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Nuclear Magnetic Resonance Studies of Chloride Binding to Proteins

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Abstract: The ^{35}Cl NMR relaxation times for chloride ions bound to a number of proteins have been analyzed in terms of a simplified model for internal motion in macromolecules. The model consists of assuming that the chloride is bound to the end of a rigid rod, which is attached to the protein at the apex of a conical hole embedded in the protein. The rod is then free to diffuse about in the conical hole. The results of the analysis show that, with one exception, the data can all be reproduced with the same correlation time for the internal motion and the same quadrupole coupling constant for the bound chloride. The only parameter that varies significantly from protein to protein is the half angle of the conical hole.

Introduction

Proteins as well as other biological macromolecules are not rigid systems but are molecules with a considerable degree of internal flexibility and there is no doubt that the internal mobility phenomena affect the biological function. Currently there is a great deal of interest in these phenomena and important experimental and theoretical approaches have been presented. (See ref 1 for a recent review and references to the literature.)

In recent years, chlorine NMR studies of chloride binding to proteins have been used to investigate a number of interesting properties associated with the proteins.[2] However, the major use of the method has generally been restricted to that of a titration indicator, since the parameters derived from the NMR relaxation of the bound ions (the quadrupole coupling constant, $\chi = e^2qQ/h$, and the correlation time, τ_c) have seemed to vary in an inconsistent manner.

Although it was recognized at an early stage[3] that these variations in the NMR relaxation parameters were probably related to internal motion at the binding site, these effects were not evaluated quantitatively. In this article we will show that the NMR relaxation data from a number of proteins, including some previously unpublished results, can all be explained in a consistent way by means of a simplified model of internal motion.[4] In this model, the chloride ion is attached to the protein via a rod which is free to move about in a conical hole embedded in the protein. By assuming that the correlation times for the motion of the proteins vary from one protein to the next according to the Debye-Perrin[5] model and that the actual χ value for the bound chloride is 3.6 MHz, then we arrive at a reasonably consistent value for the correlation time of the internal motion in these proteins. The only adjustable parameter that varies appreciably from protein to protein is the size of the conical hole, exactly as would be expected.

Furthermore, for pig heart lactate dehydrogenase (HLDH) and rabbit muscle lactate dehydrogenase (MLDH), the introduction of the coenzyme NADH to the proteins results in significant decreases in the size of the hole derived from NMR relaxation data. Since this is in agreement with crystal structure studies,[6,7] it lends support to the interpretation of these parameters as being physically significant.

Experimental Section

The ^{35}Cl relaxation times were measured using a Bruker Bkr-322s spectrometer with homemade external proton field lock. The signals were time averaged with a Varian V-71 computer using a homemade interface and program.

The longitudinal relaxation times were measured with $\pi-t-\pi/2$ pulse sequences and the transverse relaxation times with Meiboom-Gill-Carr-Purcell sequences. In each case the time constants were determined by a linear least-squares fit to a plot of the logarithm of the appropriate magnetization vs. the time. For the transverse relaxation measurements, the fits were terminated after the magnetization had decayed to 20% of its value in the first echo. The standard deviations of these fits were normally less than 3% for the longitudinal relaxation and less than 5% for the transverse relaxation. Each data point used was the average of at least three separate measurements on the same solutions.

In computer simulations of the experimental conditions (see Figure 1), it was determined that these standard deviations are compatible with the linear approximation to the relaxation rates (see below).

With the exception of the measurements on hemoglobin bound chloride,[3] the relaxation rates at a particular site were determined by taking the difference between the measured relaxation rates in the presence and in the absence of a chloride competitive ligand. Since the competitive ligands chosen exclude the chloride from a specific site, the effects due to binding at sites other than that excluded will cancel when this difference is taken.

The alkaline phosphatase was obtained from *E. coli* bacteria and kindly supplied us by Dr. Hedvig Csopak, Department of Biochenistry, University of Gothenburg, Sweden.

