221. A Model for Nicotinamide-Tryptophane Charge-Transfer Interactions: the Complexation of Nicotinamide-Ammonium Salts by a Macrocyclic Receptor Molecule Bearing Tryptophane Side Chains

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Summary

The complexation of primary ammonium salt substrates by macrocyclic polyether receptor molecules provides a general method for studying the nature and stereochemistry of intermolecular interactions. The substrates and receptors are fitted each with one of the interacting units and the resulting effects in the complex are analyzed. The method is used to study the biologically important indole-pyridinium donor-acceptor interaction. The complexes between macrocycles, bearing an indole group in side chains, and pyridinium-ammonium salts display a characteristic charge-transfer band. The absorption coefficients and stability constants have been determined. Competition experiments also provide a new method for measuring the stability constants of macrocycle-ammonium complexes in organic solvents.

Introduction. – Most biological processes involve at some stage an interaction between a small organic substrate and a localized area on the surface of a macromolecule. Elucidation of the nature and configuration of the interacting fragments is greatly facilitated if suitable models are available to which the physicochemical properties of the biological system itself may be compared. Such models may simply be mixtures of compounds containing the units X and R whose interaction is being studied, or molecules in which these groups are covalently linked. The latter can give information about the geometrical requirements of the interaction whereas the former give indications about its strength.

We have developed a general model which combines the features of both and retains the mechanistic analogy to the first step of many biological processes, namely substrate binding. Schematically, the molecular fragments X and R are attached respectively to the ligand and to a substrate which form a complex of known strength and configuration. The remarkable ability of the 18-crown-6 macrocyclic structure to complex primary ammonium salts [1] provides a suitable entry into such model systems. In particular, since the complexes of receptor mole-

[1]) ERA. 265 of the CNRS.

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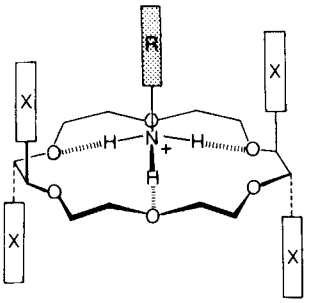


Fig. 1. *Schematic representation of the complexes of the following macrocyclic receptor molecules:*
1 X = L-CONHCH(Y)CH_2-(3-indolyl), Y = COO^-
2 X = L-CONHCH(Y)CH_2-(3-indolyl), Y = $COOCH_3$
3 X = $CONHCH_2CH_2$-(3-indolyl)
4 X = $CON(CH_3)_2$

cules derived from the bis-tartro-crown macrocycle display *lateral interactions* between X and R *(Fig. 1)* [2] [3], they should be suitable for the study of stacking phenomena [4] [5] (nucleic bases, intercalating drugs, aromatic rings, etc.) and related processes.

As models for the indole-pyridinium through space interactions, we investigated the complexation of pyridinium ammonium substrates by macrocyclic ligands bearing indole groups in side chains. Indeed pyridinium and indole nuclei form donor-acceptor (D-A) complexes and the resulting $\pi_D\pi_A^*$ charge transfer (CT) interactions have been used, for instance, to study the environment of the coenzyme NAD^+ [6], the accessiblity of tryptophanyl or tyrosyl residues in proteins [7] and the conformation of the luteinizing hormone releasing factor [8]. A number of model systems, presenting either intramolecular interaction in a linked D-A pair [4] [9–11] or intermolecular association of separate D and A molecules [4] [10] [12] have provided physicochemical support to these investigations. Unfortunately CT-interaction arising from covalently bound partners is important only when linked through a short carbon framework; furthermore since $\varepsilon^{CT}_{trans} \gg \varepsilon^{CT}_{gauche}$ for compounds like D-$(CH_2)_2$-A, additional CT interaction occurring through the linking σ-electron system has to be considered [11]. This interferring through-bond interaction present in covalent model systems is absent in intermolecular D-A complexes, but studies of the latter systems is made difficult by their low stability (especially in polar solvents).

Both problems may be overcome by using an additional binding process, not involved in the CT-interaction, for bringing together the D and A groups in an intermolecular complex, thus allowing only through-space CT-interaction to occur. Such a system may be realized by attaching one moiety (*e.g.* D) to an 18-crown-6 type macrocycle and fitting the other moiety (*e.g.* A) with a primary ammonium group; binding of the NH_3^+ group to the macrocycle is expected to bring the D and A groups into proximity *(Fig. 2)*.

