SOLVATION PROPERTIES OF NATURAL AND SYNTHETIC IONOPHORES

I. Stoichiometry of Complexes with Alkali and Alkaline Earth Cations in Aprotic Organic Solvents

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ABSTRACT Ion-solvent interactions play a very important role in the studies of stoichiometry, structure, and stability of complexes of cations with natural and synthetic ionophores. These compounds are extremely useful in study of the interaction of neutral salts with macromolecules and the mechanism of cation transport across biological membranes. Knowledge of the ionophore solvation properties enables one to choose a suitable solvent for complexation studies and to obtain detailed information on the solvent effect. We would like to present in this paper a very simple method of estimating the solvation properties of ionophores. We treat the ligand as an assembly of individual noninteracting binding sites. The solvation properties of solvents can be used to represent the solvation sites in natural and synthetic ligands. The solvation properties are represented by the Gutmann donor number (DN) of the model solvent. We can define the solvation ability of a ligand binding site to be "donor number of binding site" ($DN_{binding\ site}$), which in turn can be represented by the DN of the appropriate model solvent. The average DN of the ligand ($DN_{average}$) is defined as $[\Sigma_{i=1}^{n}(DN_{binding\ site})i]/n$, where n is the number of the ligand binding sites. Comparison of the $DN_{average}$ with the $DN_{solvent}$, together with the knowledge of the composition of the system, characterizes remarkably well the solvation properties of the ligand. This model explains (*a*) the stoichiometry of many alkali and alkaline earth cation complexes with natural and synthetic ligands in aprotic organic solvents, (*b*) the transport of alkali and alkaline earth cations across lipid bilayers, and (*c*) how polypeptides and proteins interact with neutral salts in solutions.

INTRODUCTION

Since the discovery of the specific potassium ion-binding capabilities of the antibiotic valinomycin (1), there have been many studies of the complexing abilities of naturally occurring and synthetic peptides (2–4). The preponderance of these studies have focused upon the relationship of the structure of the ionophore to its ion-binding ability; the crucial nature of the solvent in many of these reactions has often been neglected. Because we wish to understand better the contribution that the solvent makes to many fundamental properties of such complexes, we have undertaken a study of the stoichiometry of two ligands in three different solvents. Our hope is that it will be possible to rationalize our results, and the results of others, using a relatively simple model of ion solvation. The approach that we take is an extension of the method of Eisenmann and co-workers (5, 6), who showed that the solvation of ions by ion carriers can be approximated by the solvation of the same ions by appropriate solvents. We characterize the solvent by its Guttman donor number (7–9) and devise a method of assigning a Guttmann donor number to the ionophore. The two factors that then determine the possible stoichiometries of the complexes are the relative magnitudes of the mean donor number of the ligand and of the solvent and the ratio of the concentration of the cation to the ligand.

The two ligands that we have chosen are the crown ether 2,3-benzo-1,4,7,10,13-pentaoxacyclopentadeca-2-ene (benzo-15-crown-5) (Fig. 1*a*) and the linear hexapeptide Boc-(Gly-L-Pro)$_3$OBz (Fig. 1*b*). Benzo-15-crown-5 is a typical crown ether forming well-defined complexes with many alkali and alkaline earth cations (10). In these complexes the cation is located in the center of the main macrocyclic ring, more or less in the plane defined by the oxygen atoms; the forces responsible for the formation of this complex are primarily ion-dipole in nature. The ability of peptides to bind metal ions has been well documented, particularly with respect to divalent cations (4–6). We have investigated the stoichiometry of the binding of benzo-15-crown-5 to sodium ion(s) in nitromethane and in diethyl ether and of Boc(Gly-L-Pro)$_3$OBz to magnesium in acetonitrile using ^{1}H NMR as a probe of the extent of the complexation reaction.

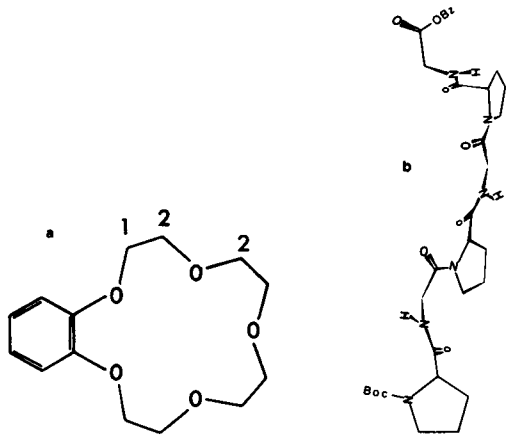

FIGURE 1 (*a*) Benzo-15-crown-5; (*b*) Boc-(Gly-L-Pro)$_3$OBz.