The pig heart lactate dehydrogenase and the aldolase were purchased from Sigma Chemical Co., St. Louis, Mo.

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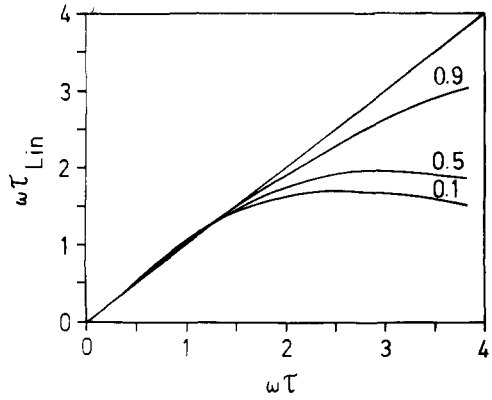

Figure 1. The correlation time obtained with the linear approximation (see text) times the resonance frequency in radians/s, $\omega\tau_{Lin}$, vs. the actual correlation time times the resonance frequency, $\omega\tau$. The numbers associated with each curve correspond to the fraction of $1/T_1$ which is associated with $1/T_{1A}$ (eq 1). The straight line corresponds to the $\omega\tau_{Lin} = \omega\tau$.

NMR Relaxation Equations

The relaxation equations for the longitudinal (along the external magnetic field) and transverse (perpendicular to the external magnetic field) NMR relaxation of a spin $^3/_2$ nucleus involved in chemical exchange have been derived elsewhere.[8] For chloride ions exchanging between an aqueous solution (site A) and a binding site (site B) on a protein, the relaxation is the sum of two exponential functions.[2,8] However, if the time constants associated with the two exponentials do not differ too much from one another, then the relaxation can be approximated as one exponential (the linear approximation), with the associated time constants T_1 and T_2 for the longitudinal and transverse relaxation, respectively. In this case, the net relaxation rates (the difference between total relaxation, and the relaxation in site A) are

$$\Delta\frac{1}{T_1} = \frac{1}{T_1} - \frac{1}{T_{1A}} = p_B\left[\frac{0.2}{T'_{1B} + \tau_B} + \frac{0.8}{T''_{1B} + \tau_B}\right] \quad (1)$$

$$\frac{1}{T'_{1B}} = \frac{1}{10}\left(\frac{e^2qQ}{\hbar}\right)^2 J(\omega) \quad (2)$$

$$\frac{1}{T''_{1B}} = \frac{1}{10}\left(\frac{e^2qQ}{\hbar}\right)^2 J(2\omega) \quad (3)$$

$$\Delta\frac{1}{T_2} = \frac{1}{T_2} - \frac{1}{T_{2A}} = p_B\left[\frac{0.6}{T'_{2B} + \tau_B} + \frac{0.4}{T''_{2B} + \tau_B}\right] \quad (4)$$

$$\frac{1}{T'_{2B}} = \frac{1}{20}\left(\frac{e^2qQ}{\hbar}\right)^2 [J(0) + J(\omega)] \quad (5)$$

$$\frac{1}{T''_{2B}} = \frac{1}{20}\left(\frac{e^2qQ}{\hbar}\right)^2 [J(\omega) + J(2\omega)] \quad (6)$$

where p_B is the probability of finding the chloride at site B, τ_B is the lifetime of the ion at site B, $e^2qQ/\hbar$ is the quadrupole coupling constant, and $J(\omega)$ is the so-called spectral density at the resonance frequency ω ($2\pi \times 8.818$ MHz, in this case). The spectral density is a measure of the effectiveness of the molecular motions in producing transitions at the resonance frequency and is given by

$$J(\omega) = \int_0^\infty C(t)\cos(\omega t)dt \quad (7)$$

where $C(t)$ is the correlation function.

