## [5] The Role of Micelles in Protein–Detergent Interactions<sup>1</sup>

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## I. Introduction

The interaction of proteins with amphiphilic ligands has received increasing attention in recent years. The practical as well as theoretical importance of these interactions are illustrated by the following examples.

1. Investigations of the molecular properties of membrane proteins and serum lipoporoteins have for the most part required the use of detergents as solubilizing agents and as probes for hydrophobic binding sites.<sup>2</sup>

2. The popular technique of identifying and cataloging polypeptides on the basis of their mobilities in sodium dodecyl sulfate-polyacrylamide gel electrophoresis is based on a specific type of detergent-protein interaction.<sup>3</sup>

3. Two-dimensional polyacrylamide gel electrophoresis using sodium dodecyl sulfate in one direction and the nonionic detergent, Triton X-100, in the other has been used to identify polypeptides containing long hydrophobic sequences or regions.<sup>4</sup> This technique relies on differences in binding characteristics between water-soluble and intrinsic membrane proteins in that the former do not in general bind nonionic detergents.

It is apparent from these few examples that an understanding of the thermodynamics of detergent-protein and detergent-detergent interactions is of central importance in many areas of research. It is the purpose of this chapter to outline the theoretical and practical aspects of these interactions with particular emphasis on the competitive effects of micelle formation and protein-detergent binding.

## II. Thermodynamic Equilibria

A. Micelle Formation. In aqueous solution amphiphilic molecules self-associate at a specific concentration (critical micelle concentration) to form well-defined interaction products. The theoretical aspects of this

<sup>&</sup>lt;sup>1</sup> This work was supported in part by National Institutes of Health Grants HL 14882 and NS 12213.

<sup>&</sup>lt;sup>2</sup> C. Tanford and J. A. Reynolds, Biochim. Biophys. Acta 457, 133 (1976).

<sup>&</sup>lt;sup>3</sup> T. B. Nielsen and J. A. Reynolds, see Vol. 48, p. 3.

<sup>&</sup>lt;sup>4</sup> A. Helenius and K. Simons, Proc. Natl. Acad. Sci. U.S.A. 74, 529 (1977).



FIG. 1. (A) The binding of detergent to protein as a function of total unbound detergent concentration where the amphiphile has the self-association properties shown in (B). Protein concentration =  $10^{-5} M$ , n = 100. (1)  $\Delta G^0 = -8.4$  kcal/mole; (2)  $\Delta G^0 = -8.21$  kcal/mole. (B) The increase in free monomer concentration (---) and micelle concentration (----) as a function of total detergent concentration. Critical micelle concentration =  $0.906 \times 10^{-6} M$ ; m = 100,  $\Delta G_{mic}^0 = -8.15$  kcal/mole monomer.

phenomenon are discussed in detail in a number of recent publications.<sup>5-7</sup> We can describe this process by means of the following equation.

$$mD \rightleftharpoons D_{\rm m}$$
 (1)

where *m* is the average association number, *D* is the concentration of monomeric amphiphile and  $D_m$  is the concentration of micelles. The association constant is

$$K = \frac{[D_{\rm m}]}{[D]^m} \tag{2}$$

Figure 1A shows the increase in concentration of D and  $D_m$  as a function of total amphiphilic concentration; where m = 100 and the free energy of micellization  $\Delta G_{mic}^0$  is -8.15 kcal/mole monomer. It is of particular im-

<sup>&</sup>lt;sup>5</sup> C. Tanford, "The Hydrophobic Effect." Wiley (Interscience), New York, 1973.

<sup>&</sup>lt;sup>6</sup> C. Tanford, J. Mol. Biol. 67, 59 (1972).

<sup>&</sup>lt;sup>7</sup> C. Tanford, J. Phys. Chem. 78, 2469 (1974).

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