -lactalbumin (14.4 kDa). Proteins were revealed by the silver [28] or by the Coomassie Blue [29] staining method.

Analytical isoelectric focusing in the pH range 2.5–7.0 (Pharmalyte; Sigma, St. Louis, MO, U.S.A.) was performed on a 5.0% (w/v) acrylamide gel slab with a LKB Multiphor electrophoresis system (Pharmacia) in accordance with the manufacturer's instructions.

N-terminal analysis of electroblotted protein

Samples of GSA-AT were electroblotted from SDS/12.5% polyacrylamide gels on poly(vinylidene difluoride) membranes (Immobilon; Millipore). Transfer was performed as described by Yuen et al. [30] in 10 mM 3-(cyclohexylamino)-1-propane-sulphonic acid (pH 11)/10% (v/v) methanol at 5 mA/cm² for 1 h at 4 °C; transferred proteins were stained with Coomassie Blue R-250. The N-terminal amino acid sequence of blotted GSA-AT was determined by stepwise Edman degradation with an Applied Biosystems–Perkin Elmer 477A pulsed-liquid protein sequencer equipped with a 120A HPLC apparatus for the online phenylthiohydantoin-amino acid identification. N-terminal analysis of GSA-AT was performed on enzyme samples purified from two different cell cultures.

Conversion of glutamate to ALA *in vitro*

Preparation of crude cell extract

Wet cells (10 g) of *S. solfataricus* were washed with 0.1 M Bicine (pH 7.9)/25 mM MgCl₂/5 mM DTT, suspended in 30 ml of the same buffer and sonicated in 3 ml portions for 150 s each (30 s intervals). This sonicate was dialysed against buffer for 5 h with two changes of buffer. *E. coli* TOP F 10 cells (1.07 g) grown in Luria–Bertani medium with 15 µg/ml tetracycline were harvested by centrifugation at 4500 *g* for 30 min at 4 °C, washed with 0.1 M Bicine (pH 7.9)/25 mM MgCl₂/5 mM DTT, suspended in 6 ml of the same buffer and sonicated in 3 ml portions for 10 min (30 s intervals). This sonicate was dialysed against buffer for 5 h with two changes of buffer.

Assay conditions

The reaction mixture contained, in a total volume of 0.25 ml, 0.1 M Bicine, pH 7.9, 25 mM MgCl₂, 5 mM DTT, 5 mM ATP, 1 mM NADPH, 5 mM LA, 20 μ M PMP and 0.2 ml of *S. solfataricus* sonicate, with 3 mM glutamate as substrate. The control contained sonicate and buffer. After 1 h at 60 °C, 25 μ l of 1 M citric acid and 0.25 ml of 10 % (w/v) SDS was added. The mixture was boiled for 2 min, cooled to room temperature and centrifuged at 14000 g for 20 min at 4 °C. The reaction conditions for *E. coli* sonicate were the same as described for *S. solfataricus* sonicate except that the reaction mixture contained 20 μ M PLP instead of PMP and was incubated for 2 h at 37 °C.

Determination of ALA

ALA synthesis was determined by the method of Weinstein and Beale [31]. The mixture was applied to a Dowex 50X 8-400 column. The column had been prewashed with 2 ml of 1 M NaOH and 2 ml of 0.05 M sodium citrate, pH 3.01; the sample was loaded and the column was washed first with 2 ml of water and then with 2 ml of citrate, pH 3.00, in 25 % (v/v) methanol. ALA was eluted with 0.5 M sodium phosphate, pH 6.8, in 1 ml fractions. Ethyl acetoacetate (50 μ l) was added to each fraction and ALA was determined as described above. To study the effect of cofactors, the sonicate was processed as described above and assayed with selective exclusions of substrate and cofactors from the incubation mixture.

RESULTS AND DISCUSSION

GSA-AT assay

Measurement of the activity of GSA-AT is difficult because the substrate, GSA, is very unstable at pH values at which the enzyme is active (pH 7.2–8.2). In fact, as reported in a detailed study by Hooper et al. [12], under these conditions, GSA degrades spontaneously, also giving rise to the production of ALA [12,32]. Because ALA is also the product of the enzymic reaction, assay conditions had to be chosen that would measure the enzymic conversion in the presence of a consistent background of chemical formation of this product. This kind of problem is even more important when an assay must be conducted at higher temperatures. Therefore the chosen pH for the assay was 6.5, at which GSA is about twice as stable than at pH 8.0 [12,32]. The duration of assay and incubation temperature were chosen after several attempts: for the *S. solfataricus* enzyme an incubation temperature of 60 °C and a 7 min reaction time were the best compromise to allow the detection of the enzyme activity to a reasonable extent and to avoid a high background value in the control.