When the lifetime of the ion at site B is very long, then the transverse and longitudinal relaxation rates are the same and the temperature dependence of the net relaxation rates is the opposite of that for the short τ_B case. It has been suggested[9] that this difference can be used to distinguish between binding at metal sites as opposed to nonmetal sites. In any case, for the binding considered here, we have assumed that τ_B is negligibly small compared to the relaxation times at site B (i.e., $\tau_B < 1$ μs), so that τ_B can be dropped from eq 1 and 4. Furthermore, one usually assumes that the correlation function can be described by a single exponential,

$$C(t) = \exp(-t/\tau_{app}) \quad (8)$$

where τ_{app} is the apparent correlation time. With these assumptions, then, the net relaxation rates can be converted into an apparent correlation time and an apparent quadrupole coupling constant, χ_{app}.

Obviously, at a given resonance frequency, τ_{app} and χ_{app} together contain the same information as T_1 and T_2 taken together, since the two sets of quantities can be converted into one another unambiguously via eq 1-8. However, at a fixed resonance frequency, representing the experimental data in terms of τ_{app} and χ_{app} has the advantage that τ_{app} depends only on the molecular motions. Consequently, for a given model of the molecular motions, we can calculate τ_{app} directly without having to consider the quadrupole coupling constants, the binding constants, or the number of binding sites. Furthermore, by representing the data in terms of τ_{app}, we can facilitate a comparison with the hydrodynamic models of motion and more easily determine whether internal motion needs to be considered. But it should be emphasized that these are only apparent quantities which, although useful, are not necessarily physically significant.

In order to investigate the applicability of the linear approximation to the relaxation, we have used the correlation function in eq 8, calculated the full biexponential relaxations, and performed least-squares analyses of the full relaxations to single exponentials. We then used eq 1-8 to recalculate the apparent correlation time and compare it with the one we started with. The results of these calculations are shown in Figure 1.

It can be seen that as long as $\omega\tau \leq 1.5$, the linear approximation is valid. Furthermore, deviations from the correct correlation time were always accompanied by sharp increases in the standard deviation of the fits to single exponentials. Consequently, as long as the relaxation appears to be exponential, the linear approximation should be valid. And this is the case for the chloride relaxation in the presence of the proteins considered here.

Internal Motion at the Binding Site

The model for internal motion to be considered here is where the chloride ion is bound to the end of a rigid rod. The rod is then attached to the protein at the apex of a conical hole embedded in the protein and is free to diffuse back and forth through the hole to the limits imposed by the size of the cone.

The transformation from the laboratory coordinate system to the coordinate system at the nucleus being relaxed can be conveniently broken down into two steps: (a) from the laboratory to the principal coordinate system of the protein and (b) from the protein to the internal rotor or rod. The correlation function associated with (a) involves the usual correlation times for the protein's overall motion and it decays to zero at infinite times. Similarly, the correlation function for (b) involves a correlation time for the internal motion. However, in this case the function does not necessarily decay to zero at infinite time, since the motion is restricted by the walls of the conical hole. Rather it decays to the square of the average value of the transformation matrix associated with (b). Owing to the cylindrical symmetries of the hole and the rod, the only nonzero contribution to this average is $\langle d_{00}(\gamma)\rangle^2 = \langle(3\cos^2\gamma - 1)/2\rangle^2$, where γ is the angle between the symmetry axis of the cone

Table I. Assumed Parameters

protein	dimensions,[a] Å	solution viscosity,[b] cP	$\tau_\perp$,[c] ns	$\tau_\parallel$,[c] ns	number of binding sites/ molecule[d]
hemoglobin	64 × 55 × 506	1.07	32	32	4
human serum albumin	144 × 45 × 22	0.836	77[e]	19[e]	6
alkaline phosphatase	60 × 60 × 60	1.56[f]	58	58	1
horse liver alcohol dehydrogenase	110 × 60 × 45	0.836	62	31	4
lactate dehydrogenase	80 × 70 × 70	0.836	54	54	4
aldolase	171 × 52 × 52	0.836	161	27	4