Results. – We synthesized the receptor molecules **1–3** in which tryptophane and tryptamine species are linked to the carboxylic acid groups of a chiral macrocyclic unit, the bis-tartro-18-crown-6 [2]. Substances **1** and **2** have been reported earlier [3], as well as **4** [2]. The tetratryptamido derivative **3** has been obtained by condensing tryptamine with the tetraacid chloride prepared from **4** [2].

When aqueous solutions of the tetratryptophanate receptor **1** and the nicotinamide-ammonium substrate $\mathbf{S_1}$ were mixed, a yellow colour developed up to a ratio

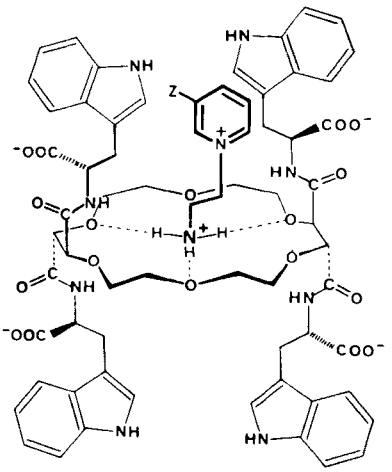


Fig. 2. *Schematic representation of the yellow* [**1**, S_1] *complex* (Z = $CONH_2$)

ligand/substrate 1:1. Subsequent addition of any other complexable cation competitively inhibited this phenomenon. Quantitative analysis of the spectral changes induced by various amounts of S_1 gave the extinction coefficient for the complex $\varepsilon^{320} = 450$ $M^{-1}cm^{-1}$ and the association constant $K_s = 2.8\ 10^3$ M^{-1}. This K_s value is equal within experimental error to those determined by potentiometric titration for S_1 and NH_3^+-$(CH_2)_2$-NH_3^+ [3]. Thus, the D-A interaction seems not to contribute significantly to the energy of complex formation. To examine the structural parameters governing both stability and CT-intensity, we measured K_s and ε for the complexation of various pyridium-ammonium salts with ligands **2** and **3** bearing respectively tryptophane methyl ester and tryptamine residues. The variation of the optical density with substrate concentration is shown in *Figure 3*. Computer-assisted least mean square adjustment to these curves yields the values given in the *Table*.

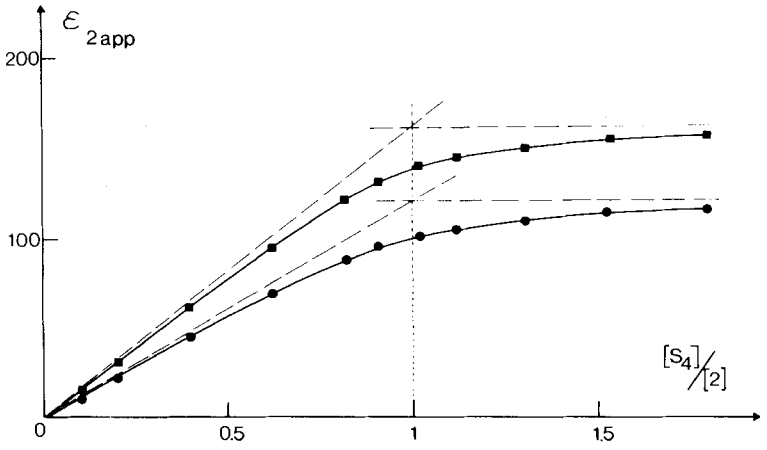


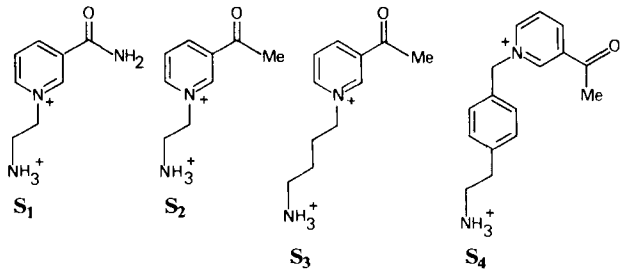
Fig. 3. *Plot of the apparent extinction coefficient for receptor* **2** *at 320* (■) *and 340* (●) *nm* versus *the* [S_4/**2**] *concentration ratio* ([**2**] = 10^{-3}M)

Table. *Stability constants* K_s (M^{-1}) *and extinction coefficients* ε ($M^{-1}cm^{-1}$) *for the complexation of ligands* **2** *and* **3** *with various substrates in acetonitrile*[a])

Substrate	**2**		**3**	
	K_s	ε^{320}	K_s	ε^{320}
S_1	$> 10^6$	255	1.10^5	370
S_2	$> 10^6$	330	$> 10^5$	430
S_3	4.10^4	240	1.10^4	370
S_4	4.10^4	160	$1.5\ 10^4$	235

[a]) Estimated errors are $K_s \pm 20\%$ and $\varepsilon \pm 5\%$.

