Volume 267, number 2, 236–238 FEBS 08598 July 1990

The principal difference in regulation of the catalytic activity of water-soluble and membrane forms of enzymes in reversed micelles

γ-Glutamyltransferase and aminopeptidase

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Received 10 May 1990

The regularities of their functioning of enzyme, water-soluble and membrane forms, in the systems of the reversed micelles of surfactants in organic solvents are compared. Using as examples γ-glutamyltransferase (in AOT reversed micelles in octane) and aminopeptidase (in Brij 96 reversed micelles in cyclohexane), the principal difference in the catalytic activity regulation of water-soluble and membrane forms is demonstrated. The catalytic activity of the membrane form depends considerably on the surfactant concentration at the constant degree of hydration, whereas the activity of the water-soluble form is constant under these conditions. The catalytic activity dependence on the surfactant concentration is regarded as a test for enzyme membrane activity.

Aminopeptidase; γ-Glutamyltransferase; Micellar enzymology; Reversed micelle

1. INTRODUCTION

The phenomenon of enzyme catalysis in reversed micelles, discovered in 1977 by Martinek et al. [1], is now under intensive study in dozens of laboratories all over the world [2–4]. Despite the rapid progress in this field, the generalization of the observed catalytic regularities [5] is limited by the confusion arising from mixing of the data obtained for two different classes of proteins – water-soluble and membrane-active.

The former do not interact with the micellar matrix and could be incorporated into the reversed micelle without considerable change of its structure [6,7]. The latter, actively participating in the micellization process, being entrapped in reversed micelles, always cause structural rearrangements [8,9].

We have demonstrated [10] the principal difference in the regulation of catalytic activity of these two classes of enzymes in reversed micelles using examples of native and stearoylated α-chymotrypsin. The former represent a typical water-soluble enzyme, the latter can be regarded as a model membrane-active enzyme. In this work the regularities of functioning in reversed micelles of soluble and membrane forms of γ-glutamyltransferase from hepatoma [11] and aminopeptidase from bovine brain [12] are compared.

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Abbreviations: AOT, aerosol OT (sodium bis(2-ethylhexyl)sulfosuccinate); Brij 96, oleyl poly(10)oxyethylene ether; Triton X-100, (4-octyl)phenol poly(9-10)ethylene glycol; NA, (4-nitro)aniline; CNA, (3-carboxy-4-nitro)aniline; LeuNA, L-leucyl(4-nitro)anilide; GluNA, γ-glutamyl(4-nitro)anilide; GluCNA, γ-glutamyl(3-carboxy-4-nitro)anilide

2. MATERIALS AND METHODS

2.1. *Enzymes*

The soluble and membrane forms of γ-glutamyltransferase ((5-glutamyl)-peptide:amino acid 5-glutamyltransferase, EC 2.3.2.2) were kindly presented by G.N. Evtushenko and N.N. Chernov (Department of Biochemistry, P. Lumumba University, Moscow). The soluble form was extracted from the entwined poorly differentiated hepatoma H-27 using the method [11] which includes the enzyme solubilization by papain. The enzyme was then purified by gel filtration on Sephadex G-150. The activity of the extracted enzyme was equal to 30 units/mg (one unit of activity liberates 1 μmol of NA from GluNA per 1 min at 25°C, pH 8.5). The membrane form of γ-glutamyltransferase was extracted from the homogenate of the hepatoma H-27 with 1% Lubrol (Sigma) [11] and used in the kinetic experiment without further purification.

The soluble form of aminopeptidase was extracted from fresh bovine cerebral cortex using the method described [12]. After ammonium sulfate precipitation, the enzyme was purified on DEAE-Sepharose CL-4B and AH-Sepharose 4B. The activity of the extracted enzyme was equal to 0.1 units/mg (one unit hydrolyzes 1.0 μmol of LeuNA to NA per 1 min at 37°C, pH 7.2). The membrane form of aminopeptidase was extracted from the homogenate of cerebral cortex with 1% Triton X-100 (Sigma) according to the modified method [12] which did not include the stage of enzyme purification at DEAE-Sepharose, CL-4B, described in [12].

The protein concentrations in the obtained enzyme preparations were measured using the Bradford procedure [13].

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2.2. *Enzyme treatment with papain*

Twenty μl of 6.3 mM papain (Sigma) solution were added to 0.5 ml of solution of membrane form of γ-glutamyltransferase (or aminopeptidase) in 50 mM Tris-HCl buffer, pH 7.5. After 1 h of incubation with papain at 20°C the enzyme was separated by gel filtration on Sephadex G-150.