The above assay conditions were useful in following the enzyme purification, and should simply be considered to be the

working conditions. The fact that the assay had to be performed at a minimum of 60 °C owing to the enzyme's extremely low activity at 37 °C (results not shown) markedly affected the reliability of this assay to provide a true assessment of catalytic properties.

Purification of GSA-AT

A scheme of enzyme purification from crude cellular extracts of *S. solfataricus* is reported in Table 1. The final purification step was anion-exchange chromatography at pH 7.0. Whereas most proteins were eluted in the void volume, GSA-AT was recovered with a saline gradient step. The active fraction contained a single protein band with a molecular mass of 56 $\pm$ 1 kDa as shown by SDS/PAGE (Figure 1). An overall purification of 172-fold was obtained. Despite the large number of known mesophilic GSA-ATs from plant and bacterial sources, this is the first direct evidence of the presence of this enzyme in a thermophilic archaean.

N-terminal amino acid sequence of the GSA-AT

The N-terminal sequence of GSA-AT from *S. solfataricus* is presented in Figure 2. The sequence is aligned with the N-terminal sequence of the enzyme from barley, *Synechococcus* and *Salmonella typhimurium*. Of the first comparable 25 amino acid residues, 16 are identical. This suggests a significant identity (68 %) of this domain within GSA-AT with *S. solfataricus* and those from other sources. It is worth noting that comparison of the N-terminal sequence of the *S. solfataricus* GSA-AT with sequences in the Swiss-Prot databank did not reveal any significant similarity with other proteins. Although they refer to a very small part of the protein sequence, these results can be considered indicative of a significant similarity in the primary structure of the plant, cyanobacterial and thermophilic archaean aminotransferases.

Physical properties of GSA-AT

The apparent molecular mass of the native GSA-AT was determined by gel-filtration chromatography. The peak of GSA-AT activity was found in a fraction corresponding to a molecular mass of 87 $\pm$ 5 kDa. The SDS/gel electrophoresis analysis revealed a single band at 56 $\pm$ 1 kDa (Table 2); considering that *Sulfolobus* proteins generally show an anomalously higher molecular mass when analysed by SDS/PAGE [33], we concluded that GSA-AT from *S. solfataricus* is a functional dimer. The purified enzyme was analysed by isoelectric focusing; the silver-stained gel revealed a single protein band localized at pH 6.2 (Table 2).

Cofactor requirements

The absorption spectrum of the freshly purified GSA-AT is typical of a transaminase containing PMP as cofactor with a λ_{max} at 340 nm (Figure 3). Although the purified enzyme is mainly in the pyridoxamine form, its activity can be increased by approx. 50 % by the addition of PMP to the assay mixture. It is worth noting that GSA-AT purified from other sources (barley and *Chlamydomonas*) is almost in its apo-enzyme form [11,34]. Although this aspect has to be studied in more detail, it seems that the affinity of *S. solfataricus* GSA-AT for the PMP coenzyme is high and similar to that observed for GSA-AT from *Synechococcus* [7]. Therefore, as regards the effect of PMP/PLP cofactors, the GSA-AT of the archaeobacterium *S. solfataricus* is more similar to the corresponding enzyme from *Synechococcus*

Table 1 Purification of GSA-AT from *S. solfataricus*

One unit of activity is defined as the amount of enzyme that causes the formation of 1 nmol of ALA at 60 °C under the assay conditions described in the Materials and methods section.

	Total activity (units)	Protein (mg)	Specific activity (units/mg)	Yield (%)	Purification (fold)
Homogenate	3843	639.9	6.0	100	1
Gel-filtration	1445	71.6	20.2	38	3.4
Mono-Q	1560	1.5	1033	40	172

Figure 1 Elution profile of gel chromatography on Mono Q of *S. solfataricus* cell extract after gel-filtration chromatography on Sephadex 200

Chromatographic conditions are described in the Materials and methods section. The peak marked C was the only one showing GSA-AT activity. The inset shows the SDS/PAGE pattern of samples after different purification steps: M, molecular mass markers; A, extract of *S. solfataricus* cells; B, active sample collected after gel-filtration chromatography on Sephadex 200; C, active sample collected after gel chromatography on Mono Q.