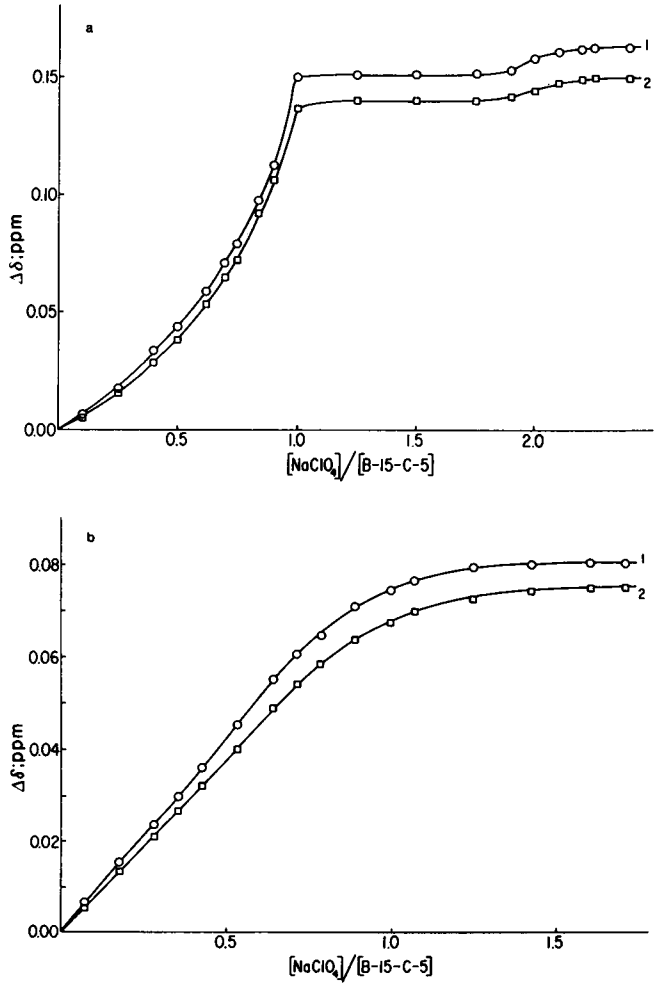

FIGURE 2 Downfield increments of chemical shifts of 'H signals of Benzo-15-crown-5 VS. [$NaClO_4$]/[Benzo-15-crown-5] ratio. (*a*) 0.02M solutions of Benzo-15-crown-5 in nitromethane. (*b*) 0.02M solutions of Benzo-15-crown-5 in Diethylether. Numbers at the right indicate signal assignment (cf. Fig. 1*a*). Full lines correspond to the computed points.

EXPERIMENTAL PROCEDURES

Boc(Gly-L-Pro)$_3$OBz was prepared according to the procedure given by Deber and Blout (11). Benzo-15-crown-5 was prepared following the method of Pedersen (10).

All solvents (nitromethane, acetonitrile, and diethyl ether) were obtained from Merck at 99.9% deuteration (Merck Chemical Div., Merck & Co., Inc., Rahway, NJ) and were dried for 48 h over molecular sieve 4A. $NaClO_4$ (Fisher Analar, Fisher Scientific Co., Pittsburgh, PA.) and $Mg(SCN)_2$ (Fisher Analar) were dried under vacuum 10^{-4} mmHg at 80°C for 48 h. Solutions were prepared under nitrogen.

^{1}H NMR spectra were recorded at 270 MHz on a spectrometer (Bruker Instruments, Inc., Billerica, MA, model WH-270) operating in the Fourier transform mode, using 5-mm sample tubes. The deuterium signals of the solvents served as internal locks and TMS (tetra methyl silane) as an internal reference. All experiments were conducted at 27°C. NMR measurements of the ^{1}H chemical shifts of the complexes were performed on solutions prepared by mixing 0.02 M solutions of the ligands with 0.02 M ligand and 0.1 M salt solutions in the same solvent. The procedure described by Reuben and co-workers (12,13), for the conditions of fast exchange of the ligand was used for the data analysis.

