BINDING OF ALKALI AND ALKALINE EARTH CATIONS AND OF PROTONS TO THE PEPTIDE GROUP

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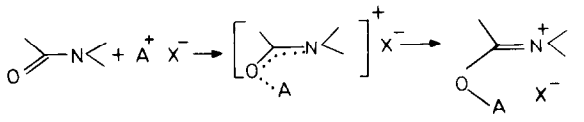
The interaction of alkali and alkaline earth metal ions, and of protons with the amide (peptide) group is of importance in the biophysical chemistry of peptides and proteins. Such interactions are involved in the conformational transitions of polypeptides, denaturation of proteins, selective ion complexation by macrocyclic antibiotic ionophores, and so on. In earlier studies [1–3] we had focussed attention on the proton transfer and protonation of amides, and on the interaction of lithium ion with amides and peptides. The general mode of such interactions may be written as:



(1)

where A^+ is the cation of interest and X^- the counterion. The site of binding is the carbonyl oxygen atom, and when A^+ is a proton or Li^+, the extreme right structure is realized. In this communication, we wish to provide electronic spectral evidence for this mechanism and point out the consequences of such interactions to peptides and proteins. This study is expected to be of relevance in light of the recent interest in the alkali metal ion complexation by ionophores, and the 'controversy' on the phenomenon of protonation of amide groups in moderately concentrated acid media.

In the first set of experiments, we studied the effect of Group IA and IIA salts on the far ultraviolet spectral bands of dimethyl formamide (DMF), lactams, and diketopiperazines. The $\pi - \pi^*$ bands of these compounds occur in the 190–197 nm region in aqueous solutions. Addition of alkali and alkaline earth salts causes a blue shift of this band maxium along with a reduction in band intensity. In the case of optically active diketopiperazines, we observed such blueshifting and hypochromism in the circular dichroism spectra as well upon the addition of these salts. Among the Group IA cations, Li^+ causes the maximum spectral perturbations, while it is Ca^{2+} among Group IIA. These spectral effects are analogous to the shifts seen in the spectra of other carbonyl compounds [4] and are consistent with Scheme I mentioned above for the mode of interaction between cations and amides.

In further analogy with carbonyl compounds, we have been able to detect the presence of a solvation equilibrium between the cations and amides in solution. Fig. 1 shows the progressive redshifts of the 225 nm band of the model amide chosen, namely benzamide, brought about by these metal cations, and also by hydrogen ions(protons). An important result in all these cases is the presence of isosbestic points near 230 nm. Similar isosbestic points have been observed with other carbonyl and thiocarbonyl donors [4] and are indicative of the presence of two equilibrating species as below:

$$M^{z+}(H_2O)_m + n \text{ amide} \rightleftharpoons M^{z+}(\text{amide})_n + m\ H_2O \qquad (2)$$

The magnitudes of the shifts, when plotted against the molarity of added electrolyte (see inset, fig. 1), point to a competition between the amide ligand and

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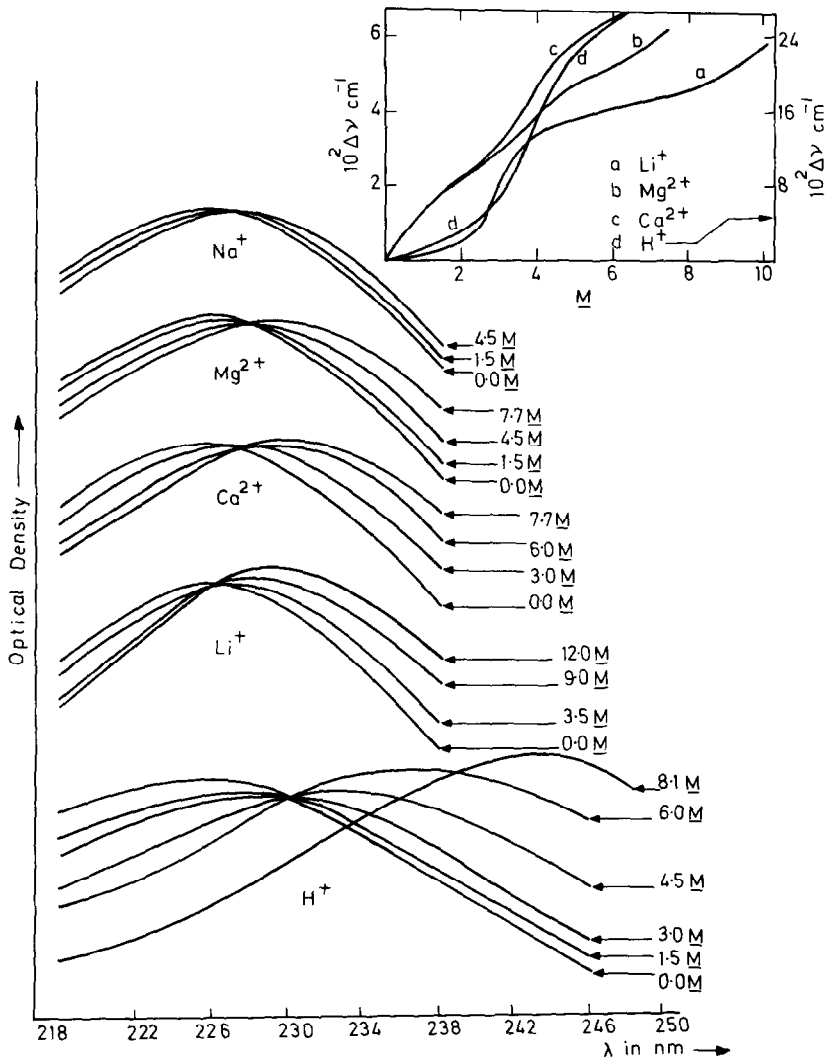

