A MODEL TO DESCRIBE BINDING DIFFERENCES BETWEEN CALCIUM AND THE LANTHANIDES IN BIOLOGICAL SYSTEMS

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Received 11 February 1974

1. Introduction

Through the use of the ultrasonic relaxation technique, it has been demonstrated that significant concentrations of inner-sphere lanthanide complexes exist in regions where the local dielectric constant is less than that of water. Under the same conditions calcium will not form these complexes. Therefore, lanthanide binding to a biochemical molecule at the calcium binding site will not occur if the region surrounding this site has a dielectric constant lower than that of water and if the cation size is crucial.

The chemical similarity of calcium and the rare earths, coupled to the latter's ease of spectroscopic observation, has led to isomorphous lanthanide substitutions to detect calcium binding sites in α-amylase [1], thermolysin [2] and bovine serum albumin [3,4]. Both types of cations have also successfully activated the conversion of trypsinogen to trypsin [5]. In some cases the lanthanides appear not to bind to a molecule that accepts calcium, and in the case of α-amylase the region of lanthanide concentration which leads to binding at the calcium site has been demonstrated to be a function of which buffer system was utilized [6,7]. Since calcium substitution by the lanthanides is becoming an important biochemical tool, it is important to determine under what experimental conditions the cations are similar or equivalent.

We have previously demonstrated that in aqueous solution the mechanism of lanthanide association occurs via a multistep process [8] involving cation-ligand bond formation as the rate-determining step [9]. Ultrasonic relaxation investigations in aqueous solutions have demonstrated that the lanthanides form both inner- and outer-sphere ion pairs with nitrate [10–13] and sulfate [9,11,13,14] but essentially only outer-sphere complexes with chloride [15]. When the solvent dielectric constant is lowered by repeating the ultrasonic measurements in aqueous alcohols, the fraction of inner-sphere nitrate complexes is increased and chloride forms inner-sphere complexes below a water mole fraction of 0.85 (solvent dielectric constant of 68 [16]). A surprising result has been obtained when the tetrahedral perchlorate ion is the ligand [17]. This ligand has traditionally been used as the anionic form of 'inert' electrolytes, with the assumption being made that no binding occurs between the cation and perchlorate in solution. The existence of inner-sphere erbium perchlorate complexes at water mole fractions below 0.9 (dielectric constant of 71) indicates that as the dielectric constant decreases below that observed in water, the lanthanide ions will tend to complex with any anions which are in near proximity.

Since the dielectric constant of the environment in the region of the calcium binding sites need not resemble that of a simple ligand in water, it is essential to determine if calcium and the lanthanides react similarly as a function of dielectric constant. We have chosen to examine the association reaction between calcium and perchlorate to determine if differences between the cations exist as a function of solvent composition and dielectric constant.

2. Experimental

A stock solution of $Ca(ClO_4)_2$ was prepared in water by adding perchloric acid to $CaCO_3$ and heating to remove the CO_2 produced. The solutions were made up to a final concentration of 0.200 M salt by dilution with the correct proportions of distilled water and methanol. The ultrasonic relaxation equipment and technique are similar to those described before [8,17]. The $Ca(ClO_4)_2$ was insoluble in the aqueous methanol below 40% water by volume. One solution was studied which was 0.100 M in the salt at a water fraction of 32%.

3. Results

If a chemical reaction exists for a system and if the characteristic relaxation time is within the frequency range of the ultrasonic equipment, then the ultrasonic absorbance increases over that observed for the background absorbance of the non-reacting solvent system alone. At a given frequency, this difference is called the excess absorbance, μ. The variation of μ as a function of frequency fits a standard curve for a single relaxation process, and systematic deviations at the high or low frequency ends are usually attributed to the existence of at least one additional relaxation process.

The ultrasonic relaxation data obtained for all of the solutions containing calcium and some methanol exhibited a small, but measurable excess absorbance within the frequency range of 10 to 210 MHz. Fig. 1 shows this excess absorbance as a function of frequency for 0.200 M Er(III) (17) and 0.200 M Ca(II) perchlorates at water mole fraction =0.58 and dielectric constant =52. The $Er(ClO_4)_3$ data cannot be fit by a single relaxation curve, and the experimental data are represented as the sum of the overlapping relaxations [17]. The high frequency peak is attributed to outer-sphere erbium perchlorate complexes and the low frequency peak to the inner-sphere ones [17]. The relaxation amplitude is a measure of the ion pair concentrations times the square of the volume change for a reaction step (assuming ΔH is small). The calcium data are interpreted in terms of only a single relaxation process, and from the relaxation frequency of 154±11 MHz, this process corresponds to the formation of the outer-sphere complex. The three lowest data points for calcium correspond to systematic deviations from the single relaxation curve and may indicate the existence of some inner-sphere complex whose relaxation frequency is lower than the experimental frequency range. Thus, at the lowest water mole fractions only, there may exist some inner-sphere calcium perchlorate complex. To check on this we reduced the calcium concentration to 0.10 M to permit solubility and the water content was decreased. The result was an increase in the relative amount of outer-sphere ion pair, and once again a low frequency tail was present. However, at water compositions of 50% or more, only one relaxation was present, and this is attributed to the existence of outer-sphere complexes. The relaxation data are summarized in table 1.