RESULTS

Complexes with Benzo-15-crown-5

Pronounced changes in the chemical shifts of the various protons were observed as a function of the ratio of salt concentration to ligand concentrations in both nitromethane and diethyl ether. These results are presented graphically in Fig. 2.

To fit the experimental chemical shifts in nitromethane with appropriate equilibrium expressions, it was necessary to hypothesize the existence of three distinct reactions:

$$Na^+ + \text{benzo-15-crown-5} \overset{K_1}{\rightleftharpoons} (\text{benzo-15-crown-5})Na^+$$

$$(\text{benzo-15-crown-5})Na^+ + \text{benzo-15-crown-5} \overset{K_2}{\rightleftharpoons} (\text{benzo-15-crown-5})_2\ Na^+$$

$$(\text{benzo-15-crown-5})Na^+ + Na^+ \overset{K_{1/2}}{\rightleftharpoons} (\text{benzo-15-crown-5})(Na^+)_2.$$

In diethyl ether, however, the third expression was found not to be required; in other words, there is no reason to postulate the existence of a "cation-sandwich" complex.

Values of the intrinsic chemical shifts of the complexes and the values of the corresponding equilibrium constants

TABLE 1
STABILITY CONSTANTS OF THE $NaClO_4$ (BENZO-15-CROWN-5) COMPLEXES

Solvent	DN	K_2	K_1	K
		(M^{-1})	(M^{-1})	(M^{-1})
Nitromethane	2.7	75 ± 15	$>10^5$	15 ± 3
Diethylether	19.2	10 ± 4	285 ± 18	none

TABLE II
THE INTRINSIC CHEMICAL SHIFTS OF THE BENZO-15-CROWN-5 PROTONS (PPM RELATIVE TO TMS)

Solvent	System	Proton	H_1	H_2	H_{Ar}
Nitromethane	C*		3.66	4.01	6.67
	C_2 $NaClO_4$		3.70 ± 0.02	4.06 ± 0.02	6.68 ± 0.01
	C $NaClO_4$		3.82 ± 0.01	4.14 ± 0.01	6.70 ± 0.01
	C $(NaClO_4)_2$		3.85 ± 0.01	4.17 ± 0.01	6.70 ± 0.01
Diethylether	C*		3.67	4.03	6.69
	C_2 $NaClO_4$		3.70 ± 0.03	4.07 ± 0.03	6.71 ± 0.01
	C $NaClO_4$		3.77 ± 0.02	4.12 ± 0.02	6.72 ± 0.01

C = Benzo-15-Crown-5
*Directly measured values.

K_1, K_2, and $K_{1/2}$, were calculated by iteration from the experimentally observed chemical shifts. The equilibrium constants are given in Table I, and the intrinsic chemical shifts given in Table II.

Complexes with Boc-(Gly-L-Pro)$_3$OBz

The changes in the observed chemical shift of the amide protons as a function of the ratio of $Mg(SCN)_2$ concentration to peptide concentration in acetonitrile are shown in Fig. 3. Now, to adequately fit this curve, it was necessary to

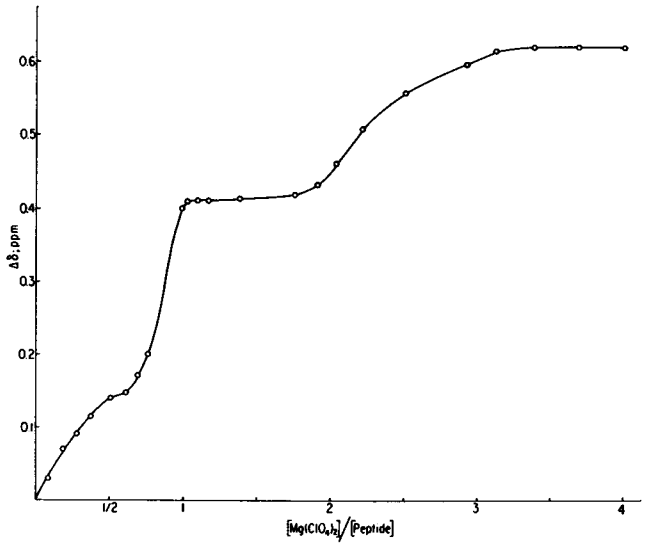

FIGURE 3 Downfield increment of chemical shifts of ^1H glycil amide signal vs. [Mg $(SCN)_2$]/[Boc (Gly-L-Pro)$_3$OBz] ratio, 2 × 10^{-2}M solutions of Boc-(Gly-L-Pro)$_3$ OBz in acetonitrile. Full lines correspond to the computed values.

