Isolation of the Peroxisomal Core from Rat Liver and Its Identification as Uricase

Kazuhisa Nakashima, Satoko Fujiwara and Sueko Hayashi

Department of Biochemistry (Chief : Prof. Tomoo Noguchi)

Kyushu Dental College, Kitakyushu, Japan

Received on June 15, 1988

Key words : *Peroxisome/Peroxisomal core/Uricase/Rat liver/Sucrose-density-gradient centrifugation*

Introduction

The degradation of purines to urate is common to all animal species, whereas the degradation of urate is much less complete in higher animals¹⁾. Enzymes involved in the degradation of purines to urate are located in the cytosol in marine fish, and those of urate to urea and glyoxylate are in the peroxisomes, suggesting that in purine metabolism, peroxisomal enzymes, e.g. uricase (EC 1.7.3.3), allantoinase (EC 3.5.2.5), and allantoinase (EC 3.5.3.4) have been lost during evolution^{2~4)}.

Uricase catalyses the initial reaction in the degradation of urate and exists as the insoluble form (core) in the peroxisomes of mammalian liver. Crystals of isolated uricase from hog liver are similar to the peroxisomal core in the electron microscopic feature, suggesting that the core consists of uricase exclusively⁵⁾. However, the peroxisomal core has been reported to be composed of uricase and other proteins^{6~8)}. Watanabe *et al.*⁹⁾ isolated the peroxisomal core from rat liver by minor modification of the method of Tsukada *et al.*¹⁰⁾ and claimed that the core was composed of uricase and a frame protein with a molecular weight of 27,000. In contrast, Antonenkov and Panchenko¹¹⁾ also isolated the core from rat liver by a similar method, suggesting that the core contained only uricase molecules. However, the isolation method of the core used in this study is based on its detergent insolubility. Furthermore, they examined the constituents of the isolated core by sodium dodecyl sulfate polyacrylamide gel electrophoresis after its gel filtration. In this method, the core-associated proteins other than uricase may be removed by repeated detergent treatments of the core and its gel filtration. Therefore, a new isolation method of the core is necessary for the identification of its constituents.

The present report describes a rapid isolation method of the peroxisomal core from rat liver without the use of detergents and its identification as uricase itself.

Materials and Methods

Peroxisomes were isolated from rat liver by using sucrose density gradient centrifugation in a vertical rotor according to the method of Osmundsen *et al.*^{1,2)}. Subsequent procedures were carried out at 0–4°C. Two milliliters of a peroxisomal suspension (2 ml) from 4 g of rat liver, free of mitochondrial, lysosomal and microsomal contamination, were diluted with the same volume of 0.01 M pyrophosphate buffer, pH 9.0, which was known to break rat liver peroxisomes⁷⁾. After being stored overnight, the suspension was layered on a 30ml discontinuous sucrose density gradient and centrifuged at $132,400 \times g$ for 70min with a vertical rotor. The gradient was prepared with the following sucrose solution in a buffer (pH 7.4) containing 5 mM 3-(N-morpholino) propane-sulfonic acid, 2 % Dextran T 10, 1 mM EDTA and 0.1% ethanol: 4.5ml each of 56, 49, 47, 45, 42, 38 and 30%. After centrifugation, fractions (1.6ml) were collected from the bottom of the tube. Core fractions (fractions 1 to 3) with uricase activity and without catalase activity were separately diluted with 4 vol. of ice cold buffer described above and centrifuged at $52,000 \times g$ for 30min. Each resulting pellet was subjected to SDS-polyacrylamide gel electrophoresis by the method of Laemmli^{1,3)}. The peroxisomal suspension (2 ml) from 4 g of rat liver was also sonicated at 10 KHz for 15 s for the disruption of the peroxisomes and subjected to sucrose density gradient centrifugation followed by the electrophoresis as described above.

Catalase and uricase were assayed by the methods previously reported^{2,3)}. A unit of enzyme activity is defined as the amount of enzymes that catalyse a decrease in substrate of 1 μ mol/min at 37°C.

