Molecular Dynamics and NMR Studies of Proteins in Ionic Solutions

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SUMMARY. The molecular dynamics of water and selected ions was studied in concentrated electrolyte solutions with, or without, proteins added. Our experimental results by multinuclear spin relaxation techniques were then compared with molecular dynamics computations for water and ions in concentrated electrolyte solutions. The mechanisms for the anionic and cationic interactions with myofibrillar proteins in aqueous solutions were investigated by nuclear magnetic resonance over a wide range of salt concentrations. The multinuclear spin relaxation data were analyzed with a thermodynamic linkage model of hydrated ion clusters of various sizes and composition. Protein amide groups were found to bind to anions with strengths in the order of the lyotropic series.

Introduction

Ion-specific effects were observed[1)] in concentrated electrolyte solutions containing proteins, such as lysozyme, myosin or soy globulins, that were analyzed with thermodynamic linkage models involving reversible binding of the appropriate, hydrated ion clusters.

Molecular Dynamics Computations

All initial structures were built using the Sybyl-Mendel molecular modeling program, and were then minimized utilizing Kollman and Tripos force-field computations. Energy minimization and molecular dynamics of the coiled/coil region of tropomyosin G resulted in a nonclassical screw-type helix (but no α-helix).

All simulations are carried out on a Unix-based Silicon Graphics workstation by employing Tripos force-field calculations for 200 molecules, with uniform boundary conditions. Molecules approached Van der Waals contact after energy-minimization up to 50 ps in 1-femtosecond steps. Molecular dynamics of concentrated solutions of proline in water show the presence of a stable proline octamer cluster with four water molecules trapped within the cluster.

The NMR relaxation data analysis by nonlinear regression with a thermodynamic linkage model, according to Wyman's theory of linked functions, allowed the determination of degree of cooperativity and apparent binding constants for ion binding to myosin and soy globulins.

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Molecular dynamics[2-12] and multinuclear spin relaxation measurements were carried out on several proteins in order to elucidate the mechanisms for the cationic and anioinic interactions with protein binding sites. Specific interactions of the anions with positively charged side chain groups of lysine, arginine and hystidine, as well as nonspecific binding to amide groups were found. Both anion and cation specific and cooperative interactions with charged side chain groups were found for myosin and tropomyosin, as well as for 7S and 11S globulins from soy.

Markedly nonlinear dependences of the ^{17}O and ^{23}Na NMR transverse relaxation rates on salt concentration were analyzed with a thermodynamic linkage model of salt-dependent solubility and hydration (ligand-induced association model), according to Wyman's theory of linked functions. nonlinear regression analysis of both ^{17}O and ^{23}Na data suggested cooperative, reversible binding of hydrated ions to myofibrillar proteins. Both ions and water were found to exchange fast, on the NMR timescale, between the binding sites of the myofibrillar proteins and the aqueous solution. At sodium chloride concentrations higher than about 0.1 grams salt/gram water, ion activities have marked effects upon the NMR relaxation rates of ions and water. A salt activity model allowed quantitative fitting of the NMR data at high salt concentrations. The effect of neglecting the ion activity in solutions of myofibrillar proteins was also estimated and compared with the ligand-induced, cooperative association model for myofibrillar proteins. The comparison between the ^{17}O and ^{23}Na results (Table 1) strongly suggests that water is exchanged as the hydrated ion species between the myofibrillar and/or myosin protein binding sites and the bulk, aqueous solution of electrolytes.

Myosin solubility and self-association were also found to be thermodynamically linked to ion-binding. Hydrophobic interactions between the tail parts of the myosin molecules are presumably the major cause of myosin self-association in the absence of salt. In addition, ionic interactions, mainly between carboxyl and ammonium or imidazolium groups, are also likely to be involved in myosin dimerization; dimer formation decreases myosin hydration, as observed in the ^{17}O NMR experiments. The heterogeneity of myosin B is reflected in its large, second virial coefficient and appears to be correlated with larger aggregate sizes and higher self-association rates than those observed for myosin A without salt.