Figure 2 N-terminal amino acid sequences of the *S. solfataricus*, *Salmonella typhimurium* [10], *Synechococcus* [7] and *Hordeum vulgare* [9] 46 kDa protein

Identical sequences are shown in bold characters.

Table 2 Comparison of kinetic and physical parameters of GSA-AT from different organisms

Abbreviation: n.d., not determined.

	<i>S. solfataricus</i>	Greening barley*	<i>Synechococcus</i> †
Molecular mass, native (kDa)	87	92	92
Molecular mass, SDS/PAGE (kDa)	56	46	46
<i>K_m</i> (GSA) (mM)	0.38‡	0.4	0.22
pI	6.2	5.1	n.d.

* References: [7,12].
† References: [14] and B. Grimm, personal communication.
‡ This value was obtained with an enzymic assay that did not provide a true assessment of catalytic properties (see text).

than to the enzyme from barley or that from *Chlamydomonas* [11].

Effect of gabaculine on GSA-AT activity

Gabaculine is considered to be a suicide inhibitor of PLP-dependent enzymes in their pyridoxal form [15,16]. When GSA-AT from *S. solfataricus* was preincubated with different amounts

Figure 3 Absorption spectrum of freshly purified GSA-AT (0.24 mg/ml) from *S. solfataricus*

of gabaculine (from 0 to 110 nM) and in the presence of a large excess of LA (which apparently serves as an amino-group acceptor, inducing the conversion of the enzyme to the PLP form) a decrease in activity was observed: 0.08 μM gabaculine was able to produce 50 % inhibition after a 20 min incubation at room temperature with the enzyme (0.03 mg/ml). Gabaculine can thus be considered an inhibitor of GSA-AT from *S. solfataricus*, as it is for the enzyme from other sources [11,12,34].

Conversion of glutamate to ALA in vitro

The conversion of glutamate to ALA in all cells that use the C₅ pathway requires glutamate as substrate, and ATP, Mg²⁺ ions, NADPH and PMP or PLP as cofactors. A cell extract of *S. solfataricus* has been proved to catalyse the formation of ALA in an assay system containing glutamate, ATP, Mg²⁺, NADPH, PMP and LA, an ALA dehydratase inhibitor [35]. Samples were incubated for 1 and 2 h at the same temperature (60 °C) used for the GSA-AT assay. Incubation of 0.20 ml of cell extract

(16 mg/ml protein) gave rise to the production of approx. 1 nmol of ALA after 1 h of incubation. No additional ALA production was observed after a further 1 h of incubation. The elimination of glutamate or of any of the cofactors except LA and PMP resulted in a nearly complete loss of activity. *E. coli* cell extract was used as a positive control for this assay procedure, with PLP instead of PMP as cofactor and incubating the assay mixture at 37 °C for 2 h [36].

Because this paper provides direct evidence of the existence of GSA-AT in *S. solfataricus*, the assay described above implies that an enzyme catalysing the reduction of glutamate to GSA has also to be present. Because of the fact that ATP, Mg²⁺ and NADPH are required, we can conclude that a Glu-tRNA reductase activity is also present in this archaeobacterium. All the results obtained, even with the selective exclusion of these cofactors, are consistent with ALA biosynthesis by the same pathway occurring in all other prokaryotes with the exception of the α -proteobacteria. These results confirm on a more concrete basis the evolutionary conclusions already drawn by Matters and Beale [22], i.e. that the ALA-synthase-catalysed condensation of glycine and succinyl-CoA was not derived from a possible eocyte ancestor but was instead acquired from the likely progenitor of mitochondria, the α -proteobacteria, which also use the ALA synthase route [22,37].