2.3. *Aminopeptidase modification with stearoylchloride [14]*

Seventy μl of 330 μM aminopeptidase solution in 0.1 M borax buffer, pH 9.5, were solubilized in 7 ml of 0.2 M Brij 96 (Sigma) solution in cyclohexane (Merck). The system was intensively shaken to achieve optical density, and then 25 μl of 80 mM stearoylchloride (Sigma) solution in cyclohexane were added to it.

The modified protein was not isolated from the reaction medium: the obtained micellar solution was used directly for the kinetic experiment. In order to obtain the micellar system with the given degree of hydration and surfactant concentration it was diluted with Brij 96 solution in cyclohexane and 50 mM Tris-HCl buffer, pH 7.5. (The independent experiment revealed that the presence in the system of the free stearic acid up to 0.1 mM concentrations do not influence the aminopeptidase activity in reversed micelles.)

2.4. *γ-Glutamyltransferase activity in reversed micelles [15]*

In a typical experiment 20 μl of 1 μM γ-glutamyltransferase and 30–360 μl of 0.5–50 mM GluCNA (Sigma) solutions in an aqueous buffer were solubilized in 2 ml of 0.3 M AOT (Merck) solution in octane (Reakhim); 25 mM Tris-HCl, pH 8.8, was used as an aqueous buffer. In order to obtain micellar systems with various concentrations of AOT the initial system was diluted with octane. The free CNA was determined spectrophotometrically (400 nm) at 25°C.

2.5. *Aminopeptidase activity in reversed micelles*

In a typical experiment 20 μl of 6 μM aminopeptidase solution in 50 mM Tris-HCl buffer, pH 7.5, and 20–240 μl of this buffer were solubilized in 2 ml of 0.2 M Brij 96 solution in cyclohexane. In order to obtain micellar systems with various Brij 96 concentrations, the initial system was diluted with cyclohexane. The reaction was started by adding 10 μl of 40–400 mM LeuNA (Boehringer) solution in acetonitrile (Reakhim). The free NA was determined spectrophotometrically (400 nm) at 25°C.

Here and above (section 2.4) the Beckman 25 spectrophotometer with thermostatic vessel section was used. The values of CNA (NA) molar absorption were measured at various degrees of hydration and AOT (Brij 96) concentrations.

The values V/E_o were determined from the Lineweaver-Burk plot under the conditions of saturation of the enzymes with GluCNA (γ-glutamyltransferase) or LeuNA (aminopeptidase).

The independent experiment revealed that Triton X-100 and Lubrol which were presented in the preparation of the membrane form of aminopeptidase and γ-glutamyltransferase do not influence the activities of these enzymes in reversed micelles.

3. RESULTS AND DISCUSSION

3.1. *Catalytic activity versus degree of hydration dependencies*

One of the most spectacular observations made in the micellar enzymology is the 'catalytic activity versus degree of hydration' profile concept. The degree of hydration, i.e. the molar ratio $[H_2O]/[surfactant]$, is a parameter determining the size of the water cavity of the reversed micelle [4].

In spite of great variety of the enzyme systems under study, the observed dependencies usually are very similar: as a rule they represent the bell-shaped curves [2–5]. The maximal catalytic activity is observed at a certain degree of hydration when the size of the inner cavity of the reversed micelle is equal to that of the protein molecule (for review, see [3]).