TABLE III
STABILITY CONSTANTS OF THE [MG$(SCN)_2$][BOC(GLY-L-PRO)$_3$ OBz] COMPLEXES IN ACETONITRILE.

K_1	K_2	$K_{1/2}$	$K_{1/3}$
(M^{-1})	(M^{-1})	(M^{-1})	(M^{-1})
(4 ± 0.2) × 10^3	250 ± 30	78 ± 13	5 ± 1

TABLE IV
THE INTRINSIC CHEMICAL SHIFTS OF THE BOC-(GLY-L-PRO)$_3$ OBz AMIDE N—H PROTONS (PPM RELATIVE TO TMS) IN ACETONITRILE

System	N—H glycines protons		
	H_1	H_2	H_3
C*	7.01	7.09	7.22
$C_2Mg(SCN)_2$	7.17 ± 0.02	7.17 ± 0.02	7.17 ± 0.02
C $Mg(SCN)_2$	7.42 ± 0.02	7.42 ± 0.02	7.42 ± 0.02
C $[Mg(SCN)_2]_2$	7.63 ± 0.03	7.63 ± 0.03	7.63 ± 0.03
C $[Mg(SCN)_2]_3$	7.71 ± 0.03	7.71 ± 0.03	7.71 ± 0.03

C = Boc-(Gly-L-Pro)$_3$OBz
*Directly measured values.

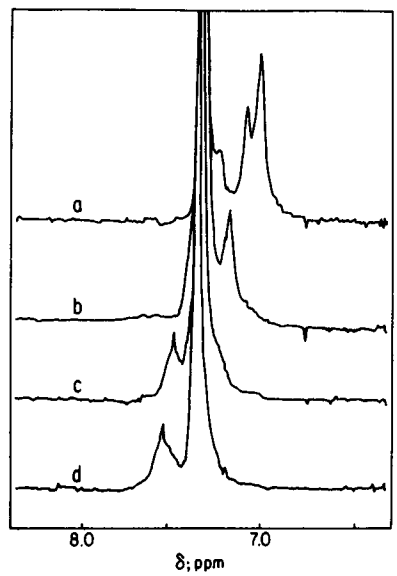

FIGURE 4 The 6.5–8.0 ppm region of 270 MHz ^1H NMR spectra of: (*a*) 2 × 10^{-2} M Boc (Gly-L-Pro)$_3$ OBz in acetonitrile; (*b*) solution of 2 × 10^{-2} M Boc (Gly-L-Pro)$_3$ OBz and 10^{-2}M $Mg(SCN)_2$ in acetonitrile; (*c*) solution of 2 × 10^{-2}M Boc (Gly-L-Pro)$_3$ OBz and 2 × 10^{-2} M $Mg(SCN)_2$ in acetonitrile; (*d*) solution of 2 × 10^{-2}M Boc (Gly-L-Pro)$_3$ OBz and 4 × 10^{-2} M $Mg(SCN)_2$ in acetonitrile.

utilize four equilibrium expressions:

$$Mg^{2+} + \text{peptide} \overset{K_1}{\rightleftharpoons} \text{peptide—}Mg^{2+}$$

$$\text{peptide—}Mg^{2+} + \text{peptide} \overset{K_2}{\rightleftharpoons} \text{peptide}_2\text{—}Mg^{2+}$$

$$\text{peptide—}Mg^{2+} + Mg^{2+} \overset{K_{1/2}}{\rightleftharpoons} \text{peptide—}(Mg^{2+})_2$$

$$\text{peptide—}(Mg^{2+})_2 + Mg^{2+} \overset{K_{1/3}}{\rightleftharpoons} \text{peptide—}(Mg^{2+})_3.$$

The observed chemical shifts were not reproduced adequately when only the first three equilibrium expressions were used; consequently, we postulated the existence of the "tri-magnesium" complex. The calculated equlibrium constants are given in Table III and intrinsic chemical shifts in Table IV.

The amide region of the 270 MHz ^{1}H NMR spectra of the free peptide and mixtures with $Mg(SCN)_2$ with relative ratios of $[Mg^{2+}]/[\text{peptide}]$ of 0.5, 1.0, and 2.0 in acetonitrile are shown in Fig. 4.

