^{35}Cl NMR STUDY OF THE INTERACTION OF CHLORIDE WITH ARGININE, HISTIDINE AND LYSINE

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1. Introduction

It is becoming increasingly clear that much can be learned about the mechanism of action of biological macromolecules from studies of the binding of properly selected ligands. In the field of protein chemistry it is well established that anion binding plays a rather general functional role [1,2]. For enzymes, this binding may take place at a protein-bound metal ion like Zn^{2+}, but it is more usual that the interaction takes place at non-metallic binding sites. In the latter case, the interaction of the anion with the positively charged amino acid residues, i.e., arginine, lysine and histidine, as well as with the amino-terminal, has to be taken into consideration; but as dispersion forces may be very important the influence of other groups should also be considered [3].

In view of the rather general functional significance of anion binding to proteins, it seems worth while to attempt to elucidate in detail the interactions involved. In so doing it is natural to compare the results obtained for proteins with those of simple well-defined reference systems where the interactions involved can be analyzed in detail. If in chosing suitable model systems, we may imitate specificity and affinity of ligand binding sites of complex biological macromolecules we have a basis for advancing hypotheses as regards interactions mechanisms.

Ligand–macromolecules interactions may be efficiently studied by a variety of nuclear magnetic resonance methods [4]; and for the case of protein–anion interactions, halide-ion quadrupole relaxation studies have been applied extensively [5]. In the communication we present a ^{35}Cl quadrupole relaxation investigation of Cl^- interaction with the three amino acids possessing positively charged side-chains, i.e., Arg, His and Lys. As will be demonstrated this method is well adapted for elucidating anion–amino acid interactions; and, on the basis of variable pH studies, the influence of different groups on the interaction can be discerned.

A second objective of the present study was to introduce the use of halide-ion NMR shielding in connection with the problem of anion binding to proteins. It has already been reported that the alkali ion binding in various colloidal systems may be successfully monitored by determining the shielding [6–9]. We have now carried out a detailed study of the ^{35}Cl chemical shifts of Cl^- in the presence of amino acids. The results demonstrate that anion–amino acid interactions may be conveniently studied in this way; and furthermore, the results show considerable promise for the use of this method in studies of peptides and proteins.

2. Experimental

The measurements were performed on a modified Varian XL-100 NMR spectrometer using the Fourier transform technique. The chemical shifts were measured with a 0.5 M NaCl solution as external reference but have been recalculated to 1.0 M NaCl solution reference in the presentation of the data. (No correction for differences in macroscopic susceptibility is necessary.) The line widths were taken as the width at half-height of the absorption peak and are related to the transverse relaxation rate ($R_2 = T_2^{-1}$)

through $\Delta\nu = R_2/\pi$. The error in both line widths and chemical shifts is estimated to be ± 1 Hz or less. All the measurements were performed at 29 ± 1°C.

The chemicals used were of analytical grade, the amino acids purchased from Sigma and the other chemicals from Merck. The total chloride concentration was kept constant at 1.0 M. pH-Values were measured to better than 0.1 pH unit and was changed by adding small amounts (dilution effects negligible) of a concentrated NaOH solution. The effect of the additional Na^+ is negligible in the case of the chemical shifts while it very slightly suppresses the observable line-narrowing [5].

3. Results and discussion

The ^{35}Cl NMR chemical shifts (δ) and transverse relaxation rates ($R_2 = T_2^{-1}$) were determined as a function of pH in the range 2–11 for solutions containing NaCl and either arginine, histidine or lysine. Furthermore, a number of other systems were studied for comparison.

In fig.1 we present the $^{35}Cl^-$ chemical shift for histidine solutions and, for comparison, the results for solutions containing imidazole. As can be seen, the ^{35}Cl chemical shift changes considerably in the pH-ranges corresponding to the titration of the

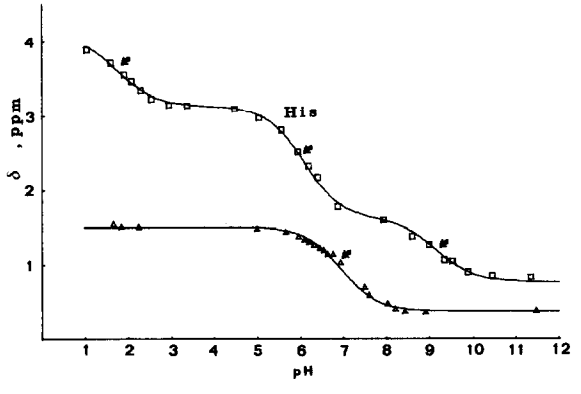

Fig.1. The pH-dependence of the $^{35}Cl^-$ chemical shift (δ) of aqueous solutions containing 1.00 M NaCl and 0.50 M histidine (□) or imidazole (△). The shifts are given with a 1.00 M NaCl solution as reference and with a positive δ denoting a downfield shift. Arrows indicate pK_a-values of the different titratable groups.