[a] The dimensions were taken from C. B. Anfinsen, Jr., et al., *Adv. Protein Chem.*, in press, and P. D. Boyer, Ed., "The Enzymes", Academic Press, New York, N.Y., 1970, but the uncertainties are such that these should be taken as assumptions. [b] Viscosities were taken to be those of the corresponding salt solutions: "International Critical Tables," Vol. 5, McGraw-Hill, New York, N.Y., 1929. [c] $\tau_\perp$ and $\tau_\parallel$ are the correlation times for the overall motion of the protein about axes perpendicular and parallel, respectively, to the protein's long axis, assuming the protein to be a symmetric top. [d] The number of Cl^- binding sites was obtained from competition experiments or from information in the literature. Cf. ref 2. [e] Calculated from ref 10. [f] 4 °C.

and the rod and $d_{00}(\gamma)$ is the Wigner rotation matrix element.

Combining the internal and overall correlations for a spherical protein, the correlation function[4] becomes

$$C(t) = (1 - A)\exp(-t(1/\tau_\perp + 1/\tau_i)) + A\exp(-t/\tau_\perp) \quad (9)$$

where $\tau_\perp$ is the overall correlation time for the protein and τ_i is the correlation time for the internal motion. When the hole is a cone with half angle ψ

$$A = \langle d_{00}(\gamma)\rangle^2 = \left[\frac{1}{1-\cos\psi}\int_0^\psi \frac{3\cos^2\gamma - 1}{2}\sin\gamma\, d\gamma\right]^2 = \cos^2\psi \sin^4\psi/[2(1-\cos\psi)]^2 \quad (10)$$

where $\psi \leq 90°$, since we are considering holes embedded in the protein. (This restriction ensures that there is a single value of ψ for each value of A. However, if we allowed $\psi > 90°$, i.e., a rod attached to the apex of a cone protruding from the surface of the protein, then for every value of A corresponding to $\psi > 78°$, there would be three solutions for ψ, one each in the ranges $78° < \psi \leq 90°$, $90° \leq \psi \leq 120°$, and $120° \leq \psi \leq 180°$.)

For a symmetric top protein, the correlation function is somewhat more complicated;[4] however, the principle is exactly the same. The only difference is that two correlation times are needed to describe the overall motion of the protein and one needs to specify the angle between the symmetry axis of the protein and that of the conical hole, θ. The result taken from ref 4 is

$$C(t) = \sum_{k,r}[(d_{kr}(\theta))^2\langle(d_{r0}(\gamma))^2\rangle\exp(-t(1/\tau_k + 1/\tau_i))] + A(1 - \exp(-t/\tau_i))\sum_k[(d_{k0}(\theta))^2\exp(-t/\tau_k)] \quad (11)$$

where θ and A are defined above and the τ_k is the overall correlation time,

$$\frac{1}{\tau_k} = \frac{(6 - k^2)}{\tau_\perp} + \frac{k^2}{\tau_\parallel} \quad (12)$$

where $\tau_\perp$ and $\tau_\parallel$ are the correlation times for the protein motion about axis perpendicular and parallel to the protein's symmetry axis.

When eq 9 or 11 is put into eq 1-7, we find that the apparent correlation time and apparent quadrupole coupling constant obtained by assuming eq 1-8 vary considerably depending upon the values of ψ and τ_i. Consequently, if we know $\tau_\perp$, $\tau_\parallel$, and the actual quadrupole coupling constant, then we can use τ_{app} and χ_{app} (or T_1 and T_2) to determine ψ and τ_i. (Of course, the results are ambiguous if we do not know both the overall correlation times and χ, but they appear to be unambiguous when both are known and when τ_{app} and/or χ_{app} are affected by the internal motion.)

A Consistent Explanation

In order to investigate whether the various apparent correlation times and quadrupole coupling constants obtained from chloride NMR relaxation measurements can be given a consistent explanation, we need to make some assumptions about the overall correlation times and the actual quadrupole coupling constants.

