# RELATIVE AFFINITIES OF ALKALI METAL IONS TO THE LIGANDS IN IONOPHORES

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## 1. Introduction

The factors governing the specificity and the mechanism of cation transport across membranes by ionophore carriers such as the cyclic peptides, depsipeptides and the macrotetrolides have been the subject of considerable current concern. The points of interest have been the nature, strength and specificity of interaction of the alkali metal ions with the liganding groups of the ionophores. A knowledge of the differential interactions of the various metal ions and the ligands involved (i.e., peptide and ester groups and water) is thus of interest in the understanding of the carrier transport process. Recently several papers have appeared which present the results of theoretical calculations on these interactions [1–3]. We have previously published [4] experimental results in detail for the amide–cation interaction system. The present paper is concerned with experimental results on the interactions of several cations with amide and ester groups in neat and in aqueous media, and presents results that support the predictions of theory. Our results show that: (1) for a given cation the interaction is stronger with the amide group than with the ester group; (2) these interactions are of the ion–dipole type; and (3) with these ligands as models, the interaction energies vary in the expected regular fashion with the metals of the I and the II group of the periodic table.

## 2. Experimental

We present results of calorimetric and infrared spectral experiments. Calorimetric measurements were done using a Dewar flask calorimeter, where the aqueous salt solution of a given molarity was added to the neat liquid amide or the ester at the same temperature. The temperature change was monitored by means of a precision mercury thermometer with a readable accuracy of 0.05°. The heat measurements were done two ways: one where the volume of the amide or the ester (the volume fraction in the total solution) was varied over a range while the salt solution was of a fixed molarity, and secondly the heats of interaction were measured between the ligand at constant volume fraction and the salt solutions of different molarities. Infinite dilution heat values were computed by graphical extrapolation. The temperature rise upon mixing in the experiments ranged from 1.0 to 30.0°, with an accuracy of ± 0.05 to 0.1°, so that the error range in the experimental determination was acceptable.

Values for the heats of interaction of salts with water, and with anhydrous amides were taken from literature.

Infrared spectra were recorded on a Perkin–Elmer model 521 instrument using CsBr windows. Spectra were run on samples containing salt: ligand of molar ratio 1:4, since in the case of amides stable adducts were isolated at this ratio. In some cases, solid adducts were formed and these were studied, but in most cases the solution of the mentioned molarity ratio was directly used.

## 3. Results and discussion

We have earlier studied in some detail the interaction between alkali metal salts and the peptide ligand [1, 4]. Molecular orbital calculations using the CNDO method, as well as spectroscopic and calorimetric experiments established that the cations interact with the peptide group in an ion–dipolar fashion, and that the cation binds to the carbonyl oxygen of the amide. As a consequence, the geometry of the amide undergoes subtle alterations. The double bond character of the central C–N bond increases, the C=O bond decreases in bond order, and the metal–oxygen bond stretching vibration is observed in the far infrared region of the spectrum (this is seen near 400 $cm^{-1}$ for the lithium–amide complexes). The C=O bond stretching frequency is redshifted upon metal ion coordination. The effects are strongest with lithium ion in group I metals. Based on these, we now interpret the far infrared bands seen in metal–ionophore complexes by Ivanov et al. [5], as due to the metal–oxygen stretching frequencies. It is also of interest to note that our calculations show the optimum bond angle of C=O . . . Li to be 120° and the O . . . Li bond length to be 2.2 Å in amide-lithium complexes. Kostetsky et al. [2] calculate these as 180° and 2 Å respectively, while Perricaudet and Pullman [3] find 145° and 1.95 Å for the corresponding sodium binding. Credence for the theoretical prediction comes from the recent crystal structure determination of the lithium complex of the cyclic decapeptide antamanide, where the O . . . Li distance is seen to be 2.11 Å [6].

Among the first group metal ions, spectroscopic and calorimetric investigations reveal that the strength of interaction with amides varies in the order $Li^+ > Na^+ \geqslant K^+$. The anion of the salts also seem important since the interactions vary with the anions of a given metal salt, with the maximum interaction occurring with perchlorates. This may be of relevance to the carriage and transport of the salt across model membranes by ionophores [7].

