ORGANIZATION OF URATE OXIDASE IN PEROXISOMAL NUCLEOIDS

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1. Introduction

Rat liver peroxisomes (microbodies) contain an electron-dense core (nucleoid) which has an ordered structure [1]. According to studies [2] of the fine structure of nucleoids isolated from rat liver, these particles contain parallel bundles of tubules, the outer diameter of which is approx. 150 Å.

On the basis of density gradient centrifugation and electron microscope observations, it was concluded [3,4] that nucleoids contain urate oxidase. The peroxisomal core could exclusively consist of this enzyme [3]. However, this was challenged [5,6].

As shown [2], the specific activity of rat liver urate oxidase increases more than 380-fold in isolated nucleoids with respect to total homogenate. The specific activity of urate oxidase in rat liver nucleoids is 1.36 units [4], 1/10th the specific urate oxidase activity of the purified preparation of this enzyme obtained [7] in a soluble form from hog liver. Rat liver nucleoids were dissolved at pH 11.0 and fractionated by Sephadex G-200 chromatography [6]. They found that the elution patterns of protein and urate oxidase were close but obtained a low specific activity as a result of inactivation during isolation of nucleoids.

In the present study we have examined the molecular organization of nucleoids from rat liver peroxisomes. Our data show that nucleoids contain only urate oxidase molecules. The enzyme soluble at pH 11.0 has mol. wt 230 000 and apparently consists of 6 equally-sized polypeptide chains.

2. Materials and methods

2.1. *Materials*

Sodium dodecyl sulfate (SDS), Tris, bovine serum albumin, ovalbumin, catalase from bovine liver were from Serva Chemical Co; Triton X-100, NAD^+, cytochrome *c* (type 3), α-chymotrypsinogen from Schuchardt, München; Sephadex G-200 from Pharmacia; EDTA disodium salt from VEB Berlin-Chemie; sucrose from Chemapol; sodium deoxycholate, alcohol dehydrogenase from yeast and horse liver, reagents for polyacrylamide gel electrophoresis from Reanal.

2.2. *Methods*

Wistar male rats, 200 g, were used. The liver fraction containing mitochondria, lysosomes and peroxisomes (λ fraction) was prepared as in [6]. This fraction was then used for isolation of purified peroxisomes [8,9]. Nucleoids were prepared by treating peroxisomes with 0.5% sodium deoxycholate in 0.05 M Tris–HCl, pH 7.5, 5 mM EDTA. Nucleoids were also purified as in [2] with a slight modification, the λ fraction was homogenized with 0.2% Triton X-100 in 0.1 M Tris–HCl, pH 9.0, 5 mM EDTA and then centrifuged at 12 500 × *g* for 35 min. This procedure was repeated 4 times.

Urate oxidase (EC 1.7.3.3) was assayed as in [6], but at pH 8.5. Catalase (EC 1.11.1.6) [10] and alcohol dehydrogenase (EC 1.1.1.1) [11] activities were measured at 25°C using a Unicam Sp-800 spectrophotometer. Protein was determined as in [12].

Nucleoids were dissolved in 0.05 M Tris–NaOH, 5 mM EDTA at pH 10.2 or pH 11.0 and 30°C. The 'soluble' form of urate oxidase was obtained by centrifugation of nucleoids, after treatment with Tris–NaOH, at 105 000 × *g* for 60 min.

Gel chromatography was done in a 2.5 × 75 cm column using Sephadex G-200. At 4 ml sample (0.7–1.0 mg protein/ml) of nucleoid material soluble at pH 11.0

was applied to a column of Sephadex G-200 equilibrated with 0.05 M Tris–NaOH, pH 11.0 and was eluted with the same buffer. Fractions, 2.7 ml, were collected with upward flow at 12 ml/h and assayed for urate oxidase activity and protein. The molecular weight of the enzyme was estimated from a calibration curve for the column using catalase (mol. wt 240 000), alcohol dehydrogenase from yeast (mol. wt 150 000) and horse liver (mol. wt 78 000), bovine serum albumin (mol. wt 68 000) and cytochrome *c* (mol. wt 11 700) as standards.

For sucrose density gradient centrifugation a solution of urate oxidase, pH 10.2 or pH 11.0, (1 mg protein/ml) was mixed with the molecular weight standards and 2 ml mixture were applied to 5–22% buffered sucrose gradients (32 ml). Centrifugation was at 4°C and 65 000 × *g* for 22 h in a UCP-60 centrifuge (USSR).

