Volume 287, number 1,2, 219–222 FEBS 10025 August 1991

ADONIS 001457939100736E

Solubilization of rat kidney lysosomes in reversed micelles of aerosol OT in octane

Alexey V. Pshezhetsky[1], Olga A. Buneeva[2] and German Ya. Wiederschain[2]

[1]*Belozersky Laboratory, Moscow State University, Moscow, USSR* and [2]*Institute of Biological and Medical Chemistry, USSR Academy of Medical Sciences, Moscow, USSR*

Received 28 May 1991

Intact lysosomes from rat kidneys were solubilized in the ternary system: surfactant (Aerosol OT)–buffer–organic solvent. According to data of laser light-scattering analysis and kinetic experiments with the lysosomal marker enzyme, *N*-acetyl-β-D-hexosaminidase (EC 3.2.1.30), the solubilization of lysosomes in this system resulted in the destruction of the lysosomes and the entrapping of their components in reversed micelles.

Lysosomal glycosidase; *N*-Acetyl-β-D-hexosaminidase; Micellar enzymology; Reversed micelle

1. INTRODUCTION

The reversed micelles of surfactants in organic solvents are widely used as the systems modeling a number of factors typical for the enzyme microenvironment in the cell [1–5]. In particular this method gives an opportunity to investigate the oligomeric structure of the enzymes [4], or their ability to associate with the lipid matrix of different structures [6,7]. Previously we have used AOT reversed micelles to study the regulation of the supramolecular organization and the catalytic activity for some lysosomal glycolipid hydrolases (glycosidases) from the human kidney: galactosialidase complex [8], hexosaminidases A and B [9] and galactocerebrosidase [10]. However, the correct interpretation of these results is difficult because of two reasons.

Firstly, there is a marked difference between the components of the enzyme's microenvironment in lysosomes and in reversed micelles. The thing is that in the above experiments we have used homogeneous or highly-purified enzyme preparations.

Secondly, the process of the isolation from tissue and purification may change the enzyme's native supramolecular organization.

Abbreviations: AOT, Aerosol OT (sodium bis(2-ethylhexyl)sulfosuccinate; BSA, bovine serum albumin; EDTA, ethylene diamine tetraacetic acid; 4-MufGal, 4-methylumbelliferyl-β-D-galactopyranoside; 4-MufClcNAc, 4-methylumbelliferyl-*N*-acetyl-β-D-glucosaminidide; 4-MufFuc, 4-methylumbelliferyl-α-L-fucoside; pNPGlcNAc, *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminidide; Triton X-100, (4-octyl)-phenol poly(9-10)ethylene glycol; Tris, tris-(hydroxymethyl)-aminomethan

Correspondence address: A.V. Pshezhetsky, Belozersky Laboratory, Moscow State University, Moscow 119 899, USSR

In this connection, direct transfer of the lysosomal content into reversed micelles by the solubilization of intact lysosomes in a ternary system: surfactant–buffer–organic solvent can provide sufficient progress in the investigation of the native supramolecular organization of the lysosomal enzymes.

2. MATERIALS AND METHODS

2.1. *Materials*

AOT supplied by Merck was used without purification. The other chemicals used were obtained from the following sources: 4-MufClcNAc, 4-MufGal, 4-MufFuc, Coomassie brilliant blue G-250 and Tris from Serva; pNPGlcNAc from Koch Light Laboratories; sucrose and BSA from Fluka; EDTA and glycine from Reanal. Ultragel AcA 34 and protein standards for gel-filtration column calibration were from Pharmacia-LKB Fine Chemicals. All other reagents were of analytical grade (Reachim, USSR).

2.2. *Isolation of lysosomes*

Isolation of lysosomes from rat kidneys and the determination of latency of lysosomal glycosidases was performed according to [11].