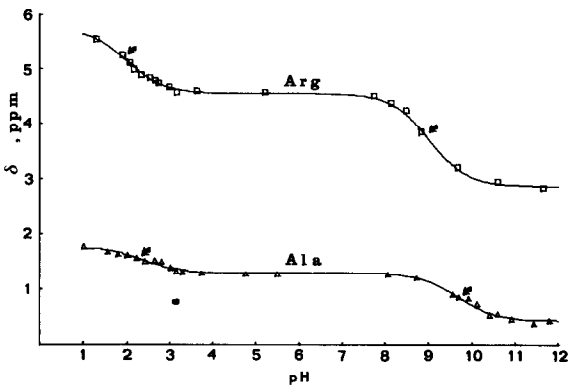

Fig.2. The pH-dependence of the $^{35}Cl^-$ chemical shift (δ) of aqueous solutions containing 1.00 M NaCl and 0.50 M arginine (□) or alanine (△). The shifts are given with a 1.00 M NaCl solution as reference and with a positive δ denoting a downfield shift. Arrows indicate pK_a-values of the different titratable groups.

different groups. (For comparison we have indicated the different pK_a-values taken from the literature [10] by arrows in figs 1–4.) In fig.2, the ^{35}Cl chemical shifts for solutions containing arginine are given with data for alanine as a reference. Again the titration of the $-COOH$ and $-\overset{+}{N}H_3$ groups is accompanied by appreciable chemical shift changes. (The high pH-region where the pK_a of the guanidinium group of arginine is located was studied in less detail. Although these results are more complex since effects other than the Cl^-–amino acid interaction influence the chemical shift at extreme pH-values, it could be established that around $pK_a = 12.48$ there is a distinct effect.) Data for lysine solutions are presented in fig.3 together with results for the reference compounds 4-aminobutyric acid and *n*-butylamine. In accordance with the other cases, pronounced changes in δ around the different pK_a-values are observed.

The effect of arginine, histidine and lysine on the ^{35}Cl transverse relaxation is depicted in fig.4 as a function of pH. As may be inferred the behaviour is analogous in the three cases in two respects, i.e., firstly deprotonation of the $-COOH$ group leads to a marked decrease in R_2 and secondly deprotonation of the α-NH_3^+ has only a small effect on the ^{35}Cl relaxation. An interesting difference is that deprotonation of the side-chain is clearly reflected in the

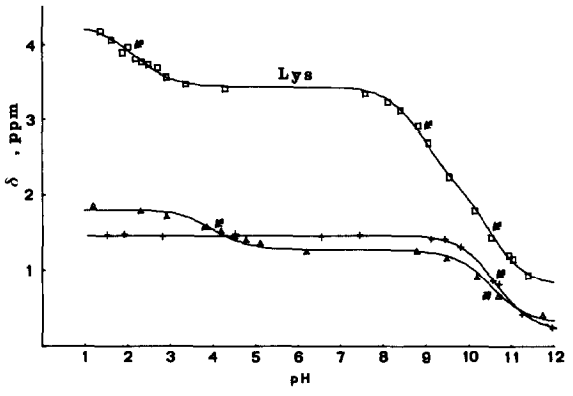

Fig.3. The pH-dependence of the $^{35}Cl^-$ chemical shift (δ) of aqueous solutions containing 1.00 M NaCl and 0.50 M lysine (□), 4-aminobutyric acid (△) or *n*-butylamine (+). The shifts are given with a 1.00 M NaCl solution as reference and with a positive δ denoting a downfield shift. Arrows indicate pK_a-values of the different titratable groups.

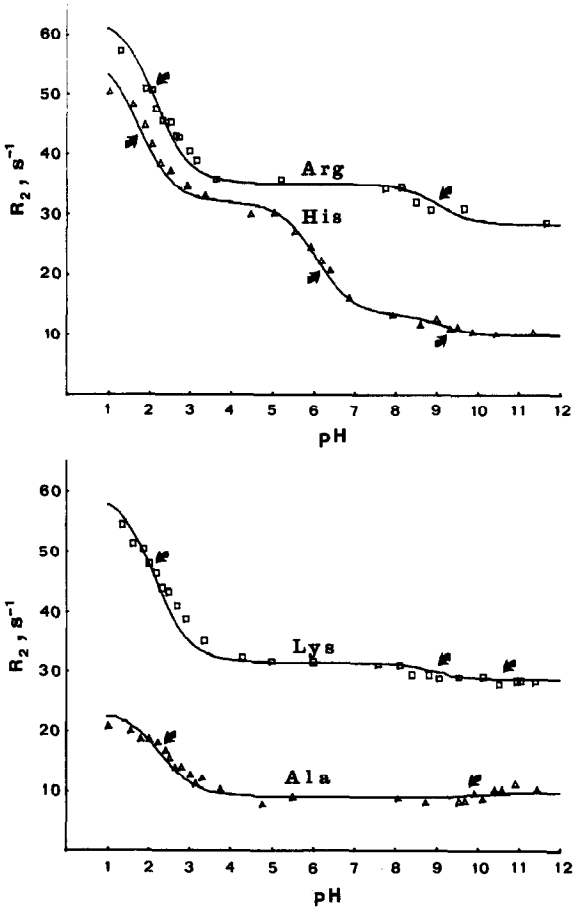

Fig.4. The pH-dependence of the $^{35}Cl^-$ transverse relaxation rate ($R_2 = T_2^{-1}$) of aqueous solutions containing 1.00 M NaCl and 0.50 M arginine (□), lysine (□), histidine (△) or alanine (△). The results are given as excess relaxation rates (R_{2ex}), i.e., as the difference of the observed relaxation rate and that of a 1.00 M NaCl solution.