We believe that reasonably reliable values for the overall correlation times of bovine serum albumin (BSA) have been obtained from dielectric relaxation measurements.[10] (These values differ from the predictions of the Debye-Perrin[5] theory by about 25%, probably as the result of a water shell that makes the protein's effective dimensions slightly larger than its actual dimensions.) Using these experimental correlation times for BSA as a base, we have calculated the overall correlation times for each of the proteins by assuming that they vary from one protein to another according to the Debye-Perrin model.[5] In each case we have taken the viscosity of the solution to be that of a 0.5 M KCl solution. (See ref 2 for a discussion of the effects of proteins on the solution's viscosity.) The results of these calculations, together with other parameters used in our calculations, are shown in Tables I and II.

In order to demonstrate the importance of the actual quadrupole coupling constant in these calculations, we have calculated a series of fits to the apparent correlation times for chloride bound to HSA at temperatures from 5 to 50 °C.[11] The overall correlation times were calculated as described above, and it was assumed that the symmetry axis of the conical hole is perpendicular to the molecular symmetry axis and that the ratio of the internal to overall correlation times is independent of the temperature. The results of these calculations are shown in Figure 2.

When there is no internal motion ($\tau_i \gg \tau$ and/or $\psi = 0$ in eq 11), then $\tau_{app} > 0.4\tau_\perp$ for this molecule, depending upon the value of θ. Consequently, the most obvious feature of Figure 2 is that the data cannot be explained if we assume that the

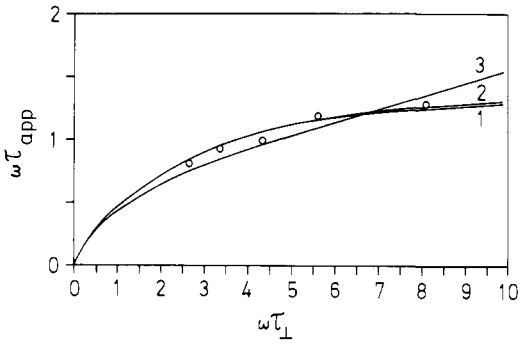

Figure 2. The apparent correlation time multiplied by the resonance frequency, $\omega\tau_{app}$, vs. $\omega\tau_\perp$ obtained as described in the text. The curves correspond to the theoretical relation with (1) $\tau_\perp/\tau_i = 10^4, \psi = 86°$, (2) $\tau_\perp/\tau_i = 113, \psi = 63.5°$, and (3) $\tau_\perp/\tau_i = 17, \psi = 48°$C. The circles correspond to the experimental data for HSA-bound chloride (ref 11) at pH 7.2 and temperatures from 5.8 to 50.4 °C. The value of τ_{app} only depends on the ratio of the transverse to longitudinal relaxation times and is therefore independent of χ. But if we introduce the magnitude of the relaxation times, then the χ values that are calculated with the above parameters correspond to (1) 1.9, (2) 3.6, and (3) 23 MHz at 28 °C.

Table II. Experimental Parameters

protein	protein concn, μM	Cl^- competitive ligand	$\Delta(1/T_1)$,[a] s^{-1}	$\Delta(1/T_2)$,[a] s^{-1}
hemoglobin[b]	*b*	none	*b*	*b*
human serum albumin[c]	73	dodecyl sulfate	43	98
alkaline phosphatase[d]	263	orthophosphate	20	28
horse liver alcohol dehydrogenase[e]	150	$Pt(CN)_4^{2-}$	78[f]	111[f]
pig heart lactate dehydrogenase[g]	31	oxamate	12	13
pig heart LDH-NADH complex[g]	31	oxamate	21	46
rabbit muscle lactate dehydrogenase[h]	60	oxamate	14	21
rabbit muscle LDH-NADH complex[h]	60	oxamate	15	47
aldolase[g]	31	fructose 1,6-diphosphate	16	32

