Soluble and Particulate Porphobilinogen-Deaminase from Dark-Grown *Euglena gracilis*

M. V. Rossetti, A. A. Juknat, and A. M. del C. Batlle

Centro de Investigaciones sobre Porfirinas y Porfirias, CIPYP, (FCEN, UBA, CONICET), Ciudad Universitaria, Pabellon II, 2do. Piso, 1428 Buenos Aires, Argentine

Z. Naturforsch. **44c,** 578–580 (1989); received July 25, 1988/April 4, 1989

Euglena gracilis, Porphobilinogen-Deaminase, Porphyrins, Soluble, Particulate

A highly efficient method employing NaSCN as a chaotropic agent was used to dissociate the membrane-bound porphobilinogen-deaminase.

The same sequence of steps was applied for purifying both soluble and membrane dissociated porphobilinogen-deaminase. The chromatographic behaviour of both proteins was quite similar. *Euglena gracilis* deaminase appears to exist in an equilibrium mixture of two active species of relative molecular masses of 40000 and 20000.

Introduction

Porphobilinogen (PBG)-deaminase (hydroxymethylbilane synthetase; EC 4.3.1.8) catalyzes the conversion of PBG into hydroxymethylbilane by a simple head to tail condensation of four substrate units, with elimination of ammonia. The final tetrapyrrol closure produces the uroporphyrinogen I [1–4]. The hydroxymethylbilane can be converted into the macrocyclic ring of uroporphyrinogen III by the uroporphyrinogen III synthase (isomerase; EC 4.2.1.75). This enzyme flips the ring D producing an asymetric arrangement and yielding the physiological intermediate after tetrapyrrole closure [1, 5].

PBG-deaminase has been isolated and purified from several sources, including bacteria, algae and animals ([6] and ref. therein [7–10]), however a simple purification method for cytoplasmic and membrane-bound PBG-deaminase had not been reported as yet.

Comparisons between the soluble and particulate deaminase-isomerase complex from dark [11] and light grown [12] *Euglena gracilis* have been already carried out.

In the present study isolation, purification and some properties of both cytoplasmic and particulate PBG-deaminase from dark-grown *Euglena gracilis* are reported.

Reprint requests to Prof. Alcira Batlle, Viamonte 1881-10 "A", 1056 Buenos Aires, Argentine.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/89/0700–0578 $ 01.30/0

Materials and Methods

Except otherwise indicated, chemicals obtained, source of enzyme and methodology were as previously indicated [11].

The enzyme unit is defined as the amount of enzyme that catalyzes the formation of one nmol uroporphyrinogen I in 4 h from PBG, under the standard incubation conditions. Specific activity is expressed as units of enzyme/mg of protein.

Results and Discussion

After several preliminary trials, the sequence finally adopted to purify the soluble PBG-deaminase was determined. Cells were disrupted by ultrasonic treatment for 30 sec and the homogenate fraction was centrifuged at $24000 \times g$ for 30 min. Glacial acetic acid was added to the $24000 \times g$ supernatant to adjust it to pH 5. After 30 min the material was centrifuged and the supernatant was fractionated with solid ammonium sulphate (30–80% saturation) and filtered through a Sephadex G-25 column.

To obtained a PBG-deaminase preparation free of isomerase and decarboxylases, the eluted protein was heated at 60 °C during 15 min. After centrifugation the supernatant was concentrated by precipitation with solid ammonium sulphate to 80% saturation. The resuspended enzyme solution was applied to a Sephadex G-100 column (2.4×59.5 cm, void volume 195 ml, flow rate 40 ml/h), previously calibrated for molecular mass determinations. Fig. 1 shows a typical elution profile. Specific activity of deaminase was associated with the mean peak

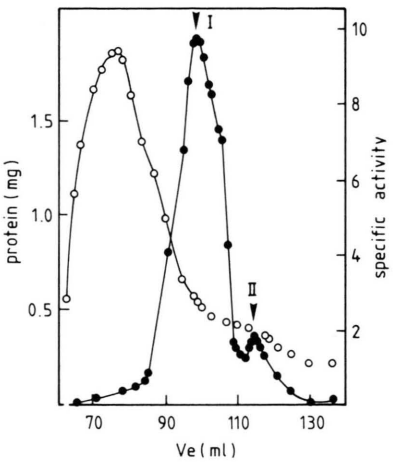

Fig. 1. Soluble *E. gracilis* deaminase. Elution profile on Sephadex G-100 of a 154-fold purified fraction. The column was equilibrated and eluted with 0.05 M sodium phosphate buffer pH 7.4. Protein content (○); specific activity (●). Other experimental conditions are as indicated in methods.

(band I) resulting in a preparation 1200-fold purified, with a 36% yield and a relative molecular mass of 40000 ± 4000. A second peak (band II), also exhibiting deaminase activity, to which a relative molecular mass of 20000 ± 2000 corresponds, was also observed.

