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Activation of acetylcholinesterase by Triton X-100

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SUMMARY

The effect of Triton X-100 on human brain acetylcholinesterase (acetylcholine hydrolase, EC 3.1.1.7) was studied with reference to its solubilization and the solubilization products which were analyzed by acrylamide gel electrophoresis. The particulate activity remained virtually constant at various Triton X-100 levels despite solubilization of the protein. In the supernatant the relative specific activity increased to 360% at 1.0% Triton X-100. The analytical and electrophoretic data indicated that the major increase in acetylcholinesterase activity after extraction with Triton X-100 is a result of an enhancement of the catalytic activity of the protein.

The imputed significance of acetylcholinesterase (acetylcholine hydrolase, EC 3.1.1.7) in the propagation of the nerve impulse has led to numerous attempts to isolate and characterize the enzyme. One difficulty in such studies arises from the fact that the enzyme is bound to cellular membranes of diverse localization such as endoplasmic reticulum, axolemma, synaptic junction, *etc.* which has necessitated the use of various procedures to "solubilize" the enzyme. Non-ionic detergents, dodecyl sulfate, butanol, enzymatic digestion and disruptive techniques are some of the techniques that have been employed for that purpose. As evidence that the method used has accomplished the desired extraction of the enzyme from the bound state, the increase in activity of the enzyme in the supernatant has been used to attest to the efficacy of solubilization. We now present some data to indicate that in the case of the utilization of Triton X-100 as a solubilizing agent the increase in supernatant enzyme activity achieved may involve other factors than simple release of the enzyme from the bound state.

Approximately 2 g of human brain which had been frozen at −60 °C were

homogenized with 4 vol. of extraction media and incubated for 30 min at 4 °C. To study the effect of Triton X-100 on the "solubilization" of the enzyme activity, 0.9% NaCl or the appropriate Triton X-100 concentration (0.2–5.0%) in a saline solution was used. The supernatant was obtained after centrifugation for 1 h at 100 000 × *g* in a Spinco Model L centrifuge. The residue that remained after centrifugation was resuspended in 12 ml water and thoroughly mixed aliquots were taken for assay. To study whether Triton X-100 had any effect on the assay procedure itself, the appropriate amount of the Triton X-100 was added to incubation media, which contained either saline-extracted enzyme or the residue of the saline extract. Acetylcholinesterase assays were performed by the method of Ellman *et al.*[1] using acetylthiocholine iodide as substrate. Blank values were obtained without the substrate and the samples were read against these controls. Protein was measured by the method of Lowry *et al.*[2] using bovine serum albumin (Pentex) as a standard All values are the averages of 3 experiments which were in close agreement.

Polyacrylamide disc gel electrophoresis was performed in a Canalco apparatus according to the method of Davis[3] as modified by Juul[4] for serum cholinesterase. The separating gel was prepared at pH 8.7, the spacer gel and the sample gel at pH 5.8. Separations were accomplished at 4 °C in the Tris–glycine buffer (pH 8.4) at 3 mA per tube for 3 h. After electrophoresis, the gels were pre-incubated in phosphate buffer at pH 6.1 without the substrate for 30 min, and then in the complete reaction mixture containing acetylthiocholine iodide ($3.2 \cdot 10^{-3}$M) and incubated for 90 min. The gels were then transferred into 3 M $(NH_4)_2SO_4$ solution at 4 °C for 24 h. At that time the gels were placed in a solution of 3 M $(NH_4)_2SO_4$ saturated with dithiooxamide to fix the enzyme and allowed to stand at 4 °C for 24 h. Destaining was accomplished with 7% acetic acid.