These data indicate that the chemistry of the lanthanide ions is fundamentally different from that of calcium when the dielectric constant is less than that for water. Under these conditions a lanthanide ion will exhibit non-specific binding with counter ions or polar regions of the macromolecule. Although lanthanide ions tend to bind through oxygen atoms

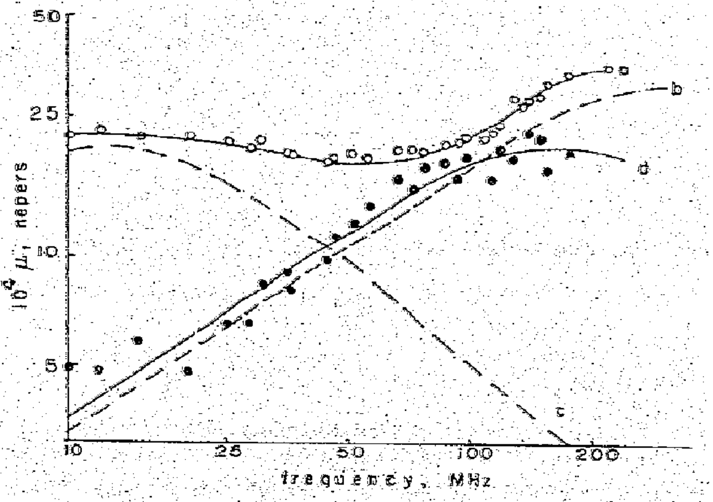

Fig. 1. Ultrasonic relaxation data for 0.200 M $Er(ClO_4)_3$ and 0.200 M $Ca(ClO_4)_2$ at a water mole fraction of 0.58 and dielectric constant of 52. a. Experimental data for $Er(ClO_4)_3$. b. The calculated curve corresponding to outer-sphere $Er\text{-}ClO_4^{2+}$ complexes. c. The calculated curve corresponding to inner-sphere $Er\text{-}ClO_4^{2+}$ complexes. d. The calculated curve for $Ca(ClO_4)_2$, which corresponds to the formation of outer-sphere complexes.

Table 1
Summary of relaxation results[a]

Water mole fraction	5×10^{-5}	0.20	0.36	0.50[e]	0.58	0.69	0.79[f]	1.00
Dielectric[b] constant	33	37	43	49	52	58	64	78.5
Relaxation data for outer-sphere complexes								NO EXCESS
Ca Amplitude[c]	Not soluble			17±1	19±1	13±0.4	11±3	
Ca Frequency[d]				108±9	154±11	132±9	122±36	
Er Amplitude[c]	38±1	21±1	21±1	–	15±1	12±1	–	
Er Frequency[d]	324±30	358±56	269±54	–	290±50	217±33	–	
Relaxation data for inner-sphere complexes								ABSORBANCE
Ca Amplitude[c] Ca Frequency[d]	Not soluble			No inner-sphere complexes[g]				
Er Amplitude[c]	289±17	1180±37	736±28	–	231±7	165±6	–	
Er Frequency[d]	9.8±0.5	7.3±0.2	8.8±0.3	–	12.1±0.4	12.3±0.5	–	

Notes

[a] The background [illegible] to be 25×10^{-17} nepers sec^2/cm. The salt concentration is 0.200 M.

[b] Reference 16.

[c] Units are 10^{17} nepers sec^2/cm.

[d] Units are MHz.

[e] Calcium perchlorate concentration is 0.100 M.

[f] Calculated Background is $(20.7 \pm 2.9) \times 10^{-17}$ nepers sec^2/cm.

[g] The magnitude of the relaxation frequency is too high for the formation of calcium inner sphere complexes in aqueous methanol. The frequencies should be equal to or less than those expected in water for inner sphere complexation.

[18], binding also occurs through other donors [19]. If the lanthanide binds to the counter ion in solution before attachment to the biochemical binding site, the reactive ion will be larger than Ca(II) and the electrostatic charge will be the same or less. Although this will not prevent lanthanide binding in some systems, it will for those in which the cation size determines if binding is possible at a given site. Under comparable conditions, calcium will not form these inner-sphere complexes, and hence the binding of the smaller solvated calcium ion will not be hindered at these sites. The binding of the lanthanide ion, but not calcium, is consistent with the observations on α-amylase where Lu(III)-activation was found to occur under different conditions depending upon whether the lanthanide ion was associated with the buffer anion [5–7].

Thus we conclude that if the effective environment surrounding the binding site is somewhat hydrophobic, then a lanthanide ion cannot bind. If the site is hydrophilic, both types of cations can bind, and due to the increased charge, tighter lanthanide binding can be expected.

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