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Fluorescence Quenching by a Brominated Detergent: Application to Diphtheria Toxin Structure[a]

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Fluorescence quenching is a powerful tool for analysis of membrane structure. Fluorescence quenching by spin-labeled lipids has been used to evaluate the depth of membrane penetration by fluorescent molecules, including polypeptides, and the composition of lipids in contact with protein.[1] Recently, brominated lipids have been introduced as additional fluorescence-quenching probes.[2,3] Now, we have further extended this technique by development of a brominated detergent. We have synthesized brominated Brij 96,[4] a detergent that is a member of the polyoxyethylene class that includes Triton X-100. We have used brominated Brij 96 to examine the effect of pH on the structure of diphtheria toxin.

Diphtheria toxin protein binds to cell membranes and penetrates the membrane subsequent to endocytosis and entry into acidic vesicles, either endosomes or lysosomes.[5,6] For the studies below, toxin was isolated as described previously.[4,7] Nicked dimers containing bound ApUp, a natural dinucleotide ligand,[8] were used in these studies. Experimental conditions are described in the figure legend

First, the intrinsic toxin protein fluorescence was characterized. There is a dramatic twofold drop in fluorescence below pH 5.5 (see TABLE 1). The λ_{max} of fluorescence shifts from 323–325 nm at higher pH to 330–333 nm at lower pH (data not shown). These changes occur in ordinary aqueous solutions as well as in the presence of ordinary Brij 96. They are paralleled by changes in the quenching of toxin fluorescence by brominated Brij 96 (FIG. 1A). Quenching is found only at lower pH, which means that detergent binding, and therefore hydrophobicity, appear only at low pH. Also, the strong quenching observed indicates that a considerable fraction of tryptophan residues are exposed to the micelle interior at low pH.

Further information on the effect of low pH comes from quenching by the aqueous quencher acrylamide. Toxin in aqueous solution is quenched more strongly at low pH (FIG. 1B). This suggests that some unfolding occurs at low pH. (Changes in tryptophan lifetime are very unlikely to be large enough to account for these effects.)

There is at least partial reversibility of low-pH-induced changes when toxin is transferred back to higher pH (TABLE 1). Intrinsic fluorescence remains weak, but quenching by brominated Brij 96 is largely reversed, indicating loss of hydrophobicity.

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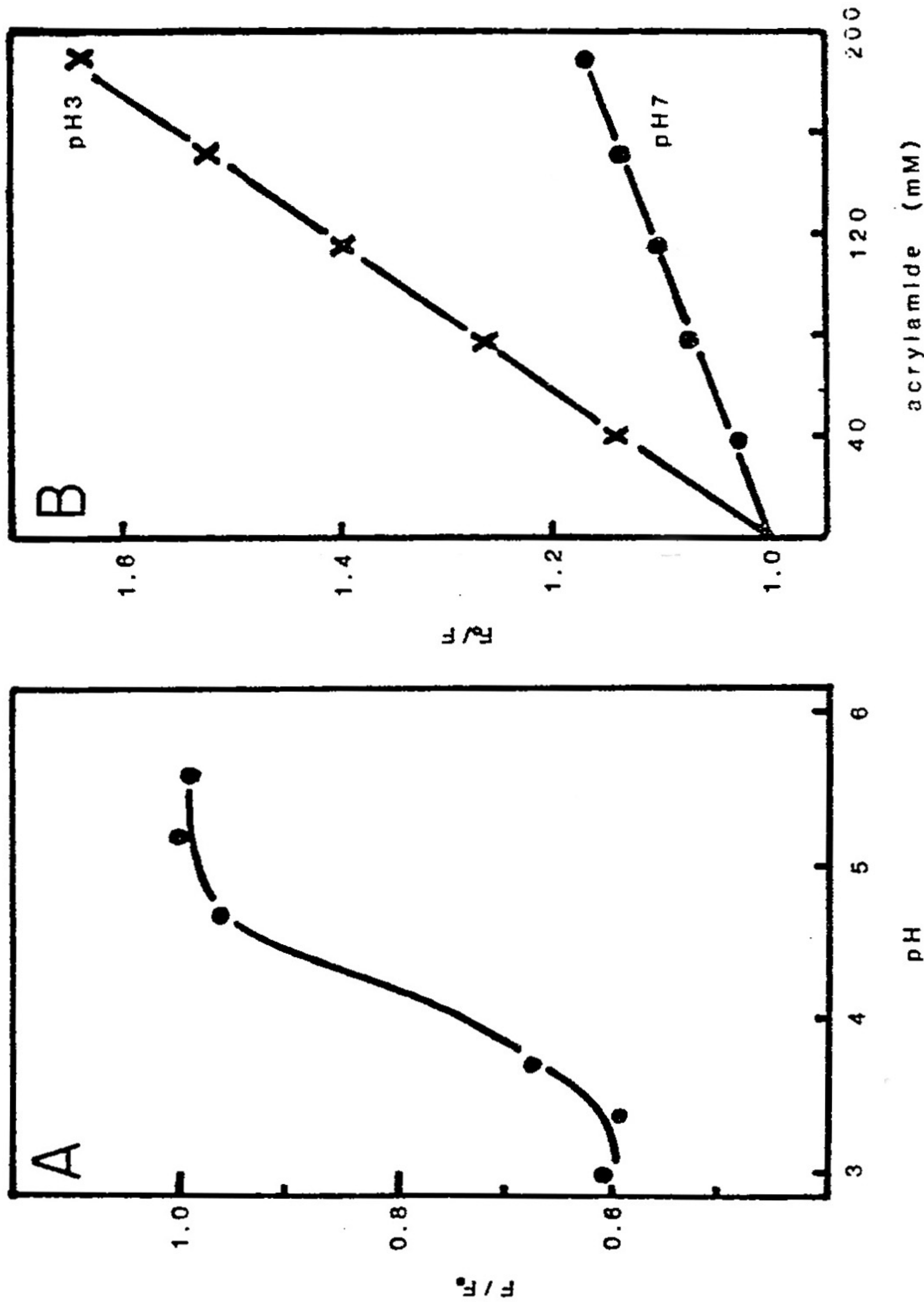

FIGURE 1. pH effect on fluorescence quenching of diphtheria toxin. (A) Quenching by brominated detergent. Samples contained 0.1 mg/ml of toxin in 10 m*M* formate (at pH < 4) or acetate (at pH > 4). F is the fluorescence in the presence of brominated Brij 96 (0.13% wt/vol) and F_o is the fluorescence in ordinary Brij 96 (0.13% wt/vol). (B) Quenching by acrylamide. Samples contained 0.005 mg/ml toxin in 150 m*M* NaCl and 5 m*M* formate at pH 3 (x) or 6.25 m*M* Tris-formate at pH 7 (●).

These experiments show that fluorescence and fluorescence quenching techniques are powerful tools for characterization of diphtheria toxin structure. It should be noted that brominated Brij 96 binding versus pH, determined by quenching, agrees with Triton X-100 binding, determined by the much more laborious method of sucrose gradient centrifugation.[9] In addition, quenching has the advantage that it can be used for kinetics. Therefore, fluorescence quenching by detergent should be a generally useful technique.

TABLE 1. Reversibility of Fluorescence Changes Induced by Low pH[a]

Conditions	F_o (Relative Units)[b]	F/F_o
pH 5.7	45	1.03
pH 3	23	.52
pH 5.7 after pH 3 incubation	28	.95

[a]Toxin concentration 0.02 mg/ml. 0.25% (wt/vol) Brij 96 or Br Brij 96 was present in all samples.

[b]F and F_o are defined in FIGURE 1.

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