In the free peptide there are three distinct chemical shifts for the N—H protons at 7.01, 7.09, and 7.29 ppm; however, the complexed peptide has only one peak, which shifts downfield with increasing magnesium salt concentration. The appearance of only one peak may well indicate that the peptide in the complex is more highly symmetric than is the free peptide. The phenyl ring protons of the benzoate group (δ = 7.36 ppm) do not shift upon complexation, suggesting that the benzoate group is unperturbed by the magnesium.

DISCUSSION

Our results give strong evidence for the existence of complexes of the form (peptide—Mg^{+2}), (peptide$_2$—Mg^{+2}), [peptide—$(Mg^{+2})_2$] and [peptide—$(Mg^{+2})_3$]. In the crown ether studies, stoichiometries of (crown—Na^+), (crown$_2$—Na^+), and [crown—$(Na^+)_2$] were observed. The particular stoichiometry observed in each case is a function both of the solvent and the ratio of cation to ligand present in solution. We propose a simple, qualitative model of the solvation properties of the ligands, solvents, and cations, which predicts remarkably well the stoichiometries of the complex species that are formed.

Current theories of ion solvation are based upon models in which the interactions between the ion and solvent dipoles and multipoles as well as the structural changes imposed on the solvent molecules by the ion are taken into account (14). Our model "averages" these complex effects into an effective interaction based on the relative Lewis acidities of the solvent and metal ions. Solvents are, of course, either Lewis acids or bases; because most metal ions are Lewis acids, interactions will take place between metal ions and base solvent molecules leading to solvated metal cations.

Gutmann (7-9) has quantified Lewis acidity and basicity by doing calormetric measurements on the interactions of a number of oxygen and nitrogen containing solvents using antimony chloride as a reference acceptor in dichloroethane: $D + SbCl_5 \rightleftharpoons D \cdot SbCl_5$. He defined the quantity $-\Delta H(D \cdot SbCl_5)$ in kcal/mol to be the donor number (DN) of the solvent D. Donor numbers and dielectric constants (ϵ) of various solvents are given in Table V (9).

The donor number is a molecular property of the solvent that is easily determined experimentally. It expresses the total interaction of the donor with an acceptor molecule, including such contributions as the dipole-dipole or ion-dipole interactions as well as the binding effect caused by the availability of a free electron pair. To some extent even steric properties of the solvent molecules are contained in the donor number. The solvation properties of a given solvent can therefore be reasonably well characterized by

TABLE V
DONOR NUMBER (DN) AND DIELECTRIC CONSTANTS (ϵ) OF CERTAIN SOLVENTS*

Solvent	DN	ϵ
1,2-Dichloroethane	—	10.1
Sulphuryl chloride	0.1	10.0
Thionyl chloride	0.4	9.2
Acetyl chloride	0.7	15.8
Benzoyl chloride	2.3	23.0
Nitromethane	2.7	35.9
Nitrobenzene	4.4	34.8
Acetic Anhydride	10.5	20.7
Benzonitrile	11.9	25.2
Selenium oxychloride	12.2	46.0
Acetonitrile	14.1	38.0
Sulpholane	14.8	42.0
Propanediol-1,2-carbonate	15.1	69.0
Benzyl cyanide	15.1	18.4
Ethylene sulphite	15.3	41.0
iso-Butyronitrile	15.4	20.4
Propionitrile	16.1	27.7
Ethylene carbonate	16.4	89.1
Phenylphosphonic difluoride	16.4	27.9
Methylacetate	16.5	6.7
n-Butyronitrile	16.6	20.3
Acetone	17.0	20.7
Ethyl acetate	17.1	6.0
Water	18.0	81.0
Phenylphosphonic dichloride	18.5	26.0
Diethylether	19.2	4.3
Tetrahydrofurane	20.0	7.6
Diphenylphosphinic chloride	22.4	—
Trimethyl phosphate	23.0	20.6
Tributyl phosphate	23.7	6.8
Dimethylformamide	26.6	36.1
N,N-Dimethylacetamide	27.8	38.9
Dimethyl sulphoxide	29.8	45.0
N,N-Diethylformamide	30.9	—
N,N-Diethylacetamide	32.2	—
Pyridine	33.1	12.3
Hexamethylphosphoramide	38.8	30.0

*Data from reference 4.

its donor number. This fact was demonstrated by Popov and co-workers in studies of ion solvation by organic solvents (15, 16).