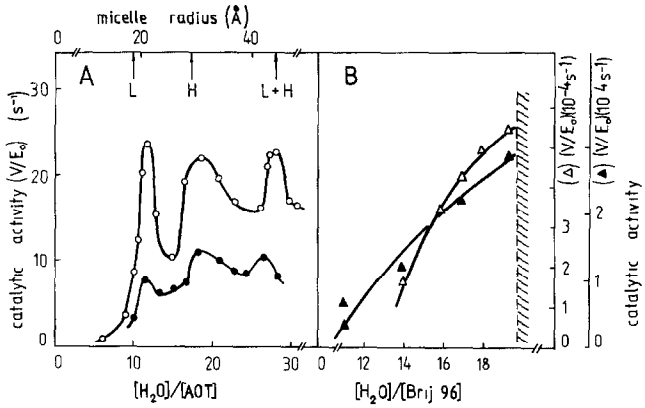

Fig. 1. Dependencies of the catalytic activities of the enzymes solubilized in the systems of reversed micelles on the degree of hydration. (A) The water-soluble (○) and membrane (●) forms of γ-glutamyltransferase in AOT reversed micelles in octane (AOT concentration is equal to 0.1 M). The scale of radii of the inner cavity of the reversed micelles is shown for comparison on which the vertical arrows indicate the values of the radii of light (L) and heavy (H) subunits and of their dimer (L + H). (B) The water-soluble (△) and membrane (▲) forms of aminopeptidase in Brij 96 reversed micelles in cyclohexane (Brij 96 concentration is equal to 0.2 M). The hatched area corresponds to the conditions of the phase separation in the system.

The profiles of γ-glutamyltransferase activity in AOT reversed micelles in octane (Fig. 1A) reveals several maxima observed at $[H_2O]/[AOT] = 11$, 17 and 26. The explanation of such unusual behavior of γ-glutamyltransferase was given recently [15]. It is based

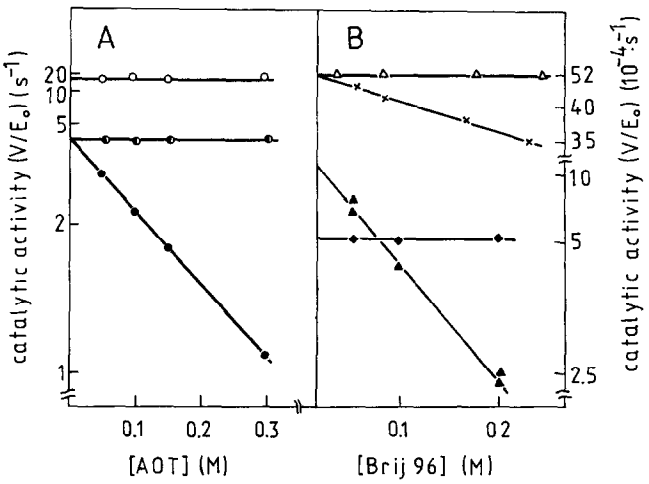

Fig. 2. Dependencies of the catalytic activity of the enzymes solubilized in the systems of reversed micelles on the surfactant concentration. (A) The water-soluble (○), membrane (●) and papain-treated membrane (◑) forms of γ-glutamyltransferase in reversed micelles in octane ($[H_2O]/[AOT]$ is equal to 17.0). (B) The water-soluble (△), stearoylated water-soluble (×), membrane (▲) and papain-treated membrane (◆) forms of aminopeptidase in Brij 96 reversed micelles in cyclohexane ($[H_2O]/[Brij\ 96]$ is equal to 19.5).

on the fact that the reversible dissociation of this heterodimeric enzyme to the catalytically active subunits proceeds in AOT reversed micelles. Therefore the observed maxima correspond to the functioning of the so-called 'light' and 'heavy' subunits of γ-glutamyltransferase and of their dimer [15].

Fig. 1B presents the 'catalytic activity-degree of hydration' dependencies for aminopeptidase solubilized in Brij 96 reversed micelles in cyclohexane. These dependencies differ from those discussed above; presumably because of the phase separation in the system, the degree of hydration corresponding to the maximum of enzyme activity is not achieved.

In any case one should conclude from Fig. 1 that there is no principal difference between the dependencies observed for water-soluble and membrane forms of γ-glutamyltransferase and aminopeptidase. (The maxima at the catalytic activity profile of the membrane form of γ-glutamyltransferase are smoothed out if compared with its water-soluble form, but this reflects rather the quantitative than the qualitative difference.)

3.2. *Catalytic activity versus surfactant concentration dependencies*

Variation of the surfactant concentration at a constant degree of hydration alters the concentration of reversed micelles in the system. In this case the radius of the inner cavity and other characteristics of reversed micelles are not changed considerably over a fairly large range of surfactant concentrations [4,5]. Thus one would expect the catalytic activity of the solubilized enzyme at a constant degree of hydration to be independent of surfactant concentration.

This was shown for water-soluble enzymes, e.g. for α-chymotrypsin [10]. However, it was demonstrated that the catalytic activity of mimetic membrane-active enzyme, stearoylated α-chymotrypsin, depends greatly on surfactant concentration [10].