The concept of Wyman's linked functions (1964) can be used to treat these processes according to Equation 1, where P is the unbound, X is the free salt, n and m are the number of X moles bound to species PX_n and PX_nX_m, and S_0, S_1, and S_2 are the solubilities of the species indicated. For this study S_1 and S_2 will be relative to S_0. The mathematical relationship representing the above stoichiometry can be represented according to Equation 2, where S_{app}

is the apparent protein solubility at a given salt concentration (X_T), $f(i)$ are the protein fractional components of species $\underline{i}_9$.

$$\underset{(S_0)}{P} + nX \xleftrightarrow{g1n} \underset{(S_1)}{PXn} + mX \xleftrightarrow{g2m} \underset{(S_2)}{PXnXm} \qquad (1)$$

For this study S_1 and S_2 will be relative to S_0. The mathematical relationship representing the above stoichiometry can be represented according to Equation 2,

$$S_{app} = S_0 f(P) + S_1 f(PXn) + S_2 f(PXnXm) \qquad (2)$$

where S_{app} is the apparent protein solubility at a given salt concentration (X_T), $f(i)$ are the protein fractional components of species $\underline{I}_9$.

$$S_{app} = \frac{S_0}{1 + \kappa_1^n X^n} + \frac{S_1 \kappa_1^n X^n}{1 + \kappa_1^n X^n} + \frac{(S_2 - S_1)\kappa_2^m X^m}{1 + \kappa_2^m X^m} \qquad (3)$$

Now, since the total salt concentration, X_T, is the sum of the free salt concentration, X, and the concentration of the bound salt of both species PX_n and PX_nX_m, it can be shown that

$$X_T = X\left[1 + \frac{n\kappa_1^n P_T X^{n-1}}{1 + \kappa_1^n X^n} + \frac{m\kappa_2^m P_T X^{m-1}}{1 + \kappa_2^m X^m}\right] \qquad (4)$$

The variation of the NMR transverse relaxation with various salt concentrations for soybean proteins is readily fitted with these equations by employing non-linear regression analysis. Representative results are shown in Figures 1 to 3 for soy proteins. These results show that the soy proteins have significant ion binding specificity for both cations and anions (Li^+ vsus Cs^+, or Cl^- vsus SO_4^{-2} , for example)

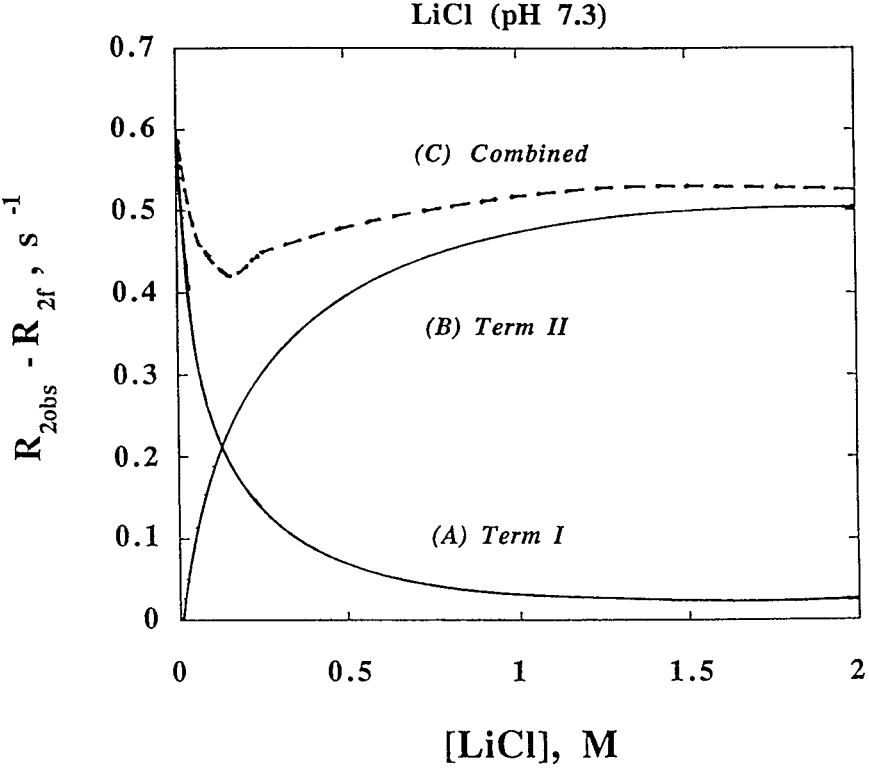

Figure 1. Graphical representation of the two terms in the protein aggregation model that was employed to fit the NMR data for varying salt concentrations at a fixed protein concentration.

