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The length of the polyoxyethylene chain in the Triton X detergents modulates the apparent activation of neurosteroid sulfatase in bovine brain

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Abstract

The effect of the Triton X series on the solubilization and enzyme activity of neurosteroid sulfatase (NSS) in the bovine midbrain was investigated. Triton X-100 and X165 stimulated NSS activity in the bovine midbrain, while Triton X-305 did not. This apparent activation was attributed to the action of the detergents, and not to the latency of the enzyme or the removal of some inhibitory substance from the microsomes. The maximum stimulation was obtained when the length of the polyoxyethylene chain of the detergent was 16. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

Detergents are widely employed to solubilize membrane proteins. However, the most difficult hurdle to overcome in the solubilization of a membrane bound protein is to choose an appropriate detergent, its concentration and ratio to the protein, and an appropriate medium. The most frequently utilized detergent for the solubilization of steroid sulfatases in liver, placenta or other tissues has been Triton X-100 [1–5]. Recently, some nonionic and amphoretic detergents have been shown to activate steroid sulfatases [6,7]. We observed similar phenomenon during the solubilization of neurosteroid sulfatases (NSS) in bovine brain microsomes. Since polyoxyethylene chains with various lengths in the Triton X series are available, it is possible to investigate the role of the polyoxyethylene moiety in enhancing NSS activity. In this paper, we report that the increase in NSS activity in bovine midbrain microsomes depends on the length of the polyoxyethylene chain of Triton X detergents.

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2. Materials and methods

2.1. Materials

Bovine brain was obtained within 2 h of sacrifice and transported on ice. Rat was killed by decapitation, and the brain was taken and kept on ice. [7-^{3}H]-dehydroepiandrosterone sulfate (DHEA-S; 25.6 μCi/mmol) was obtained from New England Nuclear Corp. (USA), and systematically purified with a column (0.6 × 15 cm) of LH-20. Triton X detergents were from Sigma Chemical Co. (USA). All other chemicals were of analytical grade.

2.2. Preparation of microsomal fraction

The procedure for preparing a microsomal fraction from bovine midbrain and murine brain was described previously [8]. Briefly, bovine midbrain and murine brain were minced and homogenized with a Teflon homogenizer in 10 mM Tris–HCl buffer, pH 7.4, containing 0.8% NaCl (1:4, w/v). The homogenate was successively centrifuged at 1,450 *g* for 10 min and 10,000 *g* for 20 min (Sorvall RC-5B, DuPont Instruments, USA), and the microsomes were obtained as precipitates by centrifuging at 105,000 *g* for 60 min

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(Sorvall combi plus ultracentrifuge, DuPont Instruments, USA). The microsomal precipitate was resuspended in 10 mM Tris–HCl buffer, pH 7.4, containing 0.32 M sucrose and stored at −70°C until use. The protein concentration was determined by the method of Lowry et al. [9]. Since Triton X-detergents interfere with protein determination by forming precipitates, the same amount of each detergent was systematically added to the standard solution. The solutions were centrifuged at 3000 *g* for 10 min before measuring their absorbance.

2.3. Enzyme assay

The method for determining NSS enzyme activity was described previously [8]. Briefly, the reaction mixture contained 46 nM [7-^{3}H]-DHEA-S (23 pmol) and 1 mg of the microsomal fraction in a total volume of 0.5 ml 10 mM Tris–HCl buffer (pH 7.4). A parallel incubation without microsomes was carried out for the control. After incubation at 37°C for 1 h, the reaction was stopped by freezing at −70°C. Two ml of ethyl acetate–isooctane mixture (1:1, v/v) were added to the incubation mixture, and the freed steroid was extracted into the upper phase. The radioactivity of free [7-^{3}H]-DHEA was measured with a liquid scintillation counter (1600TR liquid–scintillation counter, Packard, USA).

2.4. Solubilization of microsomal proteins

To solubilize the microsomal proteins, 6 mg of microsomal protein in 300 μl were mixed with various amounts of a 1.5% solution of Triton X detergents. The final volume was adjusted to 1 ml with Tris–HCl buffer. The mixture was gently agitated at 4°C for 60 min and centrifuged at 105,000 *g* for 60 min. The supernatant was recovered for the enzyme assay and protein determination.

2.5. Freezing and thawing

Freezing and thawing were repeated at −70 and 4°C, respectively. After each treatment, the microsomal fraction was immediately incubated for the enzyme assay.

3. Results and discussion

3.1. Solubilization of microsomal NSS from bovine brain and murine brain

Under our experimental conditions, 8–10% of the substrates were converted into the free form. The effect of concentration of Triton X-100 on the solubilization of microsomal NSS of bovine midbrain is shown in Fig.1. At 0.04%, no enzyme activity was observed even though almost 50% of the maximum level of solubilization was obtained. However, the enzyme activity rapidly increased at 0.1% and reached a maximum level at 0.3%. The protein solubilization did not increase above 0.1%. Therefore, we used the concentration of Triton X-100 at 0.3% in the following experiments, which is well above the critical micelle formation concentration (0.015%).

