Vol. 100, No. 2, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 29, 1981 Pages 710-715

INHIBITION OF CALMODULIN-ACTIVATED CYCLIC NUCLEOTIDE PHOSPHODIESTERASE BY TRITON X-100

Rajendra K. Sharma and Jerry H. Wang

Department of Biochemistry, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada, R3E 0W3

Received April 13,1981

SUMMARY: The activity of calmodulin-activated cyclic nucleotide phosphodiesterase in a standard reaction containing 40 ng calmodulin could be inhibited about 50% by 14 μM Triton X-100. In contrast, the calmodulin-independent phosphodiesterase required a much higher concentration of the detergent, 380 μM for 50% inhibition of its activity. The potent inhibitory effect of Triton X-100 on the calmodulin-activated phosphodiesterase reaction could be reversed by high concentrations of calmodulin. Using the gel filtration technique, it was demonstrated that ^{3}H-Triton X-100 bound to calmodulin with high affinity in buffer containing Ca^{2+}. The binding of the detergent to calmodulin was mostly eliminated in the presence of trifluoperazine, a neuroleptic drug. Among other detergents examined, including Lubrol WX, Triton QS and Triton CF, only Triton CF exhibited preferential inhibition of the calmodulin-activated phosphodiesterase. The results suggest that certain detergents in the Triton family inhibit calmodulin action by undergoing Ca^{2+}-dependent binding to the protein at the neuroleptic drug site.

INTRODUCTION

The regulatory actions of calmodulin are generally believed to involve the binding of Ca^{2+} to calmodulin to change it from an inactive to an active conformation. The active form of the protein then associates with the calmodulin-regulated proteins to modify their activities (1). A number of pharmacological agents including some neuroleptic drugs have been shown to bind to the active form of calmodulin, blocking its regulatory activity (2-4). It has been suggested that calmodulin may be the cellular target of these neuroleptic drugs (3). However, the suggestion has been questioned since inactive analogues of some of the drugs have been shown to be equally potent in the inhibition of calmodulin action (5). In the present study it is observed that Triton X-100, a nonionic detergent commonly used for the solubilization of membrane bound proteins is also a potent inhibitor of calmodulin action, and capable of undergoing Ca^{2+}-dependent association with calmodulin.

0006-291X/81/100710-06$01.00/0

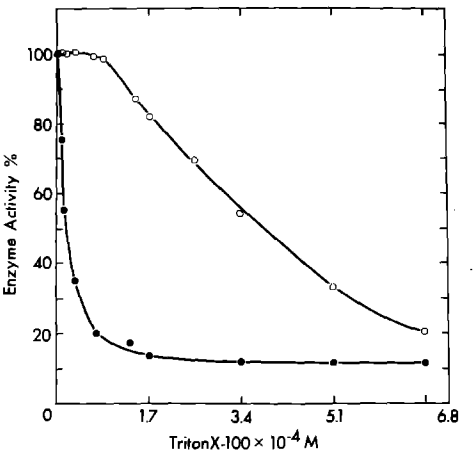


Figure 1. Effect of the Triton X-100 on different forms of cyclic nucleotide phosphodiesterase. Reactions of bovine brain calmodulin-dependent cyclic nucleotide phosphodiesterase (0.012 unit/ml) in the presence of 40 ng/ml of the calmodulin in the standard assay mixture ●—● and bovine heart calmodulin-independent cyclic nucleotide phosphodiesterase (0.013 unit/ml) O—O were carried out in the presence of various concentrations of the Triton X-100. The average molecular weight of Triton X-100 646.0 was used for the calculation of molar concentration of the detergent.

In addition to its relevance to the specificity of calmodulin drug interaction the observation indicates that detergents used for studies of membrane-bound calmodulin and calmodulin-regulated proteins should be chosen with care.

MATERIALS AND METHODS

Bovine brain calmodulin, calmodulin-dependent cyclic nucleotide phosphodiesterase and bovine heart calmodulin-independent phosphodiesterase were prepared as previously described (6-8). Phosphodiesterase and calmodulin assays have been described in detail elsewhere (6). Triton X-100, ^{3}H-Triton X-100 were obtained from Sigma and New England Nuclear respectively. Equilibrium binding of Triton X-100 to calmodulin was carried out using the gel filtration methods of Humel and Dryer (9).