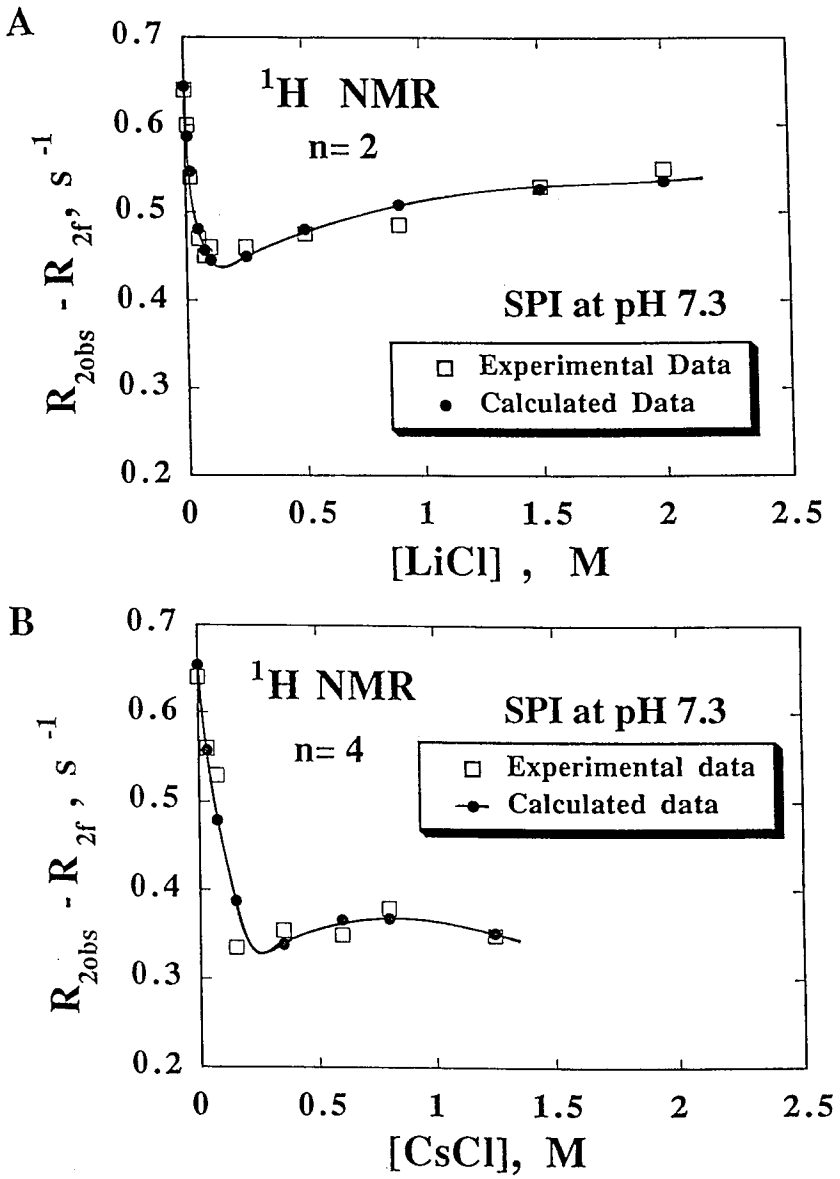

Figure 2. NMR results demonstrating the ion-binding specificity of soy protein isolates for cations.

