CONFORMATION OF THE Na^+ COMPLEX OF ANTAMANIDE IN SOLUTION

V.T.Ivanov, A.I.Miroshnikov, N.D.Abdullaev, L.B.Senyavina, S.F.Arkhipova, N.N.Uvarova, K.Kh.Khalilulina, V.F.Bystrov, and Yu.A.Ovchinnikov

Shemyakin Institute for Chemistry of Natural Products, USSR Academy of Sciences, Moscow, USSR

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Summary. The conformational states of the cyclodecapeptide antamanide and of its Na^+ complex have been studied by a number of spectroscopic methods. Two conformers were found for antamanide of which one, existing in non polar solvents has all six NH groups hydrogen bonded. A three dimensional structure of the antamanide·Na^+ complex has been proposed containing four intramolecular hydrogen bonds formed by the amide CO and NH groups. The sodium ion is held in the inner cavity by ion-dipole interaction with six amide carbonyls. The proposed structure explains the efficiency and selectivity of the antamanide complexation reaction

Wieland and collaborators[1] isolated from extracts of the poisonous Amanita phalloides antamanide (AA, Fig. 1), a new peptide which they found capable of inhibiting the toxic principles of this mushroom, namely phalloidine, the amanitins etc. In a joint work of Wieland's and our laboratories AA was shown[2] to associate in alcoholic solutions with Na^+ and K^+, forming 1:1 complexes with stability constants, respectively ~2500 and ~250 l/mol. The definitely expressed Na^+ specificity of AA is a unique property of this compound, all the known naturally occurring alkali metal complexones (valinomycin[3], enniatins[3], macrotetrolides[4] etc.) preferably complexing with larger cations (K^+ and Rb^+).

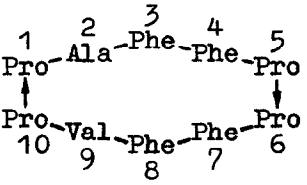

Fig.1. Antamanide

Considering that conformation plays a decisive part in the efficiency and specificity of macrocycle complexation with alkali metals[3], we have undertaken a study of the spatial structure

of AA and its Na^+ complex in solutions by the joint use of a number of physicochemical methods combined with theoretical conformational analysis. Such an approach has proved to be of quite general application being successfully employed for determining the spatial structure of model cyclohexapeptides[5,6], gramicidin S[7], the enniatins[8] and valinomycin[9]. Recently a similar approach was discussed by Gibbons et al.[10].

The sample used in the present study, obtained by a total synthesis in our laboratory[11], was identical with naturally occurring AA kindly sent to us by Wieland. The ORD curves of AA are complex and strongly dependent upon the nature of the solvent (Fig. 2). The presence of an isosbestic point at 230 nm is

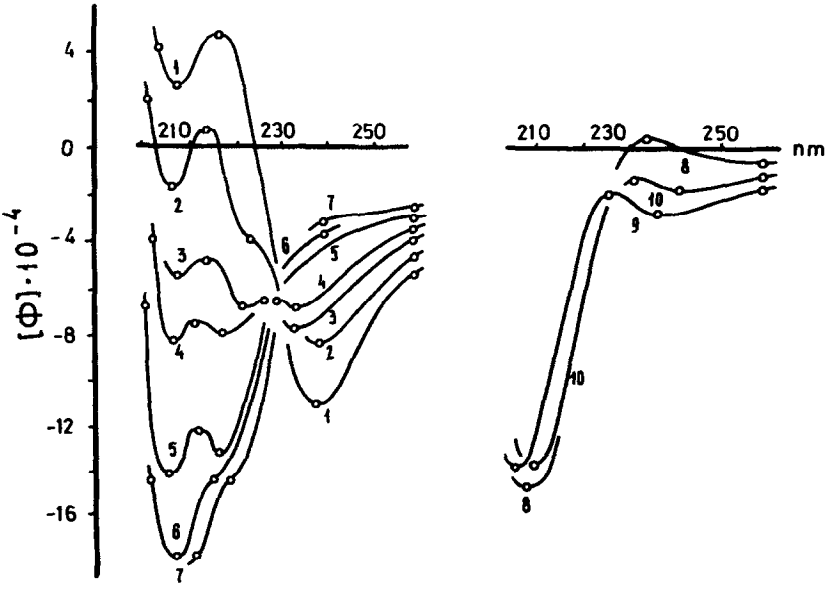

Fig. 2. ORD curves of AA and its Na^+ complex. 1 C_7H_{16}-dioxane (5:2); 2 CH_3CN; 3 C_7H_{16}-C_2H_5OH (9:1); 4 CF_3CH_2OH; 5 96% C_2H_5OH; 6 H_2O-CF_3CH_2OH (5:2); 7 H_2O-C_2H_5OH (3:1); 8 $1.4\cdot10^{-3}$ M $NaSO_3C_{12}H_{25}$ in CH_3CN (4-fold excess of salt); 9 $3.0\cdot10^{-3}$ M $NaSO_3C_{12}H_{25}$ in CF_3CH_2OH (10-fold excess of salt); 10 $2.0\cdot10^{-2}$ M NaCl in 96% C_2H_5OH (40-fold excess of salt)

strong argument in favor of the participation of only two forms of AA in the conformational equilibrium. The single NH stretching band (at ~3330 $\underline{cm}^{-1}$, Fig.3) shows that in non polar solvents all the NH hydrogens of AA participate in intramolecular hydrogen bonding (IMHB).