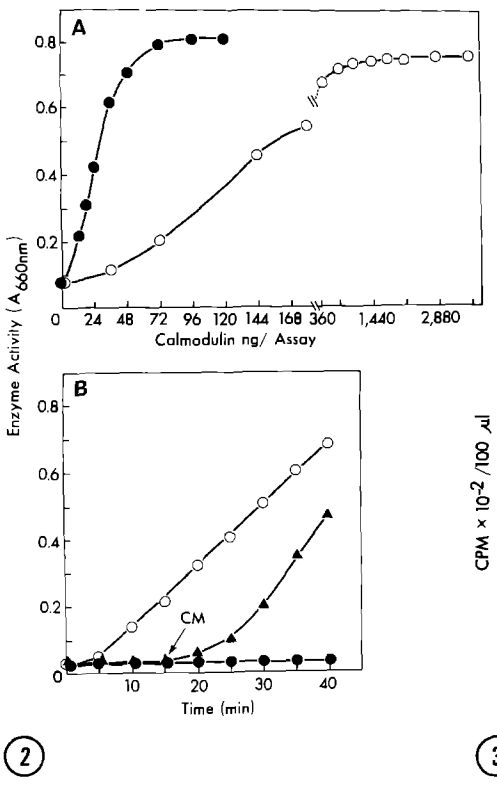
RESULTS AND DISCUSSION

Figure 1 shows that both calmodulin-dependent and calmodulin-independent cyclic nucleotide phosphodiesterase were inhibited by Triton X-100. However, the two forms of the enzyme exhibited different sensitivity to the detergent. The calmodulin-dependent phosphodiesterase activity consisted of the basal activity, representing about 12% of the total activity, and the calmodulin-

activated activity, about 88%. With increasing concentrations of Triton X-100, more than 85% of the total activity of calmodulin-dependent phosphodiesterase was rapidly inactivated at low concentrations of the detergent whereas about 10% of the activity remained even at the highest detergent concentration tested. The result suggests that Triton X-100 inhibited primarily the calmodulin-activated activity of the enzyme. This activity is highly sensitive to the detergent. The concentration of Triton X-100 required for 50% inhibition of the calmodulin-activated activity of the enzyme was 14 μM. In contrast, the calmodulin-independent form of cyclic nucleotide phosphodiesterase is much more resistant to Triton X-100. Fifty percent inhibition of this form of the enzyme by the detergent occurred at 380 μM.

The low concentration of Triton X-100 has a marked effect on the dose-response curve of the activation of phosphodiesterase by calmodulin with only a small effect on the maximal activation of the enzyme. Result of a typical experiment is shown in Fig. 2A. Thus, it appears that the inhibition of the enzyme by the detergent may be prevented by high concentrations of calmodulin. In fact, the inhibition of the enzyme by Triton X-100 could be reversed upon addition of an excess amount of calmodulin during the course of the enzyme reaction (Fig. 2B). These observations further support the notion that low concentrations of Triton X-100 affects primarily the calmodulin-activated activity of the phosphodiesterase.

The kinetic characterizations of the effect of Triton X-100 on the calmodulin-dependent phosphodiesterase reaction are similar to those of trifluoperazine on this enzyme. Trifluoperazine and several other agents (3,4) have been shown to inhibit calmodulin action by undergoing Ca^{2+}-dependent association with the protein. Figure 3 shows that ^{3}H-Triton X-100 could also bind to calmodulin in solutions containing Ca^{2+}. The concentrations of calmodulin and free Triton X-100 used in this experiment were 21 μM and 20 μM respectively. When EGTA was included in the column buffer to chelate Ca^{2+}, the binding of Triton X-100 to calmodulin was markedly decreased. Thus, the



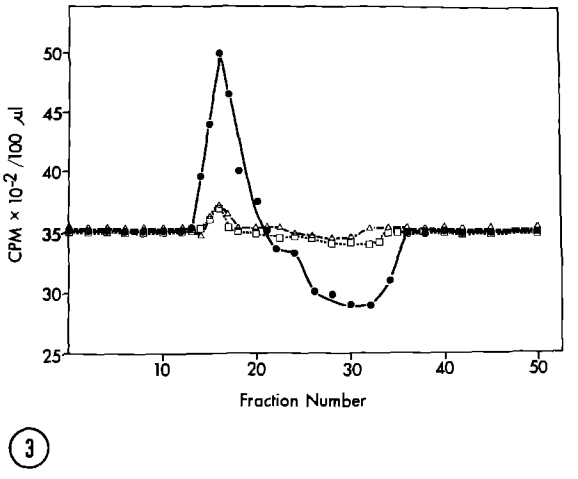


Figure 2A. Effect of calmodulin on the inhibition of calmodulin-dependent cyclic nucleotide phosphodiesterase by Triton X-100. Bovine brain calmodulin-dependent cyclic nucleotide phosphodiesterase in the absence (●—●) and presence (○—○) of 0.10 mM of the Triton X-100 was assayed at different concentration of the calmodulin.

Figure 2B. Reversal of the Triton X-100 inhibition of calmodulin-dependent cyclic nucleotide phosphodiesterase by addition of the calmodulin to the reaction. Phosphodiesterase reactions with 0.012 unit/ml of enzyme and 40 ng/ml calmodulin were carried out either in the absence (○—○) or presence of 0.15 mM of the Triton X-100 (▲—▲ and ●—●). Aliquots were removed at various time intervals as indicated for the analysis of phosphate concentration. After 15 minutes, additional calmodulin was added to a final concentration of 1.5 µg/ml to one of the Triton X-100 inhibited reaction (▲—▲) and the analysis of the progress of the enzyme reactions was then continued.