2.3. *Enzyme activity*

Enzyme activity measurements were carried out fluorometrically with 4-methylumbelliferyl substrates using a Shimadzu RF 5000 spectrofluorimeter and spectrophotometrically with *p*-nitrophenyl substrates using an Ultrospec II (Pharmacia-LKB Fine Chemicals). Enzyme activities in buffer were measured according to [12]. The hexosaminidase activity measurements in the system of reversed micelles were made according to [9]. Maximal values of the enzyme reaction rates (V) were calculated from the dependencies of the initial reaction rate in the steady state on substrate concentration by the method of non-linear regression.

2.4. *Protein concentration*

Protein concentration was assayed by the modified method of Bradford [13], using BSA as a standard.

2.5. *Geometrical dimensions of lysosomes and reversed micelles*

Geometrical dimensions of lysosomes and reversed micelles con-

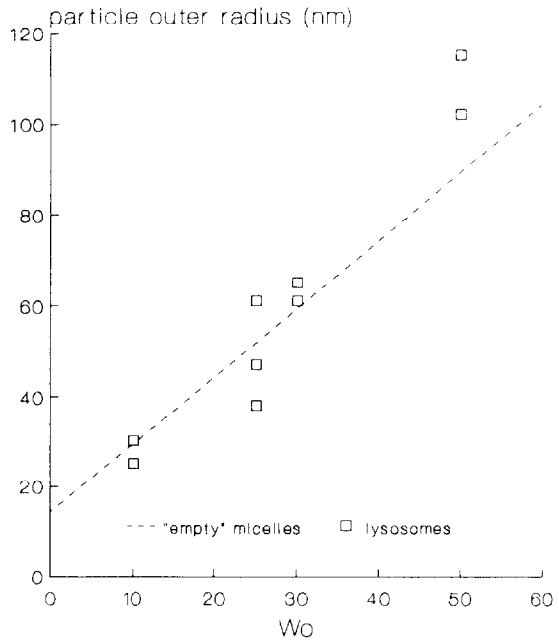


Fig. 1. Dependency of the particles' outer radius, determined by laser light-scattering, on w_0 in the system of lysosomes solubilized in AOT reversed micelles in octane. 40–500 μl of intact lysosome suspension in 0.3 M sucrose, containing 1 mM EDTA, pH shifted to 7.0 by Tris, were added to 2 ml of 0.3 M AOT solution in octane.

taining lysosomal fragments were determined by the method of laser light scattering with a light-scattering spectrometer Malvern Autosizer 2c equipped with a temperature-controlled cell holder. Experimental conditions were as follows: temperature, 310K; wavelength, 633 nm; scattering angle, 45°.

3. RESULTS AND DISCUSSION

3.1. *Solubilization of rat kidney lysosomes in the reversed micelles of AOT in octane*

The lysosomes were purified 30- to 40-fold compared with the homogenate, according to the specific activities of lysosomal glycosidases. The activities of lysosomal glycosidases (hexosaminidase, galactosidase, fucosidase) [14] were 50–90% latent within 24 h after purification. Size distribution of particles in the purified lysosome samples measured by laser light scattering was found to be polyfunctional with the dominating fraction of particles of an average radius 1.0–1.03 μm, which agrees with the data from the literature [11,14].

Addition of 10–300 μl of the lysosome preparation to 2 ml of 0.3 M AOT solution in octane and vigorous shaking of the system resulted within 0.5–1 min in an optically transparent solution. According to the laser light-scattering data this system contained particles with a sharp-dispersed size distribution. Their outer radius