Although varying widely both structurally and conformationally, the natural and synthetic ionophores that bind alkali and alkaline earth cations have a common feature: the solvation spheres of their bound cations are filled by amide, ether, ester, or other polar groups just as they are in solvation by any organic molecules containing these groups. Formation of these complexes can thus be likened to transfer of the cations from a weaker to a stronger solvating medium. Indeed, the complexes are structurally similar in many respects to the crystallosolvates that alkali metal (particularly sodium and lithium) salts form with a number of ketones and amides (4, 17), (see Fig. 5).

If the cooperative effects of ligand binding sites are ignored, one can treat the ligand as an assembly of individual binding sites. The solvation properties of commonly used solvents can then be used as models for the solvation properties of binding sites in natural and synthetic ionophores. Diethyl ether can be used to represent the etheral oxygens in crown ethers and polyethylene glycols; and tetrahydrofurane can be used to represent the etheral oxygens in natural antibiotics such as nonactin, monensin, nigericin, or dianemycin. *N,N*-dimethylacetamide and *N,N*,-dimethylformamide are models of amido groups in natural antibiotics such as valinomycin, enniatin-B, antamanide, and synthetic ionophores such as cyclopeptides and amido ethers. The solvation properties of ethyl acetate are a model for those of ester groups in natural antibiotics such as valinomycin and nonactin. The justification for this model is found in the work of Eisenman and co-workers (5, 6) (see Fig. 6). We can now define the solvation ability of a ligand binding site to be the donor number of binding site ($DN_{binding\ site}$), and we assume that $DN_{binding\ site}$ can be represented by the DN of the model solvent, i.e.,

$$DN_{ethereal\ oxygen\ of\ Crown\ ether} = DN_{dimethylether}$$

$$DN_{amido\ group\ of\ ionophore} = DN_{dimethylacetamide}$$

$$DN_{ester\ group\ of\ ionophore} = DN_{ethylacetate}.$$

One can calculate the average DN of the ligand ($DN_{average}$):

$$DN_{average} = \left[\sum_{i=1}^{n} (DN_{binding\ site})_i \right] \Big/ n$$

where n is the number of the binding sites in the ligand. $DN_{average}$ represents the ability to estimate the solvation power of a ligand when it acts as an assembly of individual binding sites, in competition with common solvents. It is assumed that the $DN_{average}$ is the lowest limit of the solvation ability of a ligand; natural and synthetic ligands possess higher solvation ability than that presented by $DN_{average}$ because of cooperative and macrocylic effects.

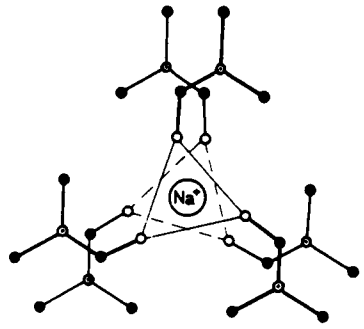

FIGURE 5 Sodium ion surrounded in the crystallosolvate $[HCON(CH_3)_2]_6$ NaI. ●, C; ⊙, N; ○, O.

Comparison of the $DN_{average}$ with DN solvent can explain many results of complexation of alkali and alkaline earth cations with natural and synthetic ligands in different solvents.

It is noteworthy that natural antibiotics (18–22), cyclic peptides (23–30), and related ligands (31–33) form sandwich complexes in solution in addition to ligand—cation (1:1) complexes. These include the following (with cation-ligand stoichiometries indicated in parentheses): ligand sandwich (1:2), and cation sandwich (2:1). From a variety of experimental studies (18–33), it appears that when the solvation number of the cation is equal to the number of ligand binding sites, the cation—ligand (1:1) complex is the most stable species regardless of the $DN_{average}$-$DN_{solvent}$ relationship. We suggest that the 1:1 complex is formed first; the [cation]/[ligand] ratio then determines the sandwich complex(es) that may be formed. This can be described by the following three equations:

$$\text{ligand} + \text{cation} = \text{ligand cation} \quad (1:1) \quad (1)$$

$$\text{ligand cation} + \text{ligand} = \text{ligand}_2 \cdot \text{cation (ligand sandwich)} \quad (1:2) \quad (2)$$

$$\text{ligand} \cdot \text{cation} + \text{cation} = \text{ligand} \cdot \text{cation}_2 \text{ (cation-sandwich)} \quad (2:1). \quad (3)$$

The suggested stoichiometry of complexes for the different $DN_{average}$-$DN_{solvent}$ relationships is summarized in Table VI.