Fig. 1.

solvent water for solvating the cation, and also to some degree of co-operativity involved in this ligand substitution process. It would appear from these results that mixed solvates of the type $[M^{z+}(H_2O)_x(\text{amide})_y]$ do not occur, contrary to the suspicion of Williams [5]. This observation, and the possible co-operativity of the ligand substitution process are of relevance to the mechanism of dehydration and complexation of alkali cations by ionophores. For example, the rate of complexation of K^+ by monactin is estimated to be $10^8 M^{-1} \cdot s^{-1}$ [6], so that ligand substitution ought to occur within 10^{-8} sec.

The interaction of protons with amides can be directly compared with those of the metal cations. As shown in fig. 1, the proton-amide interaction is also characterized by an isosbestic point and co-operativity. The mode and general features of the interaction in this case are quite similar to the alkali cations, except with a greater strength. Based on the spectral shifts produced, the strength of interaction of cations with amides is found to be in the order $H^+ > Li^+ \simeq Ca^{2+} > Mg^{2+} > Na^+ > K^+$. Molecular orbital calculations by the CNDO/2 method [7], on the interaction of ions with amides and other oxygen donor ligands predicts the strengths of interaction to be $H^+ > Li^+ > Mg^{2+} > Na^+$, consistent with the spectral data; Perricaudet

and Pullman [8] find the order of binding energies to be $Li^+ > Na^+ > K^+$.

It is interesting that the isosbestic point near 230 nm seen in low to moderate acid strengths is lost in stronger acids (see fig. 1, curve for 50% H_2SO_4), suggesting the presence of a third species which is most likely to be the protonated amide. Thus, it would appear that the interaction of protons with amides involves two stages:

(i) at low acid strengths,

$$H^+(H_2O)_m + n \text{ amide} \rightleftharpoons H^+(\text{amide})_n + m\ H_2O \quad (3)$$

and (ii) in stronger acids,

$$H^+(\text{amide})_n \rightleftharpoons [\text{amide-H}]^+ + (n-1)\ \text{amide} \quad (4)$$

where the species in the square brackets is the protonated amide.

Some comments are in order about the above equilibria. It has been repeatedly suggested that amides are protonated in acid media, at the carbonyl oxygen site. Benderly and Rosenheck [9] have recently provided far ultraviolet spectral evidence for this. However, the suggestion has been made by Liler [10] that, at low to moderate acid concentrations, the amide is protonated at the nitrogen atom, with the site changing to the oxygen at high acid media. This seems unlikely to us on two grounds: (i) Rosenheck's data suggest the amount of N-protonated species to be negligible at all acid concentrations, and (ii) we have compared the effect of varying concentrations (0–6 M) of protons and of alkali metal ions on the far ultraviolet spectrum of DMF and lactams. In both cases, the effects (blueshifts and hypochromism of the peptide $\pi - \pi^*$ band) are very similar, indicating that both these cations interact with the amide group in an identical manner, i.e., at the carbonyl oxygen site. Thus, the 230 nm isosbestic point observed in benzamide in low acid media seems to owe its origin to Equilibrium 3 rather than to N-protonation.

Equilibria 3 and 4 envisaged for the proton-amide interaction are rather subtle, with the equilibrium shifting easily depending on the nature of the interacting species and the medium. An illustration of this comes from the experiments of Zezine et al. [11], and of Steigman et al. [12], which reveal that the structural changes caused in polypeptides and proteins by concentrated acids can be reversed upon the addition of water. Also, the distinction between protonation and strong hydrogen bonding may not be too clearly discernible, particularly in low dielectric media where contact ion-pairs will be favoured. This may be the reason why the circular dichroism spectra of randomly coiled poly γ-methyl L-glutamate are nearly identical in pure H_2SO_4 and in hexafluoropropan-diol (a strongly hydrogen bonding solvent, but a weak acid of pK_a 6.37) [13].

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