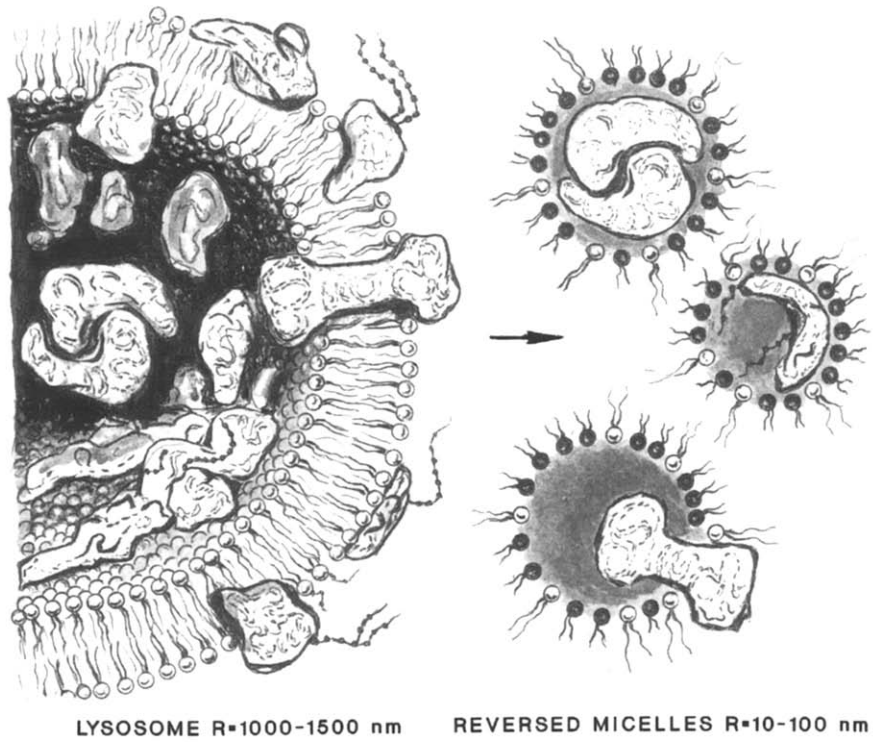


Fig. 2. Scheme of lysosome solubilization in the reversed micelles of surfactant in organic solvent.

depended on the hydration degree of the system (w_o, $[H_2O]/[AOT]$ molar ratio) (Fig. 1). While w_o changed from 5 to 50 the particles' outer radius was growing from 20 to 100 nm, which correlated with the data from [15–17] for the hydrated reversed micelles of AOT in octane (Fig. 1). Thus, the solubilization of lysosomes led to their destruction and entrapping of their components – proteins and lipids – into the reversed micelles (Fig. 2).

Recently, Luisi [18] observed solubilization of mitochondria in reversed micelles of AOT while they remained their intact structure. Such a difference of these results from our data may be connected with the marked difference between the structures of these organelles.