[a] The difference between the relaxation rate in the absence and in the presence of the competitive ligand. All values are for 0.5 M KCl solutions at 28 °C except as indicated. [b] Reference 3, 2.5 M NaCl. [c] Reference 11. [d] Present work, 4 °C. [e] Reference 14. [f] Extrapolated to infinite $Pt(CN)_4^{2-}$ concentration; see ref 14. [g] Present work. [h] Reference 15.

binding site is rigidly attached to the protein. However, with the introduction of internal motion, the data can be reproduced for a wide range of internal correlation times. (The fits in Figure 2 correspond to internal correlation times varying from 5 ns to 8 ps at 28 °C, and these parameters, together with the magnitude of the relaxation times, produce values of χ varying from 2 to 23 MHz at this same temperature.) These fits enable us to say that $\chi < 23$ MHz and $\tau_i < 5$ ns in terms of this theory, but no more. Clearly, then, an independent measure of the actual χ value is necessary in order to determine the internal correlation time.

Since we do not know of any independent determination of the quadrupole coupling constant for a chloride ion bound to a positively charged side chain of an amino acid, we have used

Table III. Derived Parameters

protein	τ_{app}, ns	χ_{app},[a] MHz	ψ, deg	internal correlation time, τ_i, ns
hemoglobin	8	2.2[b]	70[b]	1.9[b]
human serum albumin	19	1.6	64	0.7
alkaline phosphatase	8	1.4	79	0.7
horse liver alcohol dehydrogenase	9	1.7	73	1.1
pig heart lactate dehydrogenase	2[b]	3.6[b,c]	90[b]	2.1[b]
pig heart LDH-NADH complex	17	3.3[d]	53	6.7
rabbit muscle lactate dehydrogenase	10	1.6[c]	74	1.0
rabbit muscle LDH-NADH complex	26	2.1[d]	57	1.2
aldolase	16	1.8	69	1.1

[a] Calculated assuming that the binding constant for the chloride $K \gg 1$, except as indicated. [b] Probably large error; see text. [c] Assuming that the chloride binding constant $K = 1.5\ M^{-1}$, derived from measurements on 0.5 and 0.15 M KCl solutions with pig heart LDH. [d] Assuming that the chloride binding constant $K = 2.3\ M^{-1}$, derived from measurements on 0.5 and 0.15 M KCl solutions with the pig heart LDH-NADH complex.

Cohen and Reif's[12,13] electrostatic model to estimate χ. For a chloride ion bound to a NH_3^+ group, we have estimated $\chi = 3.6$ MHz. This value differs from the estimate for a guanidium group of 1.4 MHz (the value given in ref 14 is wrong by a factor of 2 owing to a computational error). But none of these estimates can be taken too literally, since a number of important parameters used, such as the dielectric constant of the medium, are not well known. However, since internal motion results in a $\chi_{app} \leq \chi$, regardless of the theory used, then it is necessary to assume that $\chi \geq 3.6$ MHz if we are to produce a consistent explanation for all of our data (see pig heart lactate dehydrogenase in Table III).

Assuming, then, that $\chi = 3.6$ MHz for all of the bound chloride ions studied, we have converted the experimental relaxation times into the values of ψ and τ_i shown in Table III, with the corresponding τ_{app} and χ_{app} given as a reference. (For nonspherical molecules, we have assumed that the axis of the cone is perpendicular to the molecular long axis.) Obviously, these values of ψ and τ_i cannot be taken too literally, since it was necessary to assume a value for χ. Furthermore, since the actual cavities at the binding sites are probably not conical and the side chains binding the chloride ions are not rigid rods, ψ is at best a semiquantitative measure of the extent of the internal motional freedom.