Samples for polyacrylamide gel electrophoresis were prepared by dissolving nucleoids in 1% SDS and 0.1% β-mercaptoethanol. Complete solubilization was achieved by heating the sample at 100°C for 1–2 min. Gel electrophoresis was performed in 10% or 5% polyacrylamide gels as in [13]. Gels were fixed and stained with Coomassie blue after electrophoresis. Densitometric measurements were made in a Gilford-240 spectrophotometer with linear scanning.

3. Results and discussion

Nucleoids were obtained from the λ fraction and from peroxisomes using 2 different methods. In both cases urate oxidase was purified about 380-fold, with spec. act. 2542 nmol/min/mg protein (table 1). Addition of EDTA prevented enzyme inactivation during nucleoid isolation and dissociation at pH 11.0.

The molecular weight of urate oxidase, solubilized at pH 11.0 was determined by gel-filtration on Sephadex G-200. Figure 1 shows that the enzyme activity elutes as one symmetrical peak, and that the elution patterns of the protein and urate oxidase activity are very similar. Gel-filtration affects the specific activity very little (table 1). The molecular weight of urate oxidase calculated from a column calibration curve is 230 000 (fig.1B).

The size of the enzyme molecules solubilized in the native from at pH 10.2 and pH 11.0 was also stud-

Table 1
Specific activity of urate oxidase in various preparations of enzyme

Enzyme preparation	Spec. act.	Rel. act.
1. Whole homogenate	6.6	1.0
2. λ fraction	29.6	4.5
3. Purified peroxisomes	284.2	45.0
4. Nucleoids isolated from λ fraction	2540	385
5. Nucleoids isolated from purified peroxisomes	2542	385
6. Urate oxidase solubilized at pH 11.0	2542	385
7. Urate oxidase activity after gel-filtration on Sephadex G-200	2550	386

Specific activity of urate oxidase is given in nmol/min/mg protein

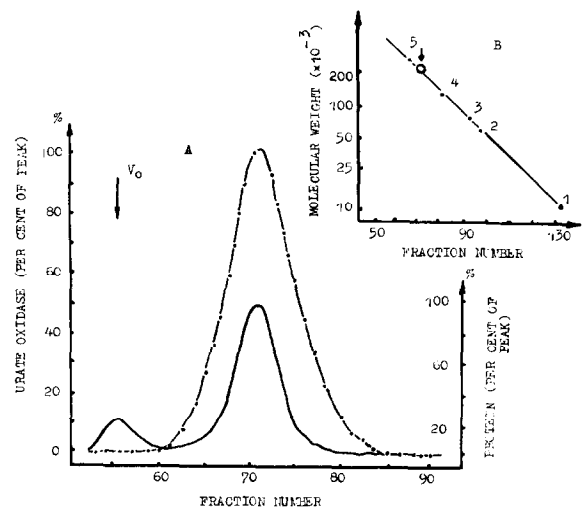

Fig.1. Gel filtration of urate oxidase on Sephadex G-200 (A). Protein: (——); urate oxidase activity (–·–·). Estimation of molecular weight of urate oxidase solubilized at pH 11.0 (B). The standard proteins were: (1) cytochrome *c*; (2) bovine serum albumin; (3) alcohol dehydrogenase from horse liver (4) from yeast; (5) catalase. The arrow indicates the elution volume of urate oxidase.

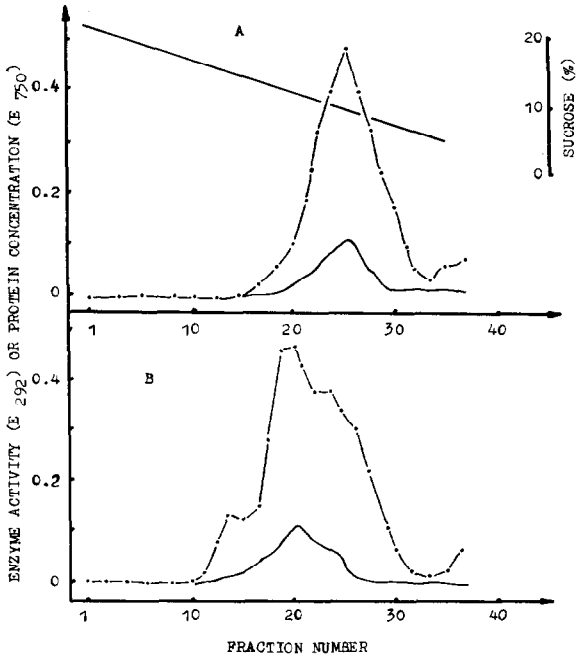

Fig.2. Sucrose gradient centrifugation of rat liver urate oxidase solubilized at pH 11.0 (A) or pH 10.2 (B). Conditions as in section 2. Enzyme activity (–·–·–) was measured in aliquots after fractionation of the gradient under the following conditions: time of incubation with 42 μM sodium urate 10 min at pH 8.5 and 37°C.