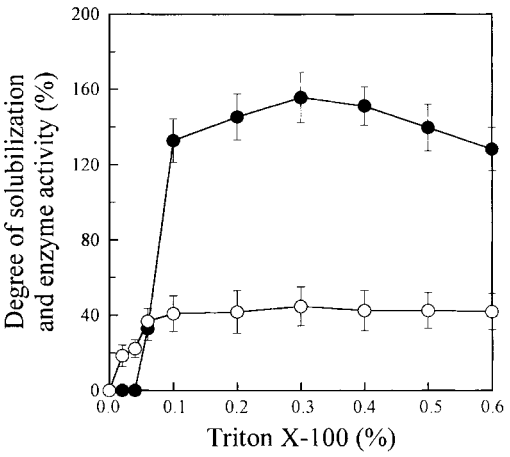

Fig. 1. Effect of concentration of Triton X-100 on the protein solubilization and enzyme activity of bovine midbrain microsomes. The protein solubilization and enzyme activity are expressed in percentage of the levels in intact microsome. Bar is the standard deviation of three independent determinations. ○: protein solubilization ●: Enzyme activity.

Triton X-100 yielded a solubilization of 48% of

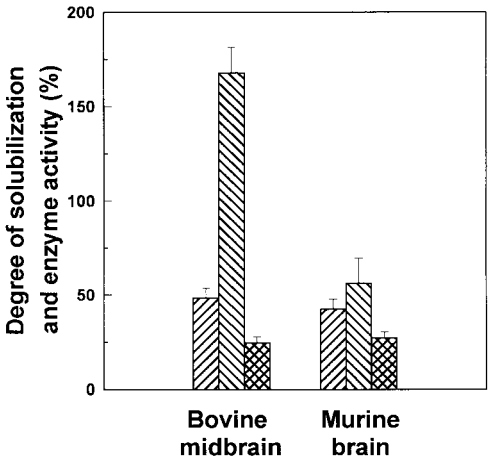

Fig. 2. Solubilization and total activity of NSS in bovine midbrain and murine brain microsomes with Triton X-100. The protein content and total enzyme activity of nontreated microsomes were taken as 100% and those of treated microsomes were expressed in relative activities. ▨: Degree of solubilization of the microsomes. z.sqsw: Total enzyme activity in the solubilized microsomes. ⊠: Total enzyme activity in the precipitates. Bar is the standard deviation of three independent determinations

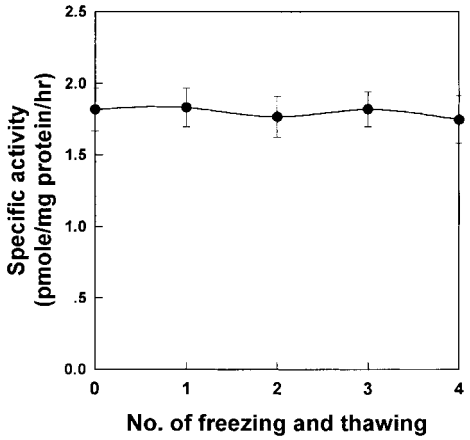

Fig. 3. Effect of freezing and thawing on the NSS activity. 0.5 mg of bovine midbrain microsomes was incubated at 37°C for 1 h with 23 pmol of [7-^{3}H]-DHEA sulfate. Bar is the standard deviation of three independent determinations.

bovine microsomes and stimulated NSS activity (Fig. 2). The total activity of the solubilized NSS from bovine brain was 1.68 times higher than that of non-treated microsomes (Fig.2), and the total NSS activity in the treated midbrain was 1.93 times higher than that of nontreated microsomes. Enhancement of the enzyme activity was not observed with murine cerebral microsomes (Fig. 2). The relative amounts of cholesterol and phosphatidylethanolamine in the microsomes isolated from rat brain [10] and bovine white matter [11] were quite similar, however, those of sphingomyelin, phosphatidylcholine and ethanolamine plasmalogen were quite varied. Additionally, SDS-PAGE profiles of solubilized microsomes of these brains were

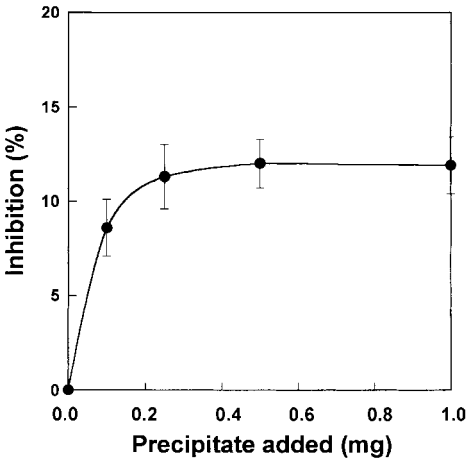

Fig. 4. Inhibition of bovine NSS activity by precipitates after treatment with Triton X-100. Indicated amounts of the precipitates were added to 0.5 mg of the solubilized NSS and incubated at 37°C for 1 h with 23 pmol of [7-^{3}H]-DHEA sulfate. The percentage of inhibition was calculated on the basis of the sum of the activities in the solubilized and the corresponding precipitates incubated separately. Bar is the standard deviation of three independent determinations.