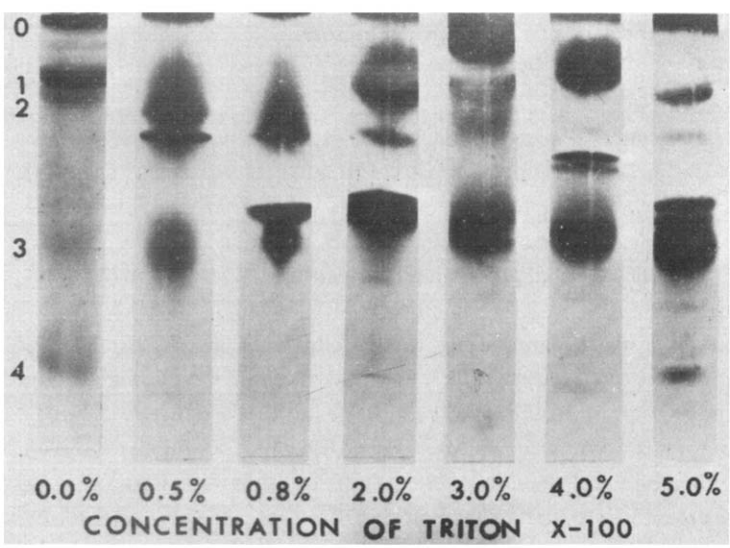

Fig. 1. Polyacrylamide gel electrophoretic patterns of human brain acetylcholinesterase after extraction at various concentrations of Triton X-100.

The gel electrophoresis patterns developed for acetylcholinesterase activity extracted at different concentrations of Triton X-100 are shown in Fig. 1. A saline extract of human brain demonstrated some enzymatic activity at the origin which apparently did not penetrate the gel under these experimental conditions and 4 additional distinct areas of acetylcholinesterase activity. With a 0.5% Triton X-100 extract areas 1 and 2 increase in density, the former evidencing a broad diffuse band. Band 3 also consists of a diffuse area while band 4 disappears. As the concentration of Triton X-100 increases, other changes in the electrophoretic pattern occur. Very little area 4 activity is demonstrable at any concentration of detergent. At a 2% Triton X-100 concentration where increase in the supernatant was shown on assay, area 3 is stained intensely while area 1 is broken up into a number of bands. At a 3% level, there is a dense staining at the origin, while at a 4% detergent concentration this activity apparently does move a short distance into the gel with areas 1 and 2 showing 2 distinct relatively sharp bands. At a 5% concentration where inhibition of activity was shown to occur, the slow moving bands are diminished in staining capacity, while a number of bands appear in area 3.

Despite the unsystematic gradations in electrophoretic patterns with increased Triton X-100 concentrations, the patterns are reproducible. In addition, the extracts were shaken with castor oil to remove the detergent and the resulting solution was subjected to electrophoresis. These extracted solutions gave patterns that were identical to the original extract. The same results were obtained if the extracts were dialyzed before electrophoresis

The effect of Triton X-100 on acetylcholinesterase assay values are shown in Table I. With various concentrations of Triton added to the assay media, neither the residue nor the supernatant showed any activation or inhibition, and the specific activities of the residue and the supernatant were approximately 2.0 and 1.0, respectively, at all detergent concentrations. Thus, the Triton X-100 *per se* does not have any effect

TABLE I

EFFECT OF TRITON X-100 ON ACETYLCHOLINESTERASE ASSAY VALUES

Residue		*Supernatant*	
Sample	*Spec. act. ($\times 10^2$)*	*Sample*	*Spec. act. ($\times 10^2$)*
Saline residue	2.0	Saline extract	1.0
+ 0.2% Triton X-100	1.9	+ 0.2% Triton X-100	1.0
+ 0.5% Triton X-100	2.1	+ 0.5% Triton X-100	1.0
+ 1.0% Triton X-100	2.2	+ 1.0% Triton X-100	1.1
+ 2.0% Triton X-100	2.2	+ 2.0% Triton X-100	1.1
+ 5.0% Triton X-100	2.2	+ 5.0% Triton X-100	1.1

Specific activity is expressed as μmoles acetylthiocholine iodide hydrolyzed/min per mg protein. Residue contained 7.4 mg protein/ml. Supernatant contained 3.4 mg protein/ml.