Discussion. – Examination of space filling models of the complexes based on the solution conformation of other complexes [2] and on the crystal structures of similar molecular fragments [13], provides a basis for rationalizing the K_s and ε sequences. The configurations assumed for the side chains of the receptors **1-3** and for the complexed substrates S_1–S_4 have been adapted from previous studies [2] and from conformational data on related substances.



bis-tetrafluoroborates

a) The strongest indole/pyridinium interaction takes place for a two carbon-long spacer between the positively charged nitrogen atoms; indeed complexes of S_2 display higher extinction coefficients than those of S_3.

b) In their complexes, the pyridinium groups of S_3 and S_4 lie at the same level above the macrocyclic cavity leading to similar stabilities (see d)) although S_4 shows weaker charge-transfer interaction. This last effect may be understood if one takes into account the fact that S_4 is *bent* at the benzylic position, so that the donor and acceptor planes are no longer parallel. Thus the geometrical requirements for optimal CT-interaction (face-to-face [10–12]) may be different from those for the H-transfer reaction between reactive macrocyclic receptor molecules and S_3, S_4 [14].

c) The CT-band is quite sensitive to the electron-attracting properties of the substituent on the pyridinium ring; complexes of S_2 (3-acetyl substitution) have higher extinction coefficients than those of the corresponding amide S_1.

d) The association constants fall roughly into two classes: the two (S_1, S_2) and the four C-atoms or similar spacer (S_3, S_4). The former complexes are more stable than the latter, probably because the stabilizing effect brought about by the macrocyclic polyether is higher in the complexation of S_1 and S_2 where the intramolecular charge-charge repulsion is the strongest. In the complexes of ligand **2** a further stabilization may come from the interaction of the carboxymethyl groups with the

pyridinium cation, especially with S_1 and S_2 where they are the closest. However this lateral interaction sterically hinders the D-A pair formation and despite having higher stabilities, all complexes of **2** show a low intensity CT-band as compared to **3**.

Apart from being a proof for the *intermolecular origin of the CT-band*, the competitive inhibition experiments may be used to *determine association constants* of indole-bearing ligands with other cations or of other ligands with pyridium-ammonium substrates. In this way, for instance, the K_s values of the Na^+ complexes of **2** and **3** have been measured (2.10^6 M^{-1} in acetonitrile) by following the spectral changes produced by addition of $NaBF_4$ to solutions containing (S_1+2) or (S_2+3); similarly, $K_s=3.10^4$ M^{-1} has been obtained for the complexation of S_3 with the tetra(dimethyl)amide-18-crown-6 **4** by adding **4** to a solution of (S_3+2) in acetonitrile.

It is clear that the method described here is not limited to the study of CT-interaction [15]. Model systems for other biologically occurring inter- or intramolecular interaction may be envisaged [4] [5] owing to the ease of structural variation in the side chains borne by the macrocycle and in the nature of the substrate. Such data should provide insight into the fundamental question of the *stereochemistry of intermolecular interactions*. More highly connected, more rigid macropolycyclic ligands are expected to allow a more refined tuning of receptor-substrate complementarity.

Experimental Part

^{1}H-and ^{13}CNMR.-spectra were taken respectively on *Varian* A60 and XL100-15 spectrometers. The chemical shifts are given in ppm downfield from internal TMS. Melting points were taken on a *Kofler* block. Unless otherwise stated, all chemicals were commercially available and used without further purification.

Synthesis of ligands and substrates. – Compounds **1**, **2** [3] and **4** [2] have been reported previously.