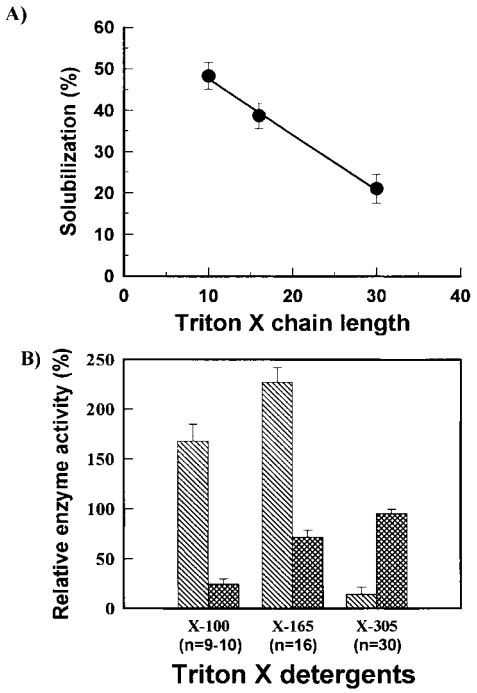

Fig. 5. Effect of the length of the Triton X polyoxyethylene moiety chain on bovine NSS activity. The concentration of various Triton X detergents was 0.3%. The protein content and the total activity of nontreated microsomes was taken as 100%, and that of treated microsomes was expressed in percentage. A) Relationship between the chain length of Triton X detergents and degree of protein solubilization of the microsomes. B) Relationship between the chain length of Triton X detergents and the total activity of NSS. ▧: Total activity of the solubilized microsomes. ▩: Total activity of the precipitates. Bar is the standard deviation of three independent determinations.

also remarkably different(data not shown). Although exact mechanism is not clear, it is quite possible that these differences contributed to apparent enhancement of NSS in bovine brain microsomes.

Since the significant increase in the activity of bovine NSS could be attributed to possible latency of NSS, we determined whether freezing and thawing increased the activity. As seen in Fig. 3, there was no increased enzyme activity with repeated freezing and thawing, suggesting that there was no latency of NSS activity in bovine midbrain microsomes. Fig. 3 also demonstrates that the neurosteroid sulfatase is quite stable with repeated freezing and thawing. A second plausible explanation for the increased NSS activity upon solubilization is removal of possible NSS inhibitors present in the unsolubilized precipitates. In fact, the inhibition of the NSS activity reached plateau at 12% with the addition of the unsolubilized precipitate to the solubilized NSS (Fig. 4). However, this degree of inhibition could not entirely explain the increase seen in Fig. 2. From the above observations, we concluded that Triton X-100 has a specific stimulatory effect on bovine NSS activity, but not on murine NSS activity.

As suggested for arylsulfatase C (ASC) in cultured human fibroblasts [6], the apparent activation of ASC may have been due to an increased accessibility of the substrate with the solubilization of the membrane. If this is the case, the micelle-forming chain length of the Triton X detergents should also affect the degree of enhancement.

3.2. Effect of the length of polyoxyethylene chain in the Triton X series on the activity of solubilized NSS

Since a series of Triton X detergents are polyoxyethylene derivatives with alkylphenyl hydrophobic groups, the length of the polyoxyethylene chain may play a role in stimulating NSS activity. We initially attempted to test Triton X-15, X-45, X-100, X-165, and X-305. However, due to the insolubility of Triton X-15 and X-45 in aqueous solution, we were only able to test Triton X-100, X-165, and X-305 at 0.3%. The solubilization capacity of microsomes was 45% with Triton X-100, 39% with X-165, and 21% with X-305 (Fig. 5(A)). Since the lengths (n) of the polyoxyethylene chain of Triton X-100, X-165 and X-305 are 9–10, 16 and 30, respectively, it is clear that the degree of solubilization was inversely proportional to the chain length (Fig. 5(A)). However, the maximum enhancement of total bovine NSS activity was 200% with Triton X-165 (n = 16), while Triton X-100 (n = 9–10) and Triton X-305 (n = 30) produced 93 and 10% increments, respectively (Fig. 5(B)). In the case of Triton X-305, most of the activity was present in the precipitate. These results suggest that the length of the polyoxyethylene moiety significantly influences the activity of bovine NSS. It is well acknowledged that the chain length of the matrix arm substantially influences the biological activity of functional proteins and enzymes by immobilizing them [12–14]. In this context, we hypothesize that a certain length of the polyoxyethylene moiety (n = 16) is necessary to attain the maximum NSS activity. However, the exact role of the polyoxyethylene moiety must be further investigated.

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