TABLE II

EFFECT OF TRITON X-100 ON RAT BRAIN ACETYLCHOLINESTERASE ACTIVITY

Extractive	*Volume of sample (ml)*		*Total protein (mg)*		*Total enzyme activity (units)**		*Relative spec. act:***		*Total enzyme activity (units)*
	Residue	*Supernatant*	*Residue*	*Supernatant*	*Residue*	*Supernatant*	*Residue*	*Supernatant*	*Supernatant + residue*
Saline 0.9%	13.4	9.5	154.2	23.7	2.0	0.30	100	100	2.30
Triton X-100 0.2%	13.2	9.6	150.5	36.5	2.3	0.98	93	260	2.97
Triton X-100 0.5%	13.3	9.2	148.9	43.2	2.0	1.19	87	280	3.18
Triton X-100 1.0%	13.0	8.6	126.0	67.0	1.9	2.18	93	360	4.04
Triton X-100 2.0%	14.3	8.9	127.2	82.7	2.1	1.94	106	230	4.04
Triton X-100 5.0%	14.2	8.8	78.1	113.5	1.4	1.36	106	120	2.76

*μmoles acetylthiocholine iodide hydrolyzed/min per ml solution.

**Saline values arbitrarily set at 100 where spec. act. = μmoles acetylthiocholine iodide hydrolyzed/min per mg protein.

on enzymatic activity after it has been extracted.

The effect of various concentrations of Triton X-100 on enzymatic activity of residue and supernatant is shown in Table II. There is redistribution of protein between the two phases and a solubilization of about 50% of the protein when the concentration of detergent is increased from 0 to 5.0%. Despite this loss of protein the total acetylcholinesterase activity remains virtually constant in the residue at Triton X-100 levels of 0.2–2% and only at a 5% level does some inhibition of activity appear to occur. In the supernatant the total enzyme increases 6–7-fold from 0.30 unit with saline extractant as compared to 1.0–2% Triton X-100. Inhibition at the 5% level is again noted. When the data are expressed as relative specific activity very little change is seen in the residue while the supernatant values increase to 360% at 1.0% Triton X-100. These results are again shown when total supernatant and residue values are calculated. In this case the total enzyme activity increases approximately 57% above the original levels.

Swanson *et al.*[5] have reported activation of (Na^+-K^+)-activated ATPase, the degree of activation being dependent on the chain length of the detergent. Roodyn[6] has noted maximum solubilization of mitochondria with Triton X (chain length=9–10) and less solubilization with longer hydrophilic chain length. Other workers have shown the activation of solubilized catalytic proteins like trypsin (EC 3.4.4.4) and plasmin (EC 3.4.4.14) by quarternary detergents[7]. Crone[8] found considerable activation associated with solubilization of acetylcholinesterase whereas Harwood and Hawthorne[9] found considerable stimulation of acetylcholinesterase with Triton X-100. DeRobertis[10] claimed only solubilization of the enzyme with Triton, though his Triton-treated mitochondrial fraction appeared to show an increased activity when compared to control. Jackson and Aprison[11] have suggested that non-ionic detergents enhance acetylcholinesterase activity by virtue of their surface-active properties. However, we did not find any enhanced activity when Triton X-100 was added to the assay media. It has been suggested that a membrane consisting of acetylcholinesterase and ATPase is an example of general subunits contributing to membrane structure[12].

The analytical and electrophoretic data indicate that the increase in acetylcholinesterase activity that is demonstrable with the use of Triton X-100 in the extractive media may not be due to greater extraction of the enzyme from the particulate to which it is bound but to changes in the properties of the catalytic protein. This is evidenced by alterations in the electrophoretic patterns obtained at the various Triton X-100 concentrations, as well as quantitative increases in supernatant activity without any commensurate changes in the activity of the residue. These changes could be due to the catalytic protein released in association with membrane fragments in varying degrees. As the membrane to enzyme attachment is altered, the size and the charge of the particle are changed and, depending on the size of attached fragments, show differing electrophoretic mobilities. Also, the nature of the binding of enzyme to membrane can be altered. This can lead either to the unmasking of hidden catalytic sites by release of an allosteric inhibitory mechanism or by conformational changes in the molecule due to the rupture of lipophilic bonds. The electrophoretic patterns obtained here at various Triton X-100 concentrations

indicate that the enzyme is released along with membrane particles in various degrees of aggregation as evidenced by the broad diffuse bands obtained as well as by the different mobilities observed for the various areas of activity.

Thus it would appear that the major increase in activity of acetylcholinesterase observed after extraction with Triton X-100 is a result of an alteration of the state of the protein which may lead to an enhancement of catalytic activity.

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