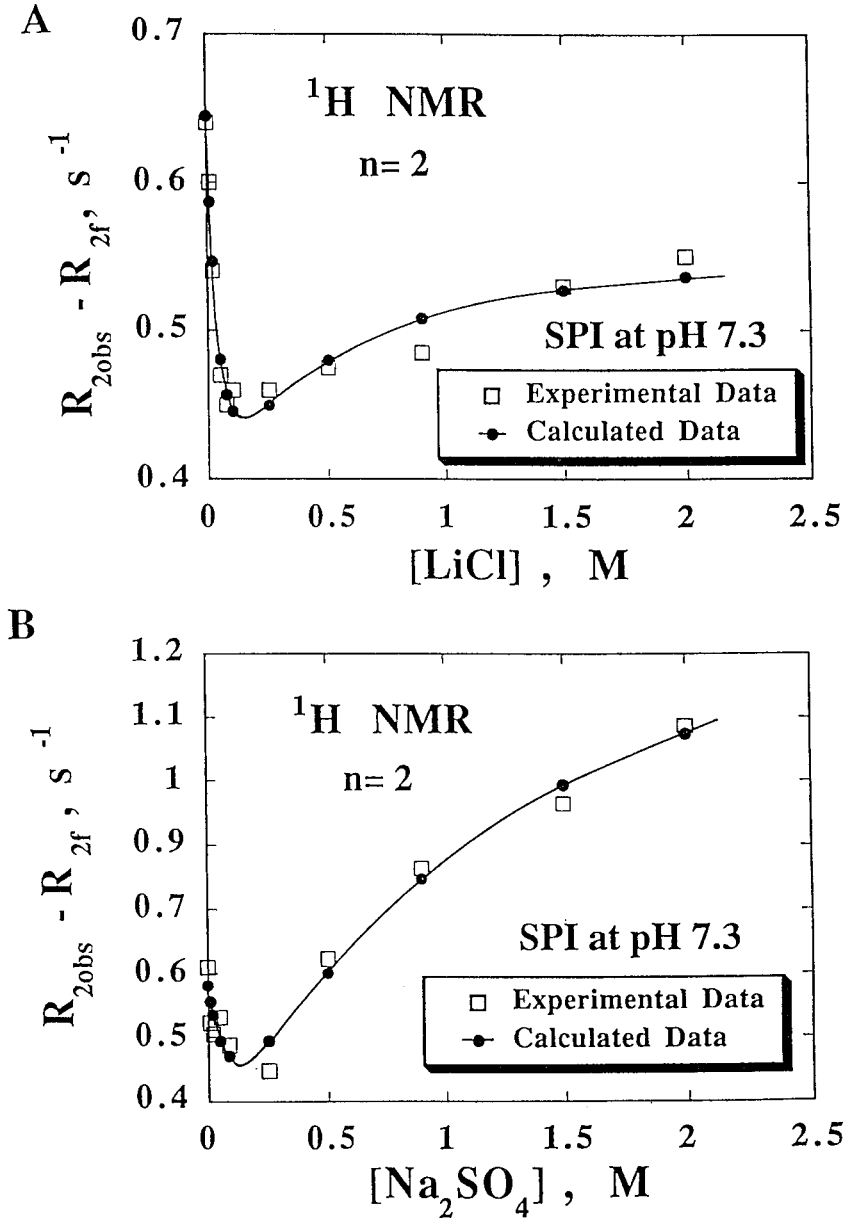

Figure 3. NMR results and calculations demonstrating the anion binding specificity of soy protein isolates at a fixed protein concentration.

Table 1. Parameters derived by Nonlinear Regression Analysis of Nuclear Spin Relaxation Data for Myofibrillar Protein Solutions with Cooperative Ion-Binding,n-mer Models (Eqs. 2 - 4).

	Sodium-23 NMR				Oxygen-17 NMR			
	n=3 Model	SD[a]	n=4 Model	SD[a]	n=3 Model	SD[a]	n=4 Model	SD[a]
R_{2B1}, s^{-1}	141	7	140.6	8	390	10	399	9
R_{2B2}, s^{-1}	109.5	3	107	3	304	5	303	4
k_{1S}	1269	239	1136	233	936	180	823	142
k_{2S}	14.5	1.1	19.9	1.6	19.3	2.5	26.5	2.8

References

[1] I.C. Baianu, Wei, T.C., and Yennerich,S. *Biophys.J.*, **212,** 152 (1991).

[2]T. Richardson, S. Oh, R. Jiménez-Flores, T.F. Kumosinski, E.M. Brown, H.M. Farrell, Jr. in *Advanced Dairy Chemistry* (Fox, P.F., ed.), Elsevier (in press)

[3]T. Clark, T. *A Handbook of Computational Chemistry: A Practical Guide to Chemical Structure and Energy Calculations*, John Wiley and Sons (1985)

[4]M. L. Allinger, *J. Am. Chem. Soc.*, **99,** 8127-8134 (1977)

[5]B. L. Kalman, *Technical Memo No. 46*, Department of Computer Science, Washington University, MO, USA (1982)

[6]G. Nemethy, H.A. Scheraga, *FASEB J.*, **4**, 3189-3197 (1990)

[7]G. Nemethy, M.S. Pottle, H.A. Scheraga, *J. Phys. Chem.*, **87**, 2361-2381 (1983)

[8]B.R. Brooks, R.E. Bruccoleri, B.D. Olafson,D.J. States, S. Swaminathan, M. Karplus, *J. Comput. Chem.*, **4,** 187-217 (1983)

[9]S.J. Weiner, P.A. Kollman, D.T. Nguyen, D. A. Case, *J. Comput. Chem.*, **7**, 230-252 (1986)

[10]C. Papostephanou, M. Frantz, in *Analytical Profiles of Drug Substances*, Vol. 7 (Florey, K., ed.), 401-422 (1978)

[11]S.R. Wilson, W. Cui, *Biopolymers,* **29**, 225-235 (1990)

[12]T-H. Lin, T.P. Quinn, D. Grandgenett, M.T. Walsh, *Proteins Struct. Funct. Genet.*, **5,** 156-165 (1989)

[13]G. W. Buchman, S. Banerjee, J.N. Hansen, *J. Biol. Chem.*, **263,** 16260-16266 (1988)

[14]S.N. Timasheff, in *The Enzymes, Vol. II* (Boyer, P.D., ed.), pp. 430-440 (1970)