(2R,3R,11R,12R)-N, N', N'', N'''-Tetrakis[2-(3-indolyl)ethyl]-1, 4, 7, 10, 13, 16-hexaoxacyclooctadecane 2,3,11,12-tetracarboxamide (**3**). A solution of 0.6 mmol of the macrocyclic tetracarbonyl tetrachloride [2] (see macrocycle in *Fig. 1*, X=COCl) in 5 ml of dry CH_2Cl_2 was added dropwise to a stirred solution of 2.5 mmol of tryptamine and 5 mmol of triethylamine in 20 ml of CH_2Cl_2. The mixture was stirred for 30 min, 2 g of alumina (*Merck* H, Typ 60/E for TLC.) and 5 ml of methanol were added and the slurry evaporated to dryness. The residue was placed on a 25×140 mm (65 g) alumina column and eluted with 6% methanol in $CHCl_3$. The fractions containing the desired product (TLC. and UV. detection) were evaporated under vacuum, and the colourless glass remaining crystallized from hot 96% ethanol as small needles (65%), m.p. 223°, $[\alpha]_D^{20}=+10.3$ ($c=0.75$, $CHCl_3$/MeOH 1:1). – ^{1}H-NMR. (acetone): 2.85 (*s*, indole NH, H_2O); 2.8–3.7 (*m*, $N(CH_2)_2$, OCH_2); 4.35 (*s*, OCH); 6.9–7.8 (*m*, indole); 10.0 (*s*, CONH). – ^{13}C-NMR. (DMSO): 24.7, 39.5 ($N(CH_2)_2Ar$); 68.8, 70.8 (OCH_2); 81.2 (OCH); 111.3, 111.8, 118.2, 120.9, 122.5, 127.3, 136.3 (Indole); 169.6 (CO).

$C_{56}H_{64}N_8O_{10}$, 3 H_2O (1063) Calc. C 63.26 H 6.63 N 10.54% Found C 63.85 H 6.77 N 10.82%

N^1-(2-Aminoethyl)nicotinamide bis-hydrotetrafluoroborate (S_1, $2BF_4^-$). To an aqueous stirred solution (5 ml) containing 1 mmol of N^1-(2-aminoethyl)nicotinamide dihydrochloride [16] was added a solution of 2.15 mmol of silver tetrafluoroborate in 5 ml of water. The precipitated silver chloride was filtered off and the clear solution evaporated to dryness under vacuum. Recrystallization from wet acetonitrile (m.p. 185°, 80%). – ^{1}H-NMR.(D_2O): 3.70 (*t*, $CH_2ND_3^+$, $^3J=6.5$ Hz); 5.0 (*t*, CH_2N^+); 8.1 (*qa*, H_5Py^+); 8.9(*t*, $H_{4,6}Py^+$); 9.2 (*s*, H_2Py^+).

$C_8H_{13}B_2F_8N_3O$ (340.8) Calc. C 28.19 H 3.85 N 12.30% Found C 28.31 H 3.91 N 12.33%

N^1-(2-Aminoethyl)-3-acetylpyridine bis-hydrotetrafluoroborate (**S_2**, *$2BF_4^-$*). A mixture of 3-acetylpyridine (20 mmol) and 2-phthalimidoethyl bromide [17] (20 mmol) in 5 ml of DMF was heated for 7 h at 110°. Ether (50 ml) was added to the cooled mixture and the resulting precipitate was filtered, dissolved in hot methanol (50 ml) and THF added until precipitation began. After cooling, the orange crystals were filtered, washed with ether and dried (65%; m.p. 198°). The phthalimido-protected **S_2** bromide (10 mmol) was refluxed with 10 ml of 48% aqueous hydrobromic acid for 12 h. The precipitated phthalic acid was filtered off and the solution evaporated to dryness. The residue was dissolved in methanol and precipitated with THF (white solid, 50%). Anion exchange as previously described gave a colourless glass which crystallized upon standing. – ^{1}H-NMR. (D_2O): 2.70 (*s*, CH_3); 3.70 (*t*, $CH_2ND_3^+$, $^3J = 6.5$ Hz); 5.0 (*t*, CH_2N^+); 8.15 (*qa*, H_5Py^+); 9.95 (*m*, $H_{4,6}Py^+$); 9.35 (*s*, H_2Py^+).

$C_9H_{14}B_2F_8N_2O$ (339.8) Calc. C 31.80 H 4.15 N 8.24% Found C 31.90 H 4.11 N 8.21%