Figure 3. Demonstration of the interaction between calmodulin and Triton X-100 by gel filtration. A column of Sephadex G-25 (1.5 x 25 cm) was prepared and equilibrated with buffer A (20 mM Tris-HCl, 1 mM magnesium acetate, 1 mM imidazole and 0.1 M NaCl pH 7.0) containing 20 µM ^{3}H-Triton X-100 and 0.01 mM $CaCl_2$. A nine hundred microliter sample volume containing 20 µM calmodulin in 0.01 mM $CaCl_2$ was applied to the G-25 column. One ml fractions were collected and radioactivity was monitored using 100 µl aliquots (●—●). For the experiment represented by ▲—▲, 20 µM Trifluoperazine was present in the equilibrating and elution buffer and in the experiment indicated by □—□, 0.1 mM EGTA instead of 0.01 mM $CaCl_2$ was present in the equilibrating and elution buffer with similar conditions as described above.

TABLE 1

Effect of detergents on different forms of cyclic nucleotide phosphodiesterase.

Addition	I_{50} (μg) [a]	
	Calmodulin-dependent cyclic nucleotide phosphodiesterase	Calmodulin-independent cyclic nucleotide phosphodiesterase
Triton X-100	9.0	245.0
Triton CF	13.0	260.0
Triton QS-15	111.0	255.0
Lubrol WX	222.0	285.0

Cyclic nucleotide phosphodiesterase was measured as previously described (6) plus various amounts of detergents. For the standard assay of calmodulin-dependent cyclic nucleotide phosphodiesterase, the reaction mixture contained 40 mM Tris-HCl, 40 mM imidazole, 5 mM magnesium acetate, 0.1 mM $CaCl_2$, 0.012 units/ml enzyme, 1.2 mM cyclic AMP and 40 ng/ml clamodulin. For the calmodulin-independent cyclic nucleotide phosphodiesterase, the activity was measured using 0.013 units/ml enzyme. Other conditions are identical to those used for the calmodulin-dependent cyclic nucleotide phosphodiesterase except 0.1 mM EGTA was used instead of 0.1 mM $CaCl_2$.

a) The I_{50} value is defined as concentration of detergents required to produce a 50% inhibition of phosphodiesterase. The values were calculated from dose response curve of the inhibition.

binding of Triton X-100 to calmodulin appears to be Ca^{2+}-dependent. The binding of the detergent to calmodulin was mostly eliminated when 20 μM of trifluoperazine was present in the column buffer (Fig. 3). The result suggests that Triton X-100 binds to the neuroleptic drug site on calmodulin.

A number of other detergents were examined for their abilities to inhibit cyclic nucleotide phosphodiesterase. Table I shows that all these detergents inhibited both calmodulin-dependent and calmodulin independent forms of the enzyme. However, only Triton CF exhibited preferential inhibition of the calmodulin-activated activity of the calmodulin-dependent phosphodiesterase.

The inhibitory activities of Triton X-100 and Triton CF toward cyclic nucleotide phosphodiesterase are nearly the same.

In conclusion, the present study shows that some of the nonionic detergents in the Triton family should be added to the list of compounds which exhibit potent inhibitory activity toward calmodulin and Ca^{2+}-dependent association with the protein. These detergents are often used to solubilize membrane bound proteins. Many of calmodulin-regulated enzymes are membrane bound and calmodulin is also partly membrane bound (10-12). It is suggested that the detergents used for the studies of these proteins, should be chosen with care.

ACKNOWLEDGMENTS

This work was supported by grant from the Medical Research Council of Canada MT 2381.

REFERENCES

1. Wang, J.H., Teo, T.S., Ho, H.C., and Stevens, F.C. (1975) Adv. Cycl. Nucl. Res. 5, 179-194.
2. Levin, R.M., and Weiss, B. (1976) Mol. Pharmacol. 12, 581-589.
3. Weiss, B., and Levin, R.M. (1978) Adv. Cyclic Nucleotide Res. 9, 285-303.
4. Hidaka, H., Yamaki, T., Naka, M., Tanaka, T., Hayashi, H., and Kobayashi, R. (1978) Mol. Pharmacol. 17, 66-72.
5. Norman, J.A. (1980) Ann. N.Y. Acad. Sci. 356, 415-416.
6. Sharma, R.K., and Wang, J.H. (1979) Adv. Cycl. Nucleot. Res. 10, 187-198.
7. Wang, J.H., and Desai, R. (1977) J. Biol. Chem. 252, 4175-4185.
8. Ho, H.C., Teo, T.S., Desai, R., and Wang, J.H. (1976) Biochim. Biophys. Acta 429, 461-473.
9. Hummel, J.P., and Dreyer, W.J. (1962) Biochim. Biophys. Acta 63, 532-534.
10. Smoake, J.A., Song, S.Y., and Cheung, W.Y. (1974) Biochim. Biophys. Acta 341, 402-411.
11. Kakiuchi, S., Yamazaki, R., Teshima, Y., Uenishi, K., Yasuda, S., Kashiba, A., Sobue, K., Ohshima, M., and Nakajima, T. (1978) Adv. in Cyclic Nucleotide Research 9, 253-264.
12. Wang, J.H., and Waisman, D.M. (1979) Curr. Top. Cell. Regul. 15, 47-107.