Nevertheless, it can be seen that ψ seems to be well behaved, changing from 90° to 53° in HLDH and from 74° to 57° in MLDH when these molecules are bound with NADH near the probable chloride binding sites. Furthermore, with the exception of hemoglobin, HLDH, and the HLDH-NADH complex, all of the internal correlation times are about 1 ns.

In hemoglobin the relaxation times were not measured in the presence of a chloride competitive ligand. Consequently, the apparent quadrupole coupling constant is probably too large, and with a smaller χ_{app}, the internal correlation time would also be smaller (compare alkaline phosphatase).

In HLDH the apparent correlation time is so small that it cannot be determined accurately. And within the error of τ_{app} and the experimental error in the net relaxation rates, this internal correlation time is also consistent with the value of 1 ns.

Thus the only deviant internal correlation time is that for the HLDH-NADH complex. However, since the crystal structure studies on this complex[6,7] show that the probable binding site[15] (the guanidinium group of arginine-109) is severely restricted by the presence of NADH, then this may reflect an actual difference.

Although it is not profitable to try to interpret the individual results until the actual quadrupole coupling constants can be determined, these calculations do show that the chloride binding studies can be given a consistent explanation in terms of internal motion at the binding sites.

Conclusion

We have shown that the chlorine relaxation data for chloride binding to several proteins can be explained in a consistent way by taking into account the effects of internal motion at the binding sites. When the actual quadrupole coupling constant for the bound ion is known, the measured NMR relaxation times can be converted into two parameters that are a measure of the extent and speed of the internal motion. And with this conversion, NMR studies of ion binding to macromolecules should prove to be a powerful tool for investigating the internal motions of charged side groups in macromolecules.

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Alkali Ion Binding to Aggregates of Amphiphilic Compounds Studied by Nuclear Magnetic Resonance Chemical Shifts

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Abstract: The NMR chemical shifts of $^{23}Na^+$, $^{35}Cl^-$, and $^{133}Cs^+$ counterions were investigated for a range of amphiphilic systems. From variable concentration studies, the intrinsic shifts, δ_m, of counterions bound to the micelles were deduced. The δ_m values are informative as regards the interactions of the counterions. From the detailed studies of δ_m of $^{23}Na^+$ it is found that counterion binding does not change appreciably with the alkyl chain length but varies very sensitively with the polar head group. The shielding of Na^+ at the micellar surface follows the sequence $-OSO_3^- >$ aryl-$SO_3^- >$ alkyl-$SO_3^- > -CO_2^-$ and this is interpreted in terms of Na^+-hydration water overlap and head group induced polarization of the Na^+ ion's hydration sphere. Chemical shifts were also found to be valuable for studies of other phases, i.e., lyotropic liquid crystals and reversed micellar solutions. For the system sodium octanoate–octanoic acid–water, a specific complex formation is inferred for the water-poor solution phase. The difference in an ion's chemical shift between H_2O and D_2O solutions is found to be useful for probing into the hydration of counterions in amphiphilic systems. The $^{133}Cs^+$ counterion is found to retain its inner hydration sphere up to high concentrations of cesium octanoate, while in the reversed micellar solutions of cesium octanoate and water in octanoic acid, the dehydration can be followed in detail. The significance of counterion water contact down to rather low water contents is suggested by these results. The chemical shift studies were complemented with investigations of the $^{23}Na^+$ quadrupole relaxation rates.

Introduction

The interest in ionic interactions in systems of synthetic and biological amphiphiles, the latter serving as models of biological membranes, has grown markedly in recent years. To study alkali ion binding to micellar aggregates and liquid crystalline phases of different types, both the quadrupole relaxation[1,2] and the quadrupole splitting methods[3-5] were introduced and these techniques are currently used by several groups.[6-11] Some time ago we proposed the study[12] of NMR chemical shifts of ions as a complement to these methods and presented an initial study giving inter alia some insight into the specificity of ion binding to micellar aggregates.[13] In the present report, we propose to present a systematic study mainly of $^{23}Na^+$ chemical shifts in solutions of a large number of

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