As is seen in Fig. 2, the analogous difference in the catalytic activity regulation of water-soluble and membrane forms is observed for γ-glutamyltransferase and aminopeptidase also. The shapes of the catalytic activity versus degree of hydration dependencies observed for the membrane forms of γ-glutamyltransferase and aminopeptidase are similar to those previously described [10] for stearoylated α-chymotrypsin (these dependencies are linearized in semi-hyperbolic coordinates; Fig. 2).

The membrane forms of the enzymes under study contain a hydrophobic peptide anchor which serves to provide in the tissues their binding with cellular membranes [16–19]. The cleavage of this anchor via papain treatment of the enzymes results in the disappearance of the observed dependencies (Fig. 2). At the same time such dependence reappears after the transformation of the aminopeptidase water-soluble form into the membrane-active state by its chemical modification with hydrophobic anchor groups (stearic acid residues) (Fig. 2B).

Thus the obtained data give evidence that the dependence of the catalytic activity on the surfactant concentration can be used as the simple and efficient test for the membrane activity of enzymes.

Acknowledgements: The authors want to thank Dr G.N. Evtushenko and Professor N.N. Chernov for presented samples of γ-glutamyltransferase; Drs E.F. Kolesanova, T.N. Rotanova and Professor V.K. Antonov for stimulating discussions and for their help in the purification of aminopeptidase; Dr N.L. Klyachko for discussion of the results of this work.

REFERENCES

[1] Martinek, K., Levashov, A.V., Klyachko, N.L. and Berezin, I.V. (1977) Dokl. Acad. Nauk SSSR (Russ.) 236, 920–923.
[2] Luisi, P.L. and Magid, L.J. (1986) CRC Crit. Rev. Biochem. 20, 409–474.
[3] Martinek, K., Klyachko, N.L., Kabanov, A.V., Khmelnitsky, Yu.L. and Levashov, A.V. (1989) Biochim. Biophys. Acta 981, 161–172.
[4] Structure and Reactivity in Reverse Micelles (Pileni, M.P. ed.) Elsevier, Amsterdam, 1989.
[5] Kabanov, A.V., Levashov, A.V., Klyachko, N.L., Nametkin, S.N., Pshezhetsky, A.V. and Martinek, K. (1988) J. Theor. Biol. 133, 327–343.
[6] Levashov, A.V., Khmelnitsky, Yu.L., Klyachko, N.L., Chernyak, V.Yu. and Martinek, K. (1982) J. Colloid Interface Sci. 88, 444–457.
[7] Shapiro, Yu.E., Budanov, N.A., Levashov, A.V., Klyachko, N.L., Khmelnitsky, Yu.L. and Martinek, K. (1989) Coll. Czech. Chem. Commun. 54, 1126–1134.
[8] Montal, M. (1984) in: Reverse Micelles (Luisi, P.L. and Straub, B.E. eds) pp. 221–229, Plenum, New York.
[9] Wirz, J. and Rosenbuch, J.P. (1984) in: Reverse Micelles (Luisi, P.L. and Straub, B.E. eds) pp. 231–238, Plenum, New York.
[10] Kabanov, A.V., Levashov, A.V. and Martinek, K. (1986) Vestn. MGU, Ser. 2, Chemistry (Russ.) 27, 591–594.
[11] Loginov, V.A., Chernov, N.N. and Berezov, T.T. (1980) Bull. Exp. Biol. (Russ.) 7, 58–60.
[12] Kolesanova, E.F., Rotanova, T.V., Amerik, A.Yu., Ginodman, L.M. and Antonov, V.K. (1986) Bioorgan. Khimiya (Russ.) 12, 340–356.
[13] Bradford, M. (1976) Anal. Biochem. 72, 248–254.
[14] Kabanov, A.V., Levashov, A.V. and Martinek, K. (1987) Ann. NY Acad. Sci. 501, 63–66.
[15] Kabanov, A.V., Nametkin, S.N., Evtushenko, G.N., Chernov, N.N., Klyachko, N.L., Levashov, A.V. and Martinek, K. (1989) Biochim. Biophys. Acta 996, 147–152.
[16] Tate, S.S. and Meister, A. (1981) Mol. Cell. Biochem. 39, 1749–1781.
[17] Tsuji, A., Matsuda, Y. and Katunuma, N. (1980) J. Biochem. (Tokyo) 87, 1567–1571.
[18] Hersh, L.B. and McKelvy, J.F. (1981) J. Neurochem. 36, 171–178.
[19] Maroux, S. and Louvard, D. (1977) Gastroenterol. Clin. Biol. 1, 377–388.