We hypothesize that the formation of cation-sandwich

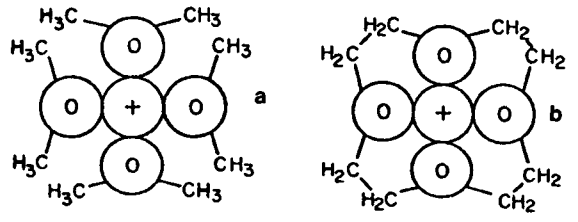

FIGURE 6 Ion solvation by methyl ether compared to "ion solvation" by a polyether. This figure illustrates schematically that the replacement of solvent molecules about an ion is analogous to the spatial array of the same ligands about the ion in an ion carrier complex. (*a*) methyl ether; (*b*) polyether.

TABLE VI
PREDICTED STOICHIOMETRY OF COMPLEXES IN THE DIFFERENT $DN_{AVERAGE}-DN_{SOLVENT}$ RELATIONS

Relation number	$DN_{AVERAGE}-DN_{solvent}$ relation	Concentration ratio (cation)/(ligand)	Equation	Stoichiometry of complexes (predicted)
		$\geqslant 1$	(1)	cation ligand (1:1)
			(3)	cation sandwich (2:1)
I	$DN_{average} > DN_{solvent}$			
		$\leqslant 1$	(1)	cation ligand (1:1)
			(2)	ligand sandwich (1:2)
		$\geqslant 1$	(1)	cation ligand (1:1)
II	$DN_{average} = DN_{solvent}$			
		$\leqslant$	(1)	cation ligand (1:1)
			(2)	ligand sandwich (1:2)
III	$DN_{average} < DN_{solvent}$	$\geqslant$ $\leqslant$	(1)	cation ligand (1:1)

(2:1) complexes is possible only when $DN_{average}$ is greater than $DN_{solvent}$. The fact that the solvation interaction with solvent molecules is weaker than the ligand interaction enables the ligand cation complex to react with a second cation to form the cation-sandwich complex if [cation]/[ligand] >1 (see Eqs. 1, 3). Similarly, in the concentration region [cation/[ligand) < 1, we expect the formation of ligand-cation (1:1) and ligand-sandwich (1:2) complexes (see Eqs. 1,2), because the cations are maximally solvated by the ligand binding sites.

When $DN_{average} \simeq DN_{solvent}$, there are several distinct stoichiometrics possible. If [cation]/[ligand] > 1, we expect only the ligand-cation (1:1) complex; but if [cation]/[ligand] < 1, we expect both the ligand-cation (1:1) and the ligand-sandwich (1:2) complexes. The cation-sandwich (2:1) complex is not expected to form with an excess of cations because the solvent more effectively binds the excess cations than does the already formed ligand-cation (1:1) complex.

When $DN_{average} < DN_{solvent}$, we expect the formation of the ligand-cation (1:1) complex only. In these solvents, the donicity of which is larger than that of the ligand binding sites, the complexing ability of the ligand is rather limited and apparently only the ligand-cation (1:1) complex may be formed if indeed any is formed at all.

The approximate $DN_{average}$ value of peptides is 27 and the DN of acetonitrile is 14.1. Thus peptides in acetonitrile obey the criterion that $DN_{average} > DN_{solvent}$. In this case we would expect to have cation-sandwich and ligand-sandwich complexes of linear and cyclo-peptides with alkali and alkaline earth cations. Our experimental data confirm this model (see Figs. 5, 6 and Tables III, IV). According to these results we suggest that the complexation of Boc(Gly-L-Pro)$_3$ OBz with Mg $(SCN)_2$ in acetonitrile can be described by the following mechanism (see Fig. 7).

In the first step, one molecule of peptide with six binding sites (amide carbonyl oxygens) solvates one magnesium ion (solvation number = 6) to form a stable (1:1) complex. When the ratio [cation] / [ligand] < 1, the 1:1 complex reacts further with the excess of peptide to form the ligand-sandwich complex; and when [cation]/[ligand] > 1, the 1:1 complex reacts further with an excess of salt to form the cation-sandwich complex. The cation-sandwich complex may react further with an excess of salt to form the tri-magnesium complex.

The values of [cation]/[binding sites] range from $1/12$ in the ligand-sandwich complex to $1/2$ in the tri-magnesium complex. There are, thus, a greater number of ion-dipole interactions of the peptide with the salt in the tri-magnesium complex. The strength of these interactions depends on the donor number of the binding site (polar group), as well as the size, charge, and polarizability of the ion.