N^1-(4-Aminobutyl)-3-acetylpyridine bis-hydrotetrafluoroborate (**S_3**, *$2BF_4^-$*). The experimental procedure for the preparation of 4-phthalimidobutyl bromide is the same as for 2-phthalimidoethyl bromide [17]. The brown crystalline compound was further chromatographed on an alumina column (activity II–III, hexane/$CHCl_3$ 1:1) and crystallized from the same solvent mixture (40%, m.p. 79°). This bromide (20 mmol) and 3-acetylpyridine (20 mmol) were heated at 130° in 4 ml of anhydrous DMF for 3 h. The cooled mixture was filtered and the residual powder washed with DMF and ether; 75%, m.p. 245° (from ethanol/methanol). Deprotection of the primary amino group and anion exchange were performed as described above. The residual glass crystallized on standing for a few weeks. – ^{1}H-NMR. (D_2O): 1.5–2.4 (*m*, CH_2CH_2); 2.70 (*s*, CH_3); 3.05 (*t*, $CH_2ND_3^+$, $^3J = 6.5$ Hz); 4.75 (*t*, CH_2N^+, $^3J = 7$ Hz); 8.25 (*qa*, H_5Py^+); 8.9–9.2 (*m*, $H_{4,6}Py^+$); 9.45 (*s*, H_2Py^+).

$C_{11}H_{18}B_2F_8N_2O$ (367.9) Calc. C 35.91 H 4.93 N 7.61% Found C 35.88 H 4.86 N 7.63%

N^1-(4-(2-Aminoethyl)benzyl)-3-acetylpyridine bis-hydrotetrafluoroborate (**S_4**, *$2BF_4^-$*). To a solution of methyl *a*-cyano-p-toluate (44 mmol) (prepared from *p*-toluic acid [18]) in anhydrous THF (40 ml) 125 ml of 1.5M diborane in THF were added dropwise with stirring over 1.5 h. The mixture was refluxed for 10 h and concentrated hydrochloric acid (15 ml) was then added cautiously. The oil remaining after evaporation of the solvent was dissolved in water (100 ml) and washed with $CHCl_3$ (50 ml). The aqueous phase was basified with 30% w/w NaOH and extracted twice with $CHCl_3$ (50 ml). The organic layer was dried (K_2CO_3), filtered and evaporated under vacuum giving *p*-(2-aminoethyl)benzyl alcohol as a colourless oil which slowly cristallized in the cold (85%, m.p. <20°). A solution of 10 mmol of this aminoalcohol in $CHCl_3$ (3 ml) and 10 ml of 40% HBr in acetic acid was stirred for 10 min, ethyl acetate (40 ml) was added and the precipitated 4-(2-aminoethyl)benzyl bromide hydrobromide filtered, washed and dried (75%, m.p. >260°). A mixture of this salt (5 mmol) and 3-acetylpyridine (5 mmol) in methanol (30 ml) was stirred at RT. for 12 h and then refluxed for 1 h. The cooled solution was filtered and the precipitated **S_4**, $2Br^-$ crystallized from methanol/ethanol (40%, m.p. >260°). Anion exchange as described for **S_1** and crystallization from 96% ethanol yields **S_4**, $2BF_4^-$ (m.p. 213°). – ^{1}H-NMR. (D_2O): 2.70 (*s*, CH_3); 3.10 (*m*, $CH_2CH_2ND_3^+$); 5.90 (*s*, CH_2N^+); 7.45 (*s*, 4 H benz.); 8.20 (*qa*, H_5Py^+); 9.10 (*m*, $H_{4,6}Py^+$); 9.45 (*s*, H_2Py^+).

$C_{16}H_{19}B_2F_8N_2O$ (428.9) Calc. C 44.80 H 4.47 N 6.53% Found C 44.72 H 4.59 N 6.52%

Measurements. – UV./VIS.-absorption spectra were recorded on a *Cary* 118 spectrometer using 1 cm optical path cells. Acetonitrile was twice distilled over P_2O_5 and stored in a quartz bottle. Stock solutions of ligands and substrates were *ca.* 10^{-2}M (the concentrations derived from known weights and from optical density measurements agreed within ±5%; compound, λ_{nm}, $\varepsilon_{M^{-1}cm^{-1}}$: **1,2,3,** 280, 20800; **S_1**, 266, 4400; **$S_{2,3,4}$**, 266, 3800) and checked for the absence of Na and K (*Varian* atomic absorption spectrophotometer); $(Na + K) < 10^{-4}$M. Typically, 1 ml of a 10^{-3}M ligand solution was titrated with increasing amounts of substrate and the UV./visible spectrum was recorded for each increment (broad absorption band extending from 300 to *ca.* 460 nm). Concentrations in the reference cell were exactly the same except that the starting acetonitrile stock solution contained excess potassium tetrafluoroborate ($5 \cdot 10^{-3}$M). At the end of the titration, excess KBF_4 was added to the measuring cell and the difference absorption spectrum returned to the baseline. Optical densities were measured at different wavelength and treated together with the ligand and substrate concentrations by a least mean square programme yielding K_s and ε for the best fit.

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