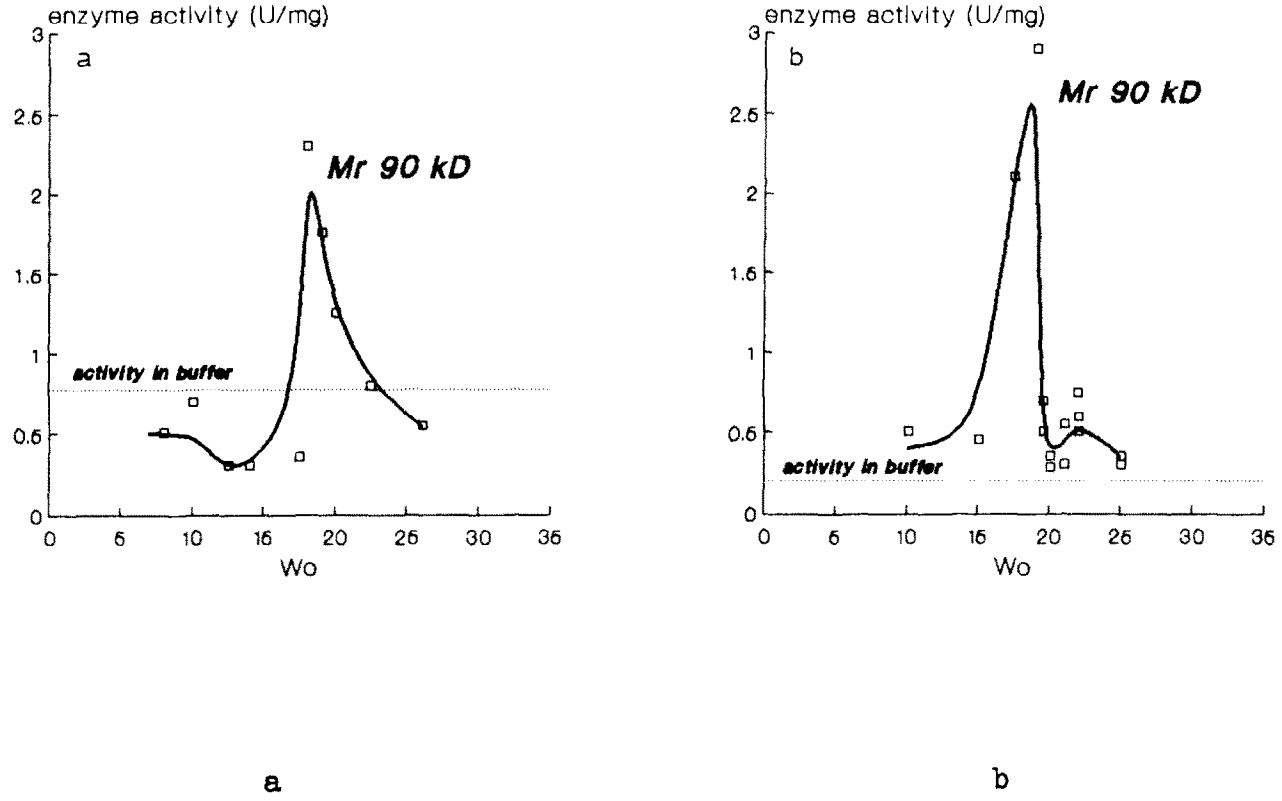
3.2. *Catalytic activity of hexosaminidase solubilized in reversed micelles*

The dependence of the hexosaminidase activity of rat kidney lysosomes solubilized in the reversed micelles of AOT on w_o is demonstrated in Fig. 3a. It is necessary to pay attention to two facts.

Firstly, the catalytic activity of the enzyme in micelles corresponds to hexosaminidase activity in the buffer solution after the destruction of lysosomal membranes by 0.2% Triton X-100 (Fig. 3a, dotted line).

Secondly, the dependence of hexosaminidase catalytic activity on w_o in the region of $w_o = 10$–25 represents a bell-shaped curve, which is typical of a great number of enzymes [1,4] and particularly of all lysosomal glycosidases investigated in our laboratory [8,9].

The analogous plots of hexosaminidase catalytic activity versus w_o (Fig. 3b) have been obtained in the case of the solubilization of a rat kidney homogenate 100000 × *g* supernatant. In both cases the enzyme revealed its maximal catalytic activity at w_o values of 18–19. The dimensions of the inner water cavities of the micelles under such conditions correspond to the size of a 80 kDa protein. As a rule the enzyme's catalytic activity reaches a maximum at values of w_o when the dimensions of the reversed micelle's inner cavity and the solubilized protein are equal [1,4]. Actually, the molecular mass of hexosaminidase (either A, or B isoform) determined by the method of gel-filtration on an Ultragel AcA 34 column (16 × 100) is 86 kDa. This value is in agreement with data from the literature [19]. The obtained data enable us to suppose that the lysosomal proteins (at least, hexosaminidase) are entrapped in individual micelles when the lysosomes are solubilized.



a b

Fig. 3. Dependency of the catalytic activity of hexosaminidase (V) in AOT reversed micelles on w_o. 5 μl of lysosomal suspension were added to 2 ml of 0.2 M AOT solution, containing 10–200 μl of 0.1 M sodium acetate buffer, pH 4.75 and 10–160 μl of 5 mM substrate in the same buffer. Temperature: 310K. The dotted lines demonstrate the hexosaminidase activity in 0.1 M sodium acetate buffer, pH 4.75. Plate a, lysosomes after destruction of the lysosomal membrane by 0.2% Triton X-100; plate b, rat kidney homogenate 100000 × *g* supernatant.