ied by gradient centrifugation (fig.2). Three peaks of enzyme activity were obtained after centrifugation at pH 10.2, whereas at pH 11.0 only one, slowly sedimenting peak was found. The molecules of urate oxidase solubilized at pH 11.0 migrated somewhat more slowly than catalase and the molecular weight of urate oxidase was estimated to be between 200 000 and 240 000. Only the approximate sizes of the higher molecular-weight forms of the enzyme were calculated. It is possible that these forms are a dimer and a trimer of the 230 000 unit. If this is so, one can conclude that the molecules of urate oxidase did not completely dissociate at pH 10.2. Additional treatment of pH 11.0 solubilized urate oxidase by anionic detergents did not decrease the molecular weight of the active 'monomer' (230 000), but this treatment inactivated the enzyme.

Electrophoresis in 10% SDS–polyacrylamide gels of nucleoids dissolved in 1% SDS at 100°C revealed

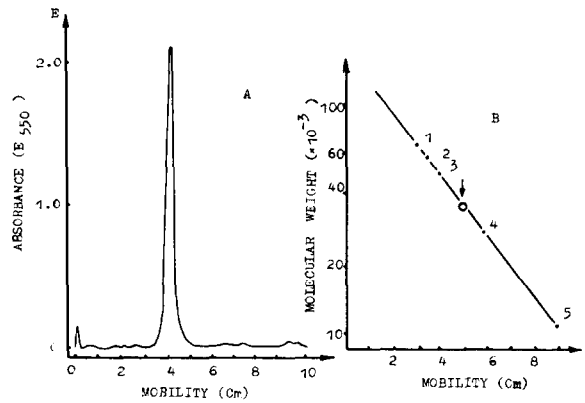

Fig.3. Dodecyl sulfate polyacrylamide gel electrophoresis of the purified peroxisomal nucleoids (A). Estimation of the polypeptide chain molecular weight (B). The standard proteins were: (1) bovine serum albumin; (2) catalase (subunit); (3) ovalbumin; (4) α-chymotrypsinogen; (5) cytochrome *c*. The arrow indicates the mobility of polypeptide chain of nucleoid.

only a single, enzymatically inactive band even at a high protein concentration (fig.3A). When this procedure was performed in the presence of marker proteins, an app. mol. wt 37 000 was determined for this polypeptide (fig.3B). β-Mercaptoethanol did not increase the mobility of the band.

Thus the results of SDS–polyacrylamide gel electrophoresis suggest that rat liver nucleoid consists only of urate oxidase monomer and does not contain other proteins. This result confirms that urate oxidase may constitute about 10% peroxisomal protein [5]. Comparing both the results from electrophoresis and gel chromatography experiments, it may be concluded that the molecular weight of a monomer (37 000) corresponds to the hexameric native structure of solubilized urate oxidase with mol. wt 230 000.

References

[1] De Duve, Ch. and Baudhuin, P. (1966) Physiol. Rev. 46, 323–357.
[2] Tsukada, H., Mochizuki, Y. and Fujiwara, S. (1966) J. Cell Biol. 28, 449–460.
[3] Hruban, L. and Swift, H. (1964) Science 146, 1316–1317.
[4] Baudhuin, P., Beaufay, H. and De Duve, Ch. (1965) J. Cell Biol. 26, 219–243.

[5] Leighton, F., Poole, B., Beaufay, H., Baudhuin, P., Coffey, J. W., Fowler, St. and De Duve, Ch. (1968) J. Cell Biol. 37, 482–513.
[6] Leighton, F., Poole, B., Lazarow, P. B. and De Duve, Ch. (1969) J. Cell Biol. 41, 521–535.
[7] Mahler, H. R., Hübcher, G. and Baum, H. (1955) J. Biol. Chem. 216, 625–641.
[8] Panchenko, L. F., Antonenkov, V. D. and Gerasimov, A. M. (1975) Bull. Exp. Biol. Med. 79, 86–87.
[9] Panchenko, L. F., Antonenkov, V. D. and Gerasimov, A. M. (1976) Int. J. Biochem. 7, 409–414.
[10] Beers, R. F. and Sizer, J. W. (1952) J. Biol. Chem. 195, 133–140.
[11] Warren, W. A. (1969) J. Biol. Chem. 245, 1675–1678.
[12] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[13] Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4409.