It may be suggested that polypeptides and proteins interact with neutral salts in aqueous solutions by the same mechanism. Polar groups (binding sites) are often found in the interior of macromolecules (e.g., the peptide bonds

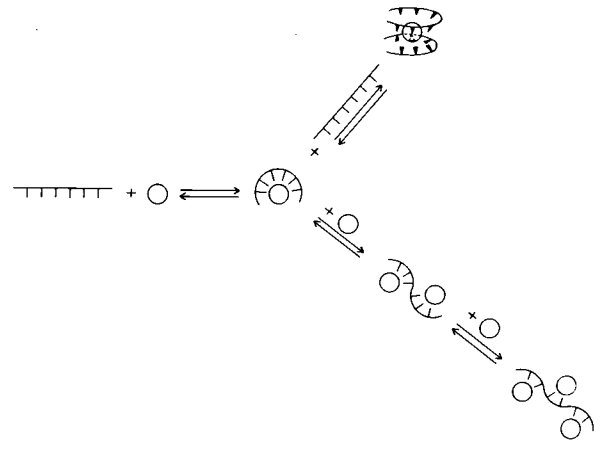

FIGURE 7 Suggested mechanism for the complexation of $Mg(SCN)_2$ by Boc(Gly-L-Pro)$_3$OBz in acetonitrile. O = $Mg(SCN)_2$; ⊤⊤⊤⊤⊤⊤⊤ = Boc(Gly-L-Pro)$_3$OBz.

connecting nonpolar, buried protein side chains), and so interactions of such groups (binding sites) with alkali and alkaline earth cations that tend to alter the free-energy difference between folded and unfolded forms of the macromolecule can certainly alter the stability of macromolecular conformations.

The complexation studies of benzo-15-crown-5 with $NaClO_4$ in nitro-methane ($DN_{average} > DN_{solvent}$) and in diethyl ether, ($DN_{average} = DN_{solvent}$) together with experimental data of other studies of ionophores complexed with alkali and alkaline earth cations analyzed according to Table VI, support the above model (18–33).

Cyclic-peptides in acetonitrile meet the criterion that $DN_{average} > DN_{solvent}$. This is consistent with the formation of cation-sandwich and ligand-sandwich complexes of cyclic peptides with alkali and alkaline earth cations and ammonium salts in this particular solvent (23–30).

Additional support for this model comes from complexation studies of $LiClO_4$ with a linear amido-ether ligand in nitro-methane and pyridine done by Olsher et al. (31). The approximate $DN_{average}$ of the amido ether ligand is 23.5 and DN of nitramethane is 2.7. In this case $DN_{average} > DN_{solvent}$ again; the cations are maximally solvated by the ligand binding sites rather than by solvent molecules. Thus we find the cation-sandwich (2:1) complex in solutions with excess salt. The DN of pyridine is 33.1. The amido ether in pyridine therefore meets the criterion that $DN_{average} < DN_{solvent}$ leading to the formation of only the cation-ligand (1:1) complex in this solvent.

Complexation studies of the natural antibiotics valinomycin (18–20, 22), enniatins (20, 21) and antamanide (18–20) also support this model. Eisenman and Margalit (32) have studied ion permeation in lipid bilayers by the amido-ether ligand (31). They found that the amido ether can act in bilayer membranes as a pure equillibrium domain carrier of monovalent cations forming ligand-cation (1:1) and ligand-sandwich (1:2) membrane-permeating complexes. If we assume that the lipid bilayer is a solvent with a very low D.N. value, then the stoichiometry of the complexes can be explained by the suggested model. $DN_{average(amido\ ether)} >> DN_{solvent(lipid\ bilayer)}$; thus we find the ligand-cation (1:1) and ligand-sandwich (1:2) complexes (see Eqs, 1, 2) in the membrane, which is a solution with excess ligand.

CONCLUSIONS

This simple model, although not accounting for the contributions of cooperative and macrocyclic effects to the complexation ability of the natural and synthetic ionophores and ignoring possible entropic contributions (*a*) reflects the important contribution of the solvation properties of the individual binding sites of ionophores, (*b*) does correctly predict the stoichiometry of many alkali and alkaline earth cation complexes with natural and synthetic ionophores in many aprotic organic solvents, and (*c*) does explain the transport of alkali and alkaline earth cations across lipid bilayers and how polypeptides and proteins interact with neutral salts in solutions.

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