THE BINDING OF ANIONIC SURFACTANTS TO HUMAN SERUM ALBUMIN STUDIED BY MEANS OF ^{81}Br NUCLEAR MAGNETIC RESONANCE.

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Received November 3, 1969

SUMMARY: A competitive binding technique has been used to investigate the binding of anionic surfactants to serum albumin. The method involves the study of the displacement of bromide ions from sites in the protein by surfactant anions by means of ^{81}Br nuclear magnetic resonance. Depending on the nature of the surfactant anion different competitive effects are observed.

The complexes formed between proteins and surfactant ions are a subject of considerable interest both from a biological and technical viewpoint.[1-3] The most extensive studies have been made on serum albumin to determine the number and nature of the binding sites of organic anions.[4-12] From equilibrium dialysis experiments Reynolds et al. found that about 10 surfactant anions are tightly bound per albumin unit with binding constants of the order of 10^4 - 10^6.[6,7] However, it is still not quite clear to what extent hydrophobic regions of the protein are involved. The results of Rosenberg et al.[13], in a study of the interaction of sodium dodecyl sulfate with phycocyanin, indicate that a considerable hydrophobic interaction occurs.

From e.m.f. measurements Scatchard et al. determined the binding of some small ions such as the halogen ions to serum albumin.[14,15] More than one class of binding sites exists, but the binding constants of the halogen ions are smaller by orders of magnitude than those of the surfactant anions.

During recent years quadrupole relaxation in nuclear resonance has been shown to provide excellent possibilities for studying ion binding to proteins.[16-26] Thus if a protein such as serum albumin is added to a solution with a large excess of bromide ions, a marked broadening of the bromine resonance signal is observed.[26] This indicates a change in relaxation of some bromide

ions, probably due to binding to specific sites in the protein. If the binding sites in the protein involved in the binding of surfactant anions are the same as those for the binding of the bromide ions, it might be possible to displace some of the bound bromide ions by the addition of an anionic surfactant due to the difference in binding strength. This would result in a line width decrease of the bromine resonance signal. As will be shown below, this is indeed the case.

A Varian V-4200 nmr spectrometer equipped with a 12 inch V-3603 magnet was used for the measurements. The ^{81}Br magnetic relaxation was measured at a magnetic field of 13.75 kG. All measurements are line width measurements, the line width being determined as the distance between the maximum and minimum slopes of the first derivative of the absorption signal. The error in the measured line width is less than 10 %. The temperature was 27.5 ± 1°C. Human serum albumin (HSA) was obtained from KABI and used without further purifications. All surfactants were of the highest purity, most of which were generous gifts from the Institute of Surface Chemistry, Stockholm. The sodium o-xylene--4-sulfonate was obtained through the kindness of Dr. Jan Bergman, Stockholm. 0.01 M and 0.1 M surfactant solutions were made by the use of distilled water doubly deionized and degassed. The initial concentration of potassium bromide was 0.167 M and of phosphate buffer 0.033 M, pH 5.6, in all series. The HSA concentration was in all cases 0.5 per cent by weight and a molecular weight of 69,000 per HSA unit has been assumed in the calculations. In no case did the dilution upon titration with surfactant solutions exceed 10% by volume.

In figures 1 and 2 the line width of the ^{81}Br resonance signal is given as a function of the total number of moles of surfactant added per mole HSA. The dashed lines pertain to "blank" solutions without HSA added.*) The addition of 0.5% HSA to the buffer-potassium bromide solution causes a line width

*)In the case of sodium dodecyl sulfate the presence of salt evidently caused both an increase in the Krafft point and a decrease in the cmc. The titration could therefore not be performed to higher concentrations than 0.001 M.

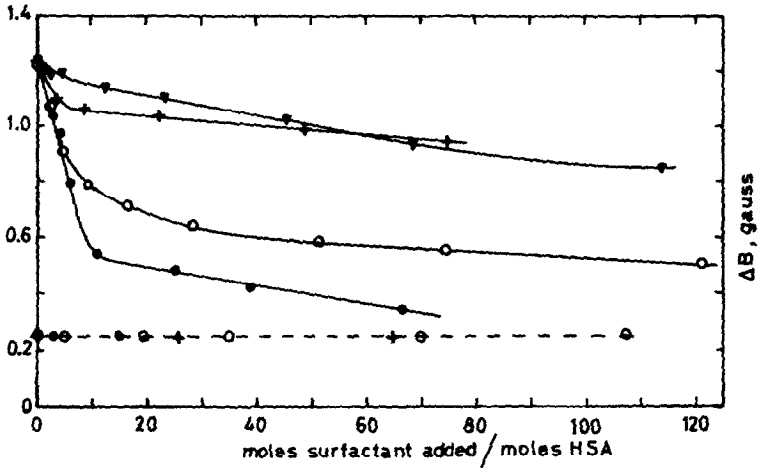
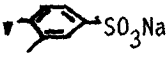


Figure 1. The line width of the ^{81}Br resonance signal as a function of total moles surfactant added/mole HSA.