Results and Discussion

Fig. 1 shows the sedimentation profile of the peroxisomes broken by pyrophosphate treatment in a sucrose density gradient. The activity of uricase as the peroxisomal core enzyme was recovered only in the bottom fractions and that of catalase as the peroxisomal soluble matrix enzyme only in the soluble top fractions. This result shows that the peroxisomes were completely broken by their pyrophosphate treatment yielding the complete separation of the core and the soluble matrix. Fig. 2 shows the results of sodium dodecyl sulfate polyacrylamide gel electrophoresis of the core fractions (fractions 1–3 of Fig. 1) from a sucrose density gradient. A single protein band was obtained in each case. When the sonicated peroxisomes were subjected to sucrose density gradient centrifugation followed by the electrophoresis of the core fractions as described above, the same result as for the pyrophosphate treated peroxisomes was obtained.

On the basis of the present results, it is concluded that the peroxisomal core of rat liver is uricase itself. It is well known that in mammalian other than primates, uricase exists as an insoluble form in the peroxisomal core. Recently we found that

Fig. 1. Sedimentation profile of rat liver peroxisomes broken by pyrophosphate treatment in a sucrose density gradient. Isolated peroxisomes from rat liver were subjected to pyrophosphate treatment and then sucrose density gradient centrifugation with a vertical rotor. After centrifugation, fractions (2 ml) were collected from the bottom. ○, uricase; ●, catalase.

uricase was also present as an insoluble form in the core of liver peroxisomes in old world monkeys (unpublished data); uricase had not been detected in new world monkeys, great apes and human¹⁴⁾. The present method for a rapid isolation of the peroxisomal core would be also usable for mammalian other than rats. It is of interest to determine by the use of the present isolation method whether the peroxisomal core is also uricase itself in all mammalian or not.

Summary

The peroxisomal core was rapidly isolated from rat liver and identified as uricase itself as follows. Rat liver peroxisomes were broken by pyrophosphate treatment or sonication followed by sucrose-density gradient centrifugation. In each case, the cores

Fig. 2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of isolated cores from rat liver peroxisomes. Core fractions 1 to 3 from a sucrose density gradient (Fig. 1) were separately subjected to the electrophoresis as described in the text. The migration is from top to bottom. Gels were stained for protein in 0.25% Coomassie brilliant Blue R 250 in methanol/acetic acid/water (5:1:5, by vol.) and destained by diffusion in 7% acetic acid.

with uricase activity and without catalase activity were recovered in the bottom fractions. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of the cores yielded a single protein band in each case. These data show that the peroxisomal core is uricase itself in rat liver. This rapid isolation method of the peroxisomal core would be also usable for other mammalian.