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REFERENCES

- Kikuchi, G., Kumar, A., Talmage, P. and Shemin, D. (1958) *J. Biol. Chem.* **233**, 1214–1249
- Gibson, K. D., Laver, W. G. and Neuberger, A. (1958) *Biochem. J.* **70**, 71–81
- Beale, S. I. and Weinstein, J. D. (1990) in *Biosynthesis of Heme and Chlorophyll* (Dailey, H. A., ed.), pp. 287–391, McGraw-Hill, New York
- Scheer, H. (ed.) (1990) *The Chlorophylls*, CRC Press, Cleveland
- Kannangara, C. G., Andersen, R. V., Pontoppidan, B., Willows, R. and von Wettstein, D. (1994) *Giba Found. Symp.* **180**, 3–25
- Kannangara, C. G. and Gough, S. P. (1978) *Carlsberg Res. Commun.* **43**, 185–194
- Grimm, B., Bull, A., Welinder, K. G., Gough, S. P. and Kannangara, C. G. (1989) *Carlsberg Res. Commun.* **54**, 67–79
- Grimm, B., Bull, A. and Breu, V. (1991) *Mol. Gen. Genet.* **225**, 1–10
- Grimm, B. (1990) *Proc. Natl. Acad. Sci. U.S.A.* **87**, 4169–4173
- Elliot, T., Avvisar, Y. J., Rhie, G. E. and Beale, S. I. (1990) *J. Bacteriol.* **172**, 7071–7084
- Jahn, D., Chen, M. W. and Söll, D. (1991) *J. Biol. Chem.* **266**, 161–167
- Hooper, J. K., Kahn, A., Ash, D. E., Gough, S. P. and Kannangara, C. G. (1988) *Carlsberg Res. Commun.* **53**, 11–25
- Bull, A. D., Pakes, J. F., Hoult, R. C., Rogers, L. J. and Smith, A. J. (1989) *Biochem. Soc. Trans.* **17**, 911–912
- Smith, M. A., Grimm, B., Kannangara, C. G. and von Wettstein, D. (1991) *Proc. Natl. Acad. Sci. U.S.A.* **88**, 9775–9779
- Rando, R. R. (1977) *Biochemistry* **16**, 4604–4610
- Soper, T. S. and Manning, J. M. (1985) in *Transaminases* (Christen, P. and Metzler, D. E., eds.), pp. 266–284, Wiley and Sons, New York
- Woese, C. R., Kandler, O. and Wheelis, M. L. (1990) *Proc. Natl. Acad. Sci. U.S.A.* **87**, 4576–4579
- Lake, J. A., Henderson, E., Oakes, M. and Clark, M. W. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 3786–3790
- Lake, J. A. (1987) *Cold Spring Harb. Symp. Quant. Biol.* **52**, 839–846
- Rivera, M. C. and Lake, J. A. (1992) *Science* **257**, 74–76
- Redlinger, R. E. and Beale, S. I. (1991) in *Photoreceptor Evolution and Function* (Holmes, M. G., ed.), pp. 151–179, Academic Press, New York
- Matters, G. L. and Beale, S. I. (1994) *Arch. Microbiol.* **161**, 272–276
- Marino, G., Nitti, G., Arnone, M. I., Sannia, G., Gambacorta, A. and De Rosa, M. (1988) *J. Biol. Chem.* **263**, 12305–12309
- Pugh, C. F., Nair, S. P., Harwood, J. L. and John, R. A. (1991) *Anal. Biochem.* **198**, 43–46
- Mauzerall, D. and Granick, S. (1956) *J. Biol. Chem.* **219**, 435–446
- Bradford, M. M. (1986) *Anal. Biochem.* **72**, 248–254
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Henkeshoven, J. and Dernick, R. (1988) *Electrophoresis* **9**, 28–32
- Zehr, B. D. and Savin, T. J. (1989) *Anal. Biochem.* **182**, 157–159
- Yuen, S., Hunkapiller, M. W., Wilson, K. J. and Yuan, P. M. (1986) *Appl. Biosyst. User Bull.* no. 25
- Weinstein, J. D. and Beale, S. I. (1985) *Arch. Biochem. Biophys.* **237**, 454–464
- Pugh, C. E., Nair, S. P., Harwood, J. L. and John, R. A. (1991) *Anal. Biochem.* **198**, 43–46
- Dianoux, A. C., Stasia, M. J., Garin, J., Gagnon, J. and Vignais, P. V. (1992) *Biochemistry* **31**, 5898–5905
- Houghton, J. D., Brown, S. B., Gough, S. P. and Kannangara, C. G. (1989) *Carlsberg Res. Commun.* **54**, 131–143
- Friedmann, H. C. and Thauer, R. K. (1986) *FEBS Lett.* **207**, 84–88
- Li, J. M., Brathwaite, O., Cosloy, S. D. and Russell, C. S. (1989) *J. Bacteriol.* **171**, 2547–2552
- Avissar, Y. J., Ormerod, J. G. and Beale, S. I. (1989) *Arch. Microbiol.* **151**, 513–519