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TRITON X NONIONIC SURFACTANTS REVERSIBLY INHIBIT THE EDTA/KCl ATPase ACTIVITY OF MYOSIN

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SUMMARY
The nonionic octylphenoxy polyethoxy series of surfactants, Triton X, reversibly inhibited the EDTA/KCl ATPase activity of purified rabbit skeletal muscle myosin at concentrations at or below their reported critical micelle concentrations. The maximum degree of enzyme inhibition increased with ethoxy content to 88% (with Triton X 102 average ethoxy content, 12.5 per molecule). The results suggest that binding of the surfactant to the myosin molecule occurs below the critical micelle concentrations and that the hydrophilic ethoxy chain forms a diffusion barrier against approach of ATP to the enzyme's active site. This model has implications for the organization of myosin in the plasma membrane of phagocytes.

Nonionic surfactants provide a means of examining the association of proteins to membranes. Intrinsic membrane proteins are extractable by nonionic surfactants (1,2) and denatured proteins can be renatured by incorporation into micelles (3). Myosin, which forms with actin filaments the main components of the contractile system of amoeboid cells (4), is an anisotropic molecule which aggregates tail to tail to form bipolar filaments (5) and has a strong attachment for the cytoplasmic face of the plasma membrane of polymorphonuclear neutrophils (6).

To understand more about the association of myosin with plasma membranes I have determined the effect of a series of nonionic surfactants on myosin ATPase activity. The evidence presented

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suggests that myosin adsorbs surfactant molecules at concentrations at or below their critical micelle concentrations, in such a way that the hydrophilic polyethoxy groups create a diffusion barrier around the active site which prevents access to the substrate ATP.

MATERIALS AND METHODS

Triton X surfactants were a gift from Rohm and Haas Company, Philadelphia, PA. CMC's were interpolated from published figures for a normally distributed population of surfactant molecules (8).

Myosin was prepared from the back muscle of a healthy New Zealand white rabbit by the method of Tonomura *et al.* (9) and stored at -20°C in 50% v/v glycerol.

ATPase activity was assayed as described previously (10). The enzyme was incubated in 0.6 ml of substrate solution containing 1 mM ATP, 0.6 M KCl, 2 mM EDTA, in imidazole chloride buffer 15 mM pH 7.0 containing the appropriate concentration of surfactant at 37°C. After 15 minutes 0.3 ml of 10% trichloroacetic acid was added to stop the reaction and precipitate protein which was pelleted at 1000 x G for 10 minutes at 4°C. The supernatant, 0.6 ml, was transferred to another tube and inorganic phosphate determined by the method of Fisk and Subbarow (11) measuring the optical density of the reduced phosphomolybdate blue complex at 700 nm. Sodium dodecyl sulfate, was added to final concentration of 1% in order to clarify the turbidity produced in the color reaction at the higher nonionic surfactant concentrations (12). Its presence tended to increase optical density by about 15% which was corrected by using appropriate standard curves containing both surfactants.

Protein was determined using the method of Lowry *et al.* (13).

RESULTS

ATPase activity determined in the presence of 0.1% Triton X 100 was reduced by 72% compared with the control containing no surfactant. After pretreatment with 0.1% Triton X 100 followed by dilution to a final concentration of 0.01% the enzyme activity was nearly all restored (Table 1).

Myosin ATPase activity was determined over a range of surfactant concentrations using a homologous series varying in ethoxy content from 5 to 16 groups per molecule. Each surfactant inhibited enzyme activity to a maximum degree at a specific concentration above which further inhibition was either slight or zero (Fig. 1). In most cases incipient inhibition was seen at surfactant concentrations below the published critical micelle concentrations

TABLE 1

Effect of Triton X 100 on myosin ATPase activity

Treatment	Specific activity (μmoles/min/mg protein)	Inhibition (%)
None	2.75	-
0.1% TX 100	0.77	72
Pretreatment with 0.1% TX 100 followed by 1/10 dilution	2.42	12

(Table 2). The maximum degree of inhibition was slight with the lowest content of ethoxy groups (TX 45) and increased with increasing ethoxy content to a maximum at 12.5 ethoxy groups per molecule (TX 102) (Fig. 2). Inhibition was slightly less with 16 ethoxy groups (TX 165).