—— 0.5 % HSA, 0.167 M KBr, 0.033 M phosphate buffer, pH 5.6

--- 0.167 M KBr, 0.033 M phosphate buffer, pH 5.6

+ $C_4H_9SO_4Na$, o $C_8H_{17}SO_4Na$, • $C_{12}H_{25}SO_4Na$, ▼ 

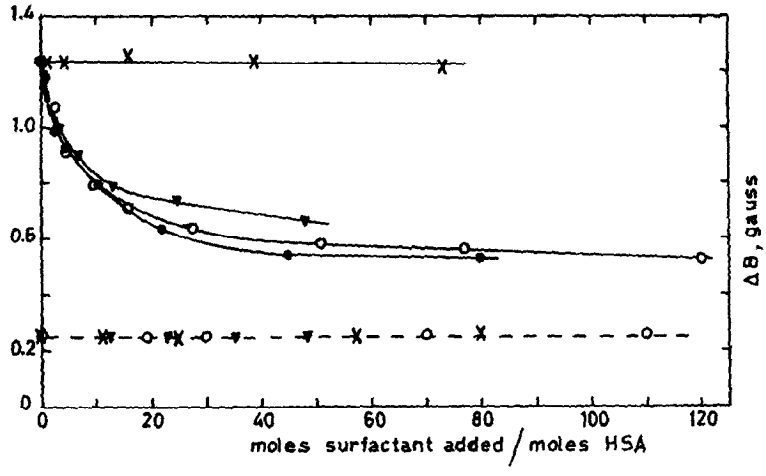


Figure 2. The line width of the ^{81}Br resonance signal as a function of total moles surfactant added/mole HSA.

—— 0.5 % HSA, 0.167 M KBr, 0.033 M phosphate buffer, pH 5.6

--- 0.167 M KBr, 0.033 M phosphate buffer, pH 5.6

x $C_9H_{19}NMe_3Br$, ▼ $C_8H_{17}SO_3Na$, o $C_8H_{17}SO_4Na$, • $C_7H_{15}COONa$

increase of about 1 gauss. If we make the assumption that there exist 1 different types of bromine sites and that the case of rapid exchange is valid for all sites*) the observed line width should be given by the following expression

$$\Delta B_{obsd} = \sum_{1}^{1} P_i \Delta B_i + P_o \Delta B_o \qquad (1)$$

P_i is the fraction of bromide ions located at sites of type $\underline{i}$, i.e. if there are n_i sites of type $\underline{i}$ occupied per molecule protein then $P_i = n_i/n$, where n = total moles bromide/mole HSA in the solution. ΔB_i is the ^{81}Br line width characterizing bromide ions at sites of type $\underline{i}$. P_o is the mole fraction of unbound bromide ions and ΔB_o the corresponding line width.

The addition of anionic surfactant causes a decrease in the line width in all cases (Figs. 1 and 2), while the cationic surfactant ($C_9H_{19}NMe_3Br$) does not affect the line width of the bromine resonance signal. This indicates, that, if there exists any interaction (e.g. a hydrophobic one, cf. refs. 3 and 5) between the HSA and the cationic surfactant at pH 5.6, no structural changes are imposed thereby which affect the binding sites of the bromide ions. The decrease in the line width of the bromine resonance signal on the addition of anionic surfactant could either depend on a decrease in P_i or ΔB_i (Eqn. 1). Reynolds et al.[7] found that the addition of surfactant caused small increases in the viscosity. This indicates that the observed line width decrease should result from a decrease in P_i i.e. the bromide ions are displaced from their sites by surfactant anions. The fact that the titration curves of the various anionic surfactants are not coincident, proves that the competitive binding technique used in this work is sensitive enough to reveal differences in binding strength of the anionic surfactants. A comparison between the displacement ability of the C_4-, C_8- and C_{12}-sulfates (Fig. 1) indicates that the hydrocarbon moiety of the surfactant anion plays an important role in determining the affinity of the surfactant anion for the protein: the

*) Some very preliminary results on ^{79}Br indicate that this may not be the case for the first 1 or 2 binding sites blocked by the anionic surfactants.

greater the chain length the stronger the binding. A change in the anionic groups of the surfactant gives much smaller effects (Fig. 2).

If more than one class of sites is present and if the corresponding association constants are very different, plots of the changes in line width as a function of moles surfactant per mole HSA would give straight lines (one for each class of sites) with different slopes and intersecting at the equivalence points.[20] These intersections give directly the number of anions bound in each class and from the slopes of the lines the ΔB_i:s (i.e. measures of the binding strength) can be calculated. This idealized behaviour is best approximated in the case of sodium dodecyl sulphate (Fig. 1). For the other anionic surfactants a more careful analysis is needed to prove the existence of more than one class of sites. A more elaborate evaluation will be presented at a later date. Our estimated values of n and ΔB_i from the present data are given in the table. The competitive binding technique gives results in good accordance with those obtained by the equilibrium dialysis technique.[7]

Table. Estimated number of sites (n_i) per HSA unit and corresponding ^{81}Br line width (ΔB_i) in gauss.

Surfactant:	C_4-SO_4Na	C_8-SO_4Na	C_8-SO_3Na	C_7-COONa	C_{12}-SO_4Na
n_i [ΔB_i]:	4 [100]	7 to 8 [185]	6 to 7 [170]	8 to 9 [185]	9[185];85[8]

Acknowledgements.

Thanks are due to Dr. Björn Lindman for his most valuable comments on this work and to Dr. Robert Carter for revising the English.

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