That the site of attachement of the metal cation to ester ligands is also the carbonyl oxygen is suggested by the infrared spectral study of stoichiometric solutions (1:4 salt: ester) of alkali metal salts in dry esters such as methyl acetate or ethyl acetate. Similar to the amide cases, the carbonyl bond stretching vibration frequency of the free ester (occurring at 1740 $cm^{-1}$ in vapour or dilute solution phase) shifts to the red as follows: LiBr: ethyl acetate (1:5 ratio) 1718; saturated LiBr: ethyl acetate (about 1:3) 1696; and $Mg(ClO_4)_2$: ethyl acetate 1696 $cm^{-1}$. These shifts of about 40 $cm^{-1}$ upon metal coordination to the easter parallel, but are somewhat lower than, the redshifts of about 55 $cm^{-1}$ seen when LiBr coordinates to amides such as *N*-methyl acetamide. We also notice that while $Mg^{2+}$ causes the same order of effect as $Li^+$, $K^+$ is practically inert since the shift of the band is small here. To the extent that infrared bandshifts are an index, $K^+$ binds to both amide and ester groups with much less strength than $Li^+$. We also looked for the presence of the vibrational band in the 400 $cm^{-1}$ region in the Li:ester and Mg:ester systems, a band predicted by theory to arise due to O . . . Li and O . . . Mg stretches and observed in complexes of these metals with other oxygen donor ligands including amides [1, 4, 8]. While the amide complexes of Li and Mg display there bands clearly near 375 and 385 $cm^{-1}$ respectively, we could observe these bands with some difficulty in the case of these metals bound to esters. Ethyl acetate itself shows absorptions in this region. With methyl acetate as the substrate (transparent by itself in this region [9]), and LiBr as the dissolved salt, bands were found at 380 (weak) and 402 (shoulder) $cm^{-1}$, while with $Mg(ClO_4)_2$ as solute, bands were seen at 380 (w), 391 (sh), and 400 $cm^{-1}$. These bands may arise due to the metal oxygen stretch, but this interpretation in the case of esters is at the moment not completely unambiguous due to the weakness of the bands and to the inherent weakness of complexation compared to the amides.

Data on the heats of mixing of aqueous salt solutions with amides, and with esters are presented in table 1. Notice that the strength of metal ion interactions with amides is in the sequence: Li > Na > K, and Li > Mg > Ca, at comparable concentrations. It is also seen that the heats of interactions of any given salt with amides is considerably larger than with the ester. The amide ligand is the stronger one. Values for the heat of mixing could not be obtained with salts of high molarities and ester due to salting out. The order of interaction of salts with ester follows the sequence: Li > K > Na; and Li > Ca. This reversal of the K,Na order with esters is not explicable at present. However, these calorimetric results reinforce the earlier suggestion that the peptide group binds the alkali metal ions stronger than the ester group. This point is of considerable relevance to the phenomenon of metal com-

Table 1
Heats of interaction of salts with amides and esters

| Ligand | Salt | $\Delta H$ in cals/mole ligand |
|---|---|---|
| Methyl acetate | 1 M NaCl | – 1500 |
| Methyl acetate | 1 M KCl | – 1700 |
| Methyl acetate | 1 M $CaCl_2$ | – 1650 |
| Methyl acetate | 1 M LiCl | – 1800 |
| Dimethyl formamide | 4 M NaCl | – 3000 |
| Dimethyl formamide | 4 M LiCl | – 3700 |
| Dimethyl formamide | 8 M $NaClO_4$ | – 2700 |
| Dimethyl formamide | 8 M LiCl | – 4500 |
| Dimethyl formamide | 4 M $MgCl_2$ | – 3800 |
| Dimethyl formamide | 4 M $CaCl_2$ | – 3500 |
| *N*-methyl acetamide | 10 M KCNS | – 2500 |

plexation by ionophores, since it suggests that homodetic peptides ought to bind metal ions stronger than the corresponding depsipeptides. Gratifyingly, it has been experimentally shown recently that the homodetic analogue of valinomycin has a larger binding constant for KBr than valinomycin itself [10]. Likewise, the replacement of the peptide bond by the ester group in enniatin B decreases the complex stability [11]. These facts have been noted earlier by Perricaudet and Pullman [3], who did SCF ab initio MO calculations on the affinities of these two ligands to Na and K, and predicted that the affinity for metal ion binding is in the order: amide > water > ester. Our present results support these predictions.

Values are also available for the heats of solution of these salts in water, and in dry liquid amides such as dimethyl formamide. The heats of solution of LiBr, NaBr and KBr, at 25°C in dry dimethyl formamide are –21.3, –7.39, and –3.89 kcal/mole respectively [12]. The heats of solution, at 25°C in water, for LiBr, NaBr and KBr are: –11.67, –0.144 and +4.75 kcal/mole respectively [13]. These values show that amide is a better ligand than water, and also the order of salts is the same in both solvents. No data is available for the heat of solution of salts in esters to afford comparison.

We conclude therefore, that the basis of interaction with ligands of interest is the ion–dipole mechanism, and the interaction with the amide group is significantly larger than with esters. This has its basis in the decreasing values of the dipole moments as we go from amide to water to esters. Also, the strength of interaction decreases roughly as we go down the alkali metal group, and the alkaline earth metal ions appear to display the same strength as lithium. We wish to point out, however, that model studies of this kind do not offer any insight into the specificity of complexation for a particular metal by ionophores such as valinomycin. This feature may have its origin most likely in the cavity size of the ionophore, the radius of the ion and the 'snugness of fit' in the inclusion adduct.

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