When the 80% ammonium sulphate precipitate was applied to a Sepharose 4B column (2.6 × 40 cm, void volume 195 ml, flow rate 38 ml/h) practically 95% of the enzyme activity was associated with the mean active band, to which a relative molecular mass of 40000 corresponds, with a purification degree of about 400-fold and 65% yield. Obviously, resolution of low molecular mass species was better on Sephadex G-100 than using Sepharose gels.

Protein peaks from Sephadex and Sepharose columns were subjected to electrophoresis and the values of relative molecular masses already found were confirmed.

On the other hand, in order to obtain the particulate PBG-deaminase, the 24000 × *g* pellet was carefully washed, resuspended in 0.5 M NaSCN and disrupted by ultrasonic treatment for 30 sec. The suspension was centrifuged at 105000 × *g* for 90 min and the NaSCN was removed applying the 105000 × *g* supernatant to a Sephadex G-25 column.

Purification of the membrane dissociated PBG-deaminase follows the sequence of steps described above for the soluble enzyme.

When the 80% saturated ammonium sulphate precipitate corresponding to the particulate protein was resuspended and applied to a Sephadex G-100 column, two active peaks of relative molecular masses of 40000 (I) and 20000 (II), which were confirmed by electrophoresis, were observed (Fig. 2). Moreover, starting from the pellet fraction we also get a highly purified preparation of deaminase (1200-fold for peak I).

It should be noted that the last fraction (Sephadex G-100) resulting either from the soluble or from the membrane bound preparation was not homogeneous on sodium dodecylsulphate polyacrylamide gel electrophoresis.

It is worth noting at this point that results here reported were obtained measuring the enzyme activity under anaerobiosis; however, parallel assays were performed in aerobic conditions, revealing that whether or not oxygen was present, the activity of both the soluble and the particulate purified PBG-deaminase was the same.

Therefore we have found that the main molecular form of deaminase is that of molecular mass 40000, in good agreement with the molecular masses reported for the enzyme of other sources ([8] and ref. therein). It was also evident that *E. gracilis* deamin-

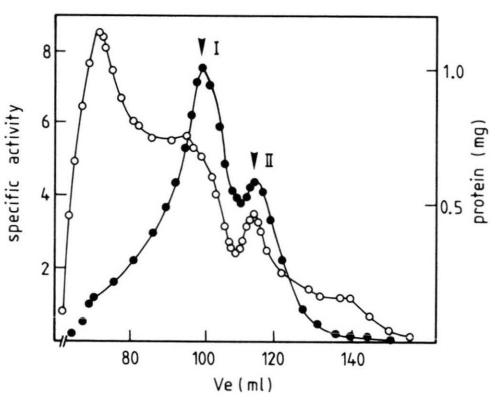

Fig. 2. Particulate *E. gracilis* deaminase. Elution profile on Sephadex G-100 of a preparation of a 112-fold purified fraction. The same column as that described in Fig. 1 was used. Protein content (○); specific activity (●). Other experimental conditions are as indicated in methods.

ase would appear to exist in an equilibrium mixture of two active species of molecular masses 40000 and 20000.

As far as the soluble and particulate PBG-deaminase is concerned, we do not know yet if both enzymes are the same protein or not, so far we have just found that their chromatographic behaviour is rather similar but further studies are required to assess the protein identity of both the cytoplasmatic and membrane bound enzyme.

[1] A. R. Battersby, C. J. R. Fookes, G. W. J. Matcham, E. McDonald, and K. E. Gustafson-Potter, J. Chem. Soc. Chem. Commun. **1979,** 316.
[2] A. R. Battersby, C. J. R. Fookes, E. McDonald, and G. W. J. Matcham, Bioorg. Chem. **8,** 451 (1979).
[3] G. Burton, P. E. Fagerness, S. Hosozawa, P. M. Jordan, and A. I. Scott, J. Chem. Soc. Chem. Commun. **1979,** 202.
[4] A. I. Scott, G. Burton, P. M. Jordan, H. Matsumoto, P. E. Fagerness, and L. Pryde, J. Chem. Soc. Chem. Commun. **1980,** 384.
[5] P. M. Jordan, G. Burton, H. Nordlov, M. M. Schneider, L. M. Pryde, and A. I. Scott, J. Chem. Soc. Chem. Commun. **1979,** 204.
[6] A. M. del C. Batlle and M. V. Rossetti, Int. J. Biochem. **8,** 251 (1977).
[7] D. C. Williams, Biochem J. **217,** 675 (1984).
[8] S. A. Fumagalli, M. L. Kotler, M. V. Rossetti, and A. M. del C. Batlle, Int. J. Biochem. **17** (4), 485 (1985).
[9] M. L. Kotler, S. A. Fumagalli, A. A. Juknat, and A. M. del C. Batlle, Comp. Biochem. Physiol. **87B** (3), 601 (1987).
[10] E. Smythie and D. C. Williams, Biochem. J. **251,** 237 (1988).
[11] M. V. Rossetti, M. L. Lombardo, A. A. Juknat de Geralnik, L. S. Araujo, and A. M. del C. Batlle, Comp. Biochem. Physiol. **85B** (2), 451 (1986).
[12] A. A. Juknat, D. Dörnemann, and H. Senger, Z. Naturforsch. **44c,** 81–94 (1989).