There was no apparent discontinuity in the inhibition curves at the CMC's and there was no correlation between the concentration at which either incipient or maximum inhibition occurred and the CMC's.

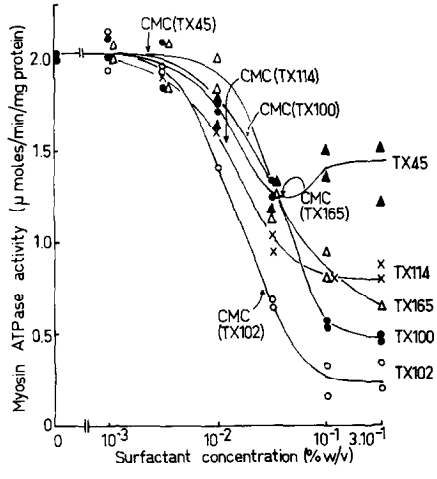

Fig. 1. Effect of nonionic surfactants on skeletal muscle myosin EDTA/KCl ATPase activity. CMC's for each member of the series were interpolated from published figures assuming that the surfactants as supplied were a normally distributed mixture of polymers.

TABLE 2

Properties of the nonionic surfactants

Type	Average ethoxy* group content	CMC** (% w/v)	Maximum degree of inhibition (%)
TX 45	5	.0049	38
TX 114	7.5	.011	60
TX 100	9.5	.018	76
TX 102	12.5	.026	88
TX 165	16	.036	68

* Data from Rohm and Haas Handbook (7)

** Data interpolated from Crook *et al.* (8) and converted into concentrations in % w/v

DISCUSSION

The results presented show that the series of Triton nonionic surfactants interact with myosin in a relatively mild concentration dependent fashion to inhibit the enzyme's ATPase activity. There is apparently no relationship between inhibition and the formation of micelles. Since inhibition tended to become constant at the higher concentrations studied it is apparent that saturating conditions existed.

Two simple explanations for this inhibition occurred to me.

(i) Myosin is sterically distorted by the binding of surfactant

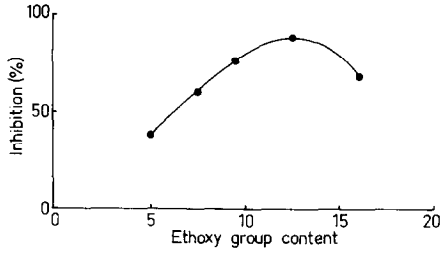

Fig. 2. Effect of increasing ethoxy group content on the maximum degree of inhibition of ATPase activity obtained by each surfactant.

to hydrophobic areas of the molecule which leads to a drop in activity. This model requires that hydrophobic adducts should bind more tightly than hydrophilic ones and by inference, produce a greater degree of inhibition. However, the most hydrophobic member of the series, TX 45 produced the least amount of inhibition. (ii) Myosin binds the hydrophobic octylphenoxy end of the surfactant molecule to specific hydrophobic sites leaving the hydrophilic ethoxy groups to surround the enzyme. This ethoxy "cloud" acts as a diffusion barrier to the ATP substrate which has difficulty attaining the active site. This model is supported by the fact that the degree of inhibition increases with increasing ethoxy group content of the surfactants.

It would seem therefore that myosin interacts with these nonionic surfactants in much the same way as do the intrinsic membrane proteins discussed by Clarke (3). These proteins bind surfactant below their CMC's and the surfactant monomers supposedly bind to the same hydrophobic sites occupied by lipid when the protein is contained within a membrane. This would explain why TX 165 did not display greater inhibition than it did. This member of the series is significantly more hydrophilic than the others (8) and its greater water solubility would militate against tight binding to protein.

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