relaxation rate for histidine and to a smaller extent for arginine (not shown in fig.4) while within our experimental error there is no effect for lysine. It should be remarked in connection with the presentation of the experimental data that in the absence of aminoacid, no pH-dependence of either R_2 or δ is observed in the range presented.

The magnitude of the changes in $^{35}Cl^-$ chemical shift or relaxation rate due to a certain Cl^-–amino acid interaction is determined by the binding constant characterizing the interaction as well as on the intrinsic ^{35}Cl shielding or relaxation rate of the complex [5]. It is now generally accepted that the shielding of alkali and halide ions depends mainly on the overlap between the outer orbitals of the ion and outer orbitals of surrounding ions for molecules and that species in direct contact with the ion studied have a dominating influence [5]. For the quadrupole relaxation of monoatomic ions, the electrostatic approach of Hertz [10,11] has been found to successfully account for experimental results in a wide range of situations [5]. In this theory, the cause of relaxation is the time-modulated field gradients from ionic point charges and molecular point dipoles which perform a translational or rotational motion with respect to the studied ion. The rate of relaxation increases with increasing magnitude of the field gradient as well as of the correlation time of the motion. One important result of Hertz's is that, when the ion is in an environment of cubic or near-cubic symmetry the field gradient may be totally or partly quenched.

For the rapid exchange conditions applicable in the present case, the observable chemical shifts (δ) and transverse relaxation rates (R_2) will constitute population-weighted averages of the different environments occupied by the chloride ion. Under the conditions of figs 1–4 it was established that only a small

fraction of the Cl^--ions is bound to the amino acids. It is then suitable to examine the excess quantities, δ_{ex} and R_{2ex}, respectively, which are defined as the differences of the observed values and those of the corresponding solutions without amino acid. To rationalize the results, the change with pH of the concentration of a certain group binding Cl^- as well as interactions between different groups must be taken into account. The following formula for the chemical shift is obtained:

$$\delta_{ex} = \Sigma\, p_i\, \delta_i = \Sigma\, \frac{b_i\, \delta_i}{1+10^{pH-pK_{ai}}} \qquad (1)$$

and a completely equivalent equation applies for R_{2ex}. Subscript i denotes a particular Cl^- binding site on the amino acid where a fraction p_i of the Cl^--ions is found. $b_i = b_i\,(A_T, Cl_T, K_i)$ where A_T and Cl_T are the total concentrations of amino acid and Cl^-, respectively, and K_i the binding constant characterizing site i. The curves of figs 1–4 constitute least-squares fits to eq. (1) using pK_a values taken from the literature [10]. As can be seen, the experimental results are very well represented by the model adopted and thus a separation of the contributions from different groups may be possible. Studies at different Cl^- and amino acid concentrations directed towards the determination of δ_i or R_{2i} and K_i are in progress. As indicated by the results in figs 1–3 a fruitful way of understanding these quantities will be by the investigation of suitable reference systems.

Although we will postpone a more elaborate analysis and interpretation of these data to a later date, we can conclude that both the ^{35}Cl chemical shift and relaxation rate are most appropriate for monitoring the interactions between chloride ions and amino acids. From the chemical shift data it is evident that all the titratable groups markedly influence the interaction. No chemical shift study of Cl^--binding to proteins has yet been reported, the main reason being that for proteins one is greatly hampered by the considerable line-broadening. The present results indicate that attempts will be worth while and that at least studies of low molecular weight proteins and peptides at high magnetic fields should be informative. From the magnitude and pH-dependence of the chemical shift of a certain protein anion binding site, significant information on the protein–ligand interaction should be obtainable via a comparison with the results for aminoacid solutions.

For the ^{35}Cl relaxation rates, a comparison between results for proteins and model systems is made difficult due to difficulties in accounting for the correlation time. As regards the relaxation rates an important observation is that the $-\overset{+}{N}H_3$ group produces only small effects, the effect decreasing in the series Arg > His > Lys > Ala for the α-$\overset{+}{N}H_3$ group. Possibly by hydrogen-bonding this group may fit into the water structure in such a way that an approximately symmetric Cl^--surrounding is formed. This symmetry may be destroyed by adjacent groups, since substitution on the nitrogen may lead to an appreciably increased relaxation rate.

A detailed account of $^{35}Cl^-$ NMR in the presence of amino acids will be presented later as well as of studies in progress on the interaction of Cl^- with suitably chosen small peptides as well as with polypeptides.

Acknowledgements

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