References

- 1) White, A., Handler, P. and Smith, E.L. : Catabolism of purine nucleotides. In : *Principles of biochemistry*. 5th ed., McGraw-Hill, New York, 1974, 714—719.
- 2) Noguchi, T., Takada, Y. and Fujiwara, S. : Degradation of uric acid to urea and glyoxylate in peroxisomes. *J. Biol. Chem.* **254** : 5272—5275, 1979.
- 3) Takada, Y. and Noguchi, T. : The degradation of urate in liver peroxisomes. Association of allantoinase with allantoinase in amphibian liver but not in fish and invertebrate liver. *J. Biol. Chem.* **258** : 4762—4764, 1983.
- 4) Noguchi, T., Fujiwara, S. and Hayashi, S. : Evolution of allantoinase and allantoinase involved in urate degradation of liver peroxisomes. A rapid purification of amphibian allantoinase and allantoinase complex, its subunit locations of the two enzymes, and its comparison with fish allantoinase and allantoinase. *J. Biol. Chem.* **261** : 4221—4223, 1986.
- 5) Hruban, Z. and Swift, H. : The localization in hepatic microbodies. *Science* **146** : 1316—1317, 1964.
- 6) Leighton, F., Poole, B., Beaufay, H., Baudhuin, P., Coffey, J.W., Fowler, St, and De Duve, C. : The large-scale separation of peroxisomes, mitochondria, and lysosomes from the livers of rats injected with Triton WR-1339. *J. Cell Biol.* **37** : 482—513, 1968.
- 7) Leighton, F., Poole, B., Lazarow, P.B. and De Duve, C. : The synthesis and turnover of rat liver peroxisomes. I. Fractionation of peroxisome proteins. *J. Cell Biol.* **41** : 521—535, 1969.
- 8) Hayashi, H., Suga, T. and Ninobe, S. : Studies on peroxisomes. VI. Relationship between the peroxisomal core and urate oxidase. *J. Biochem.* **79** : 1029—1034, 1976.
- 9) Watanabe, T., Suga, T. and Hayashi, H. : Studies on peroxisomes. VIII. Evidence for framework protein of the cores of rat liver peroxisomes. *J. Biochem.* **82** : 607—609, 1977.
- 10) Tsukada, H., Mochizuki, Y. and Fujiwara, S. : The nucleoids of rat liver cell microbodies. *J. Cell Biol.* **28** : 449—460, 1966.
- 11) Antonenkov, V.D. and Panchenko, L.F. : Organization of urate oxidase in peroxisomal nucleoids. *FEBS Letters* **88** : 151—154, 1978.
- 12) Osmundsen, H., Neat, C.E. and Norum, K.R. : Peroxisomal oxidation of long chain fatty acids. *FEBS Letters* **99** : 292—296, 1979.
- 13) Laemmli, U.K. : Cleavage of structural proteins during assembly of the head of bacteriophage. T4. *Nature* **227** : 680—685, 1970.
- 14) Christen, P., Peacock, W.C., Christen, A.E. and Wacker, W.E.C. : Urate oxidase in primates phylogenesis. *Eur. J. Biochem.* **12** : 3—5, 1970.

ラット肝臓ペルオキシソームのコアの単離と ウリカーゼとしての同定

九州歯科大学学生化学教室

中 島 和 久・藤 原 智 子・林 寿 恵 子

プリンの尿酸までの分解は全動物種に共通して存在するが、尿酸以降の分解は種により異り、高等動物ほど不完全である。我々はある種の魚類では、プリンから尿酸までの分解酵素群がシトソールに存在するのに対し、尿酸の尿素とグリオキシル酸までの分解酵素群がペルオキシソームに局在することから、動物の進化において、ペルオキシソームの酵素（ウリカーゼ、アラントイナーゼ、アラントイカーゼ）が選択的に脱落したことを示唆してきた。ウリカーゼは尿酸分解系における初発反応を触媒する。この酵素を持つ哺乳類の肝細胞には、結晶性のコア（ヌクレオイド）があり、その組成は未だはっきりしていません。ウリカーゼのみという報告と他のタンパク質も含まれるという報告がある。本研究はラット肝臓からコアを単離し、その組成を明らかにしようとしたものである。まず、ラット肝臓からペルオキシソームを

Osmundsen らの方法により単離し、その等張懸濁液をピロリン酸緩衝液で処理した（等量ピロリン酸-KOH 緩衝液を加え、pH を 9.0 に調整し、4°C で一夜放置）。次にこれをバーチカルローターを用いて、蔗糖密度勾配（30%—56%）遠心法により分画したところ、遠心管の底部近くに、ウリカーゼ活性を持ち、カタラーゼ活性を持たないコアが沈降した。そこで、このコアを集め、SDS-電気泳動を行ったところ、単一のタンパクバンドが得られた。またペルオキシソームの等張懸濁液を超音波処理後、同様に蔗糖密度勾配遠心法により分画したところ、同一の結果が得られた。以上の結果はコアがウリカーゼそのものであることを示している。なお、この非常に簡単なコア（ウリカーゼ）の単離法は他の哺乳動物の肝臓のコアの単離にも適用できるものと思われる。