range of ring sizes have led to some general as well as special effects concerning solute–solvent interactions of the open chain ground states and/or cyclic transition states.

Among the former, short bifunctional chains appear to suffer to varying extents from inhibition of solvation effects as it can be deduced from the entropies of activation. Furthermore, a systematic investigation of a ring forming reaction series provides a method to estimate the contribution of solvation to the entropy term for a given pair of polar end groups as functional groups of the intramolecular reaction.

Peculiar special effects can be revealed using effective molarity data as a diagnostic tool. An example is the formation of thiophenophan(1)ones by intramolecular acylation which is thought to suffer from a marked steric hindrance of solvation in the transition state due to the geometry requirements of the reaction mechanism and the perturbation effect of the bridging hydrocarbon chain over the heteroaromatic π-electron system.

NMR Studies of Calcium and Magnesium in Biological Systems

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Ions and ionic interactions play an important role in biochemical systems. For one thing, the physiological processes in our bodies take place in an electrolyte solution containing, as the major, simple ions: Na^+, K^+, Mg^{2+}, Ca^{2+} and Cl^-. These ions are by no means innocent bystanders to the cellular processes but play a very active biological role. It has, for example, become increasingly clear that Ca^{2+} ions in cells function as a kind of 'second messenger', often in parallel with cyclic AMP. The ions mentioned above have no suitable optical spectroscopic transitions that make them amenable to study. They have, however, all the magnetic nuclei that make NMR spectroscopic studies possible. Their spins, I, are greater than ½ and quadrupolar effects largely determine their NMR properties. Direct observation of NMR signals from the quadrupolar nuclei firmly bonded to a large slowly tumbling macromolecule is exceedingly difficult. It is, however, possible to obtain biochemically relevant information when there is a sufficiently fast chemical exchange between the macromolecular binding site(s) and the free solvated ion [1, 2].

Whereas NMR studies of $^{23}Na^+$ and $^{35}Cl^-$ are favoured by a reasonably high NMR sensitivity, studies of $^{25}Mg^{2+}$ and $^{43}Ca^{2+}$ in millimolar concentrations have only recently become feasible. The use of widebore high field superconducting magnets in combination with solenoid-type probes has been of particuar advantage. Some illustrative applications of ^{25}Mg and ^{43}Ca NMR to the study of calcium binding proteins, like troponin C, phospholipase A_2, hemocyanin and calmodulin, will be presented.

The properties of calcium in calcium binding proteins may also be studied indirectly by replacing Ca^{2+} with $^{113}Cd^{2+}$. The ionic radii of Ca^{2+} and Cd^{2+} are very similar and ^{113}Cd has spin I = ½ which allows direct observation of NMR signals from cadmium substituted calcium binding protein [3, 4]. ^{113}Cd NMR has proved to be a very sensitive probe of the nature and coordination geometry of the ligands. Recent results from ^{113}Cd NMR studies of the calcium binding proteins troponin C and calmodulin will be discussed.

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Direct NMR Studies of Ionic Solvation

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The nuclear magnetic resonance of quadrupolar ions provides a wealth of information about solvation thermodynamics and dynamics. The talk will deal with two main topics:

a) Use of cobalt(III) complexes.

Cobalt-59 (I = 7/2) has an enormous range of chemical shifts, due to low-lying excited states. The types of ion-solvent interactions to be monitored can be tailored at will simply by changing the nature of the ligands coordinated to the central Co^{3+} cation and/or changing the counter ions.

For instance, the $Co(CN)_6^{3-}$ ion measures the polarity of dipolar solvents, and will rank protic solvents according to their hydrogen-bond donor ability. Conversely, an ion such as $Co(NH_3)_6^{3+}$ will interact with solvents depending upon their strength as hydrogen-bond acceptors. Changes in net ionic charge can also be exploited to reveal details of the interactions between these cobalt complexes and other solute and solvent molecules.

b) Investigation of sodium cation solvates.

Sodium-23 ($I = 3/2$) nmr has good enough sensitivity that it can be applied to rather dilute (c*M*/m*M*) solutions of sodium salts. The observed changes in *both* the ^{23}Na chemical shifts and linewidths, in binary solvent mixtures, are successfully interpreted on the basis of competition for tetracoordination of the sodium cation by organic solvents such as THF, glymes, pyridine and other unidentate or polydentate amines. We have applied, to account *jointly* for the chemical shift and the relaxation rate data, the Hill formalism: mathematically, preferential solvation of a tetracoordinated ion, and binding of a ligand to one of four equivalent sites in a biomolecule, constitute one and the same problem. The powerful Hill formalism allows the determination of the magnitude of the chelate effect present for certain bidentate and polydentate ligands. It also leads to fairly accurate measurement of microscopic equilibrium constants for the successive steps in the replacement, in the sodium coordination sphere, of a solvent A by another solvent B.

Metalloenzymes and Model Systems. Carbonic Anhydrase: Solvent and Buffer Participation, Isotope Effects and Activation Parameters

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It is a general dilemma in biophysical chemistry that although small perturbations are theoretically most desirable, they tend to produce small effects which in turn are difficult to interpret, especially when systems are as large and complex as those encountered in the study of enzyme catalysis. In this regard the replacement of H_2O by D_2O is slowly becoming a powerful tool, especially in hydration–dehydration studies, because it gives measurable results from a modest perturbation of those molecular properties which are sensitive to mass. The ubiquity of CO_2 has prompted us to investigate one of life's most fundamental reactions, the reversible hydration of CO_2. We find that many general bases, and especially metal ion–hydroxo complexes catalyze this reaction, but the most potent of these catalysts is the zinc metalloenzyme carbonic anhydrase (carbonate hydro-lyase EC 4.2.1.1) from red blood cells. The mammalian enzyme is especially versatile catalyzing not only the reversible hydration of CO_2 but also that of aliphatic aldehydes, pyridine carboxaldehydes, pyruvic acid and alkyl pyruvate esters. The enzyme is also an efficient esterase, catalyzing the hydrolysis of certain carboxylate, sulfonate, phosphate and imidate esters. Substitution of zinc(II) by cobalt(II) gives an active enzyme which is being studied by observing its response to perturbations induced by substrates, buffers and inhibitors. We have recently studied the effects of pH, buffer concentration, steric hindrance, solvent composition (H_2O *vs.* D_2O) and inhibitor binding on CO_2 hydration, HCO_3^- dehydration and ^{18}O exchange in the CO_2–HCO_3^- system at four temperatures ranging from 7° to 35 °C. Our findings allow us to eliminate a number of anomalous mechanisms pertaining to isotopic exchange and provide us with a suitable framework for transition state characterization. Furthermore, our work shows that the Haldane relation is obeyed at every pH (pD), buffer and inhibitor concentration studied. Compliance with this relation shows that the system meets all the criteria required for the attainment of a chemical equilibrium.

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New Insights into the Host-Guest-Solvent Interaction of Some Inclusion Complexes

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Cyclodextrin (α, β- or γ-) is known to accommodate appropriate guest molecule(s) in its cavity in aqueous solution, forming an inclusion complex. Major driving force to form this inclusion complex