Thus, we have demonstrated the possibility of lysosome solubilization in systems of surfactant reversed micelles accompanied by the destruction of lysosomes and the incorporation of their components into individual micelles. Varying the reversed micelle dimension (w_0) makes it possible to investigate different lysosomal protein complexes. Such experiments are now in progress in our laboratory.

REFERENCES

[1] Martinek, K., Levashov, A.V., Klyachko, N.L., Khmelnitsky, Yu.L. and Berezin, I.V. (1986) Eur. J. Biochem. 155, 453–468.

[2] Martinek, K., Klyachko, N.L., Kabanov, A.V., Khmelnitsky, Yu.L. and Levashov, A.V. (1989) Biochim. Biophys. Acta 981, 161–172.

[3] Luisi, P.L. and Magid, L.J. (1986) CRC Crit. Rev. Biochem. 20, 409–474.

[4] Khmelnitsky, Yu.L., Kabanov, A.V., Klyachko, N.L., Levashov, A.V. and Martinek, K. (1989) in: Structure and Reactivity in Reverse Micelles (Pileny, M.P., ed.) Elsevier, Amsterdam, pp. 230–261.

[5] Luisi, P.L. (1989) in: Structure and Reactivity in Reverse Micelles (Pileny, M.P., ed.) Elsevier, Amsterdam, pp. 262–270.

[6] Klyachko, N.L., Levashov, A.V., Pshezhetsky, A.V., Bogdanova, N.G. and Martinek, K. (1986) Eur. J. Biochem. 161, 149–154.

[7] Pshezhetsky, A.V., Klyachko, N.L. and Levashov, A.V. (1990) Biocatalysis 4, 185–198.

[8] Pshetzhetsky, A.V., Levashov, A.V. and Wiederschain, G.Ya. (1991) Biochimiya (Russ) 56, 655–665.

[9] Pshezhetsky, A.V., Buneeva, O.A. and Dvorkin, V.M. (1991) Eur. J. Biochem., in press.

[10] Pshezhetsky, A.V. (1990) Proceedings of the 6-th International Conference on Organic and Bioorganic Chemistry, Prague, pp. 97–98.

[11] Maunsbach, A.B. (1974) Methods Enzymol. 31, 330–339.

[12] O'Brien, J.S. (1989) in: The Metabolic Basis of Inherited Diseases (Scriver, C.C., Beaudet, A.L., Sly, W.S. and Valle, D., eds.) McCraw-Hill, New York, pp. 1797–1806.

[13] Spector, T. (1978) Anal. Biochem. 86, 142–146.

[14] Biological Membranes – A Practical Approach (Fildlay, J.B.C. and Evans, W.H., eds) (1987) IRL Press, Oxford, pp. 22–23.

[15] Zulauf, M. and Eicke, M.F. (1979) J. Phys. Chem. 83, 480–486.

[16] Fletcher, P.D.I. (1984) in: Surfactants in Solution (Mittal, K.L. and Lindman, B.N., eds.) Vol. 3, Plenum Press, New York, 1745–1758.

[17] Ravey, J.C. and Buzier, M. (1984) in: Surfactants in Solution (Mittal, K.L. and Lindman, B.N., eds) Vol. 3, Plenum, New York, pp. 1759–1779.

[18] Hochkoeppler, A. and Luisi, P.L. (1989) Biotechnol. Bioeng. 33, 1477–1481.

[19] Beccari, T., Bellachioma, G.L., Counstauri, E., Stirling, J.L. and Orlacchio, A. (1990) Proc. 20th FEBS Congress, 20th Meeting of the Fed. Eur. Biochem. Soc., Budapest 1990, p. 201.