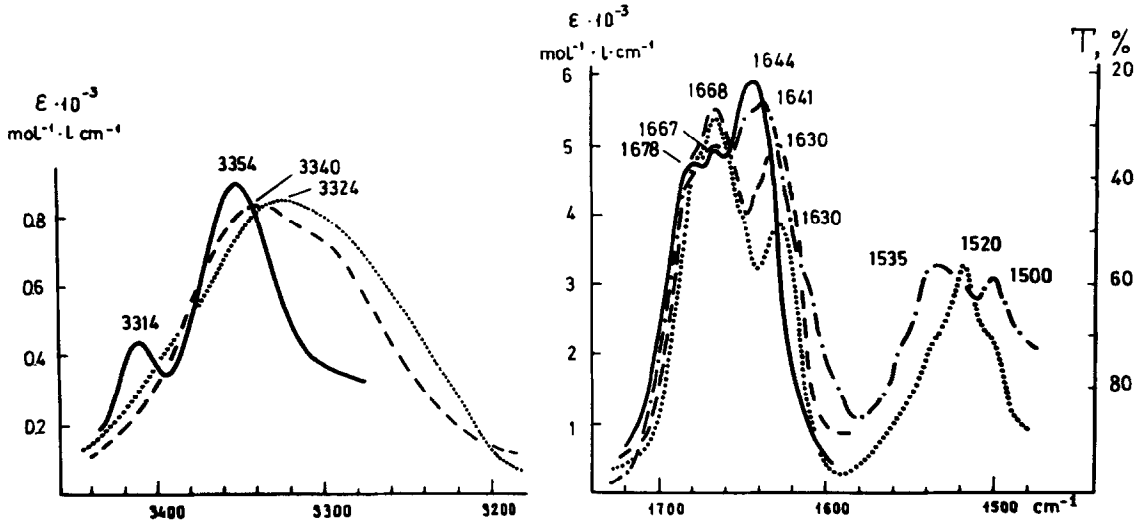

Fig.3. IR spectra of AA and its Na^+ complex. ····· $CHCl_3$; --- CCl_4-CH_3CN (2:1); —— $1.5 \cdot 10^{-2}$ M NaNCS in CCl_4-CH_3CN (2:1) (5-fold excess of salt); -·- crystalline complex, KBr disc (transmission)

Our main attention was next devoted to the AA "complexing" conformation. Contrary to the ORD curves of the free substance those of the Na^+ complex are only weakly solvent dependent, indicating rigidity of this conformation. The structure of the Na^+ complex was deduced largely from its NMR spectra, particularly from analysis of the NH proton signals, three differing pairs of doublets in the downfield region (Fig. 4, Table 1), each pair being very similar in both the chemical shifts and the $^3J_{NH-CH}$ constants. Taking into account the primary structure of AA, particularly its possession of two conformation-restricting[12,13] Pro-Pro fragments separated by two "normal" tripeptide fragments,

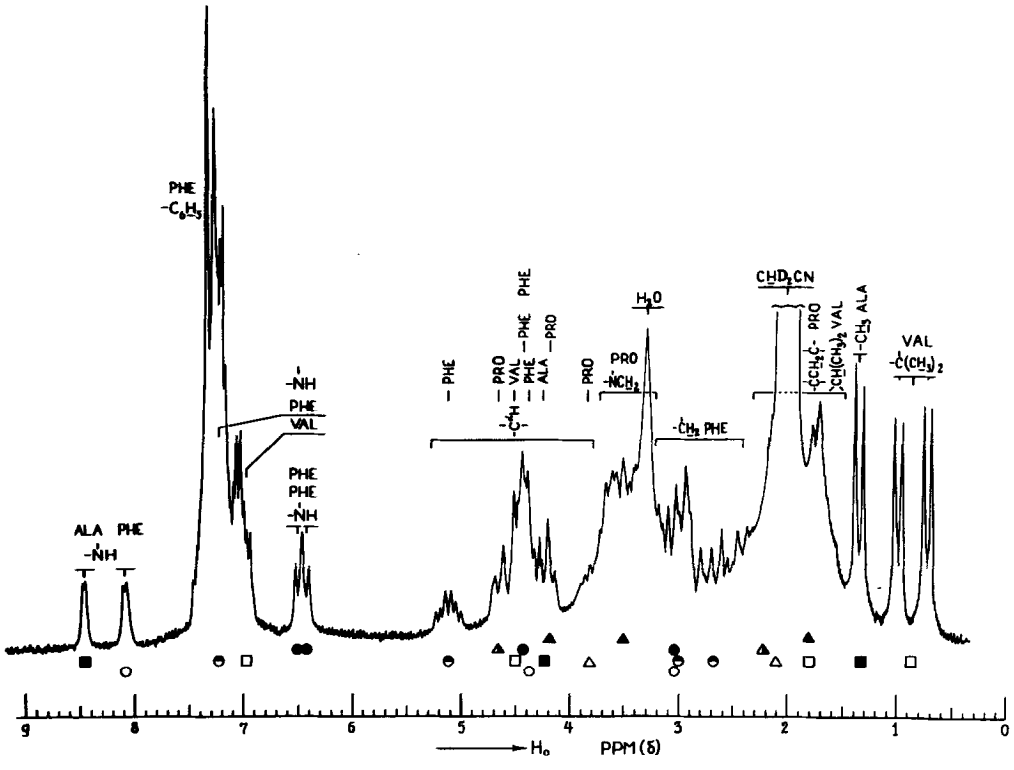

Fig. 4. 100-MHz NMR spectrum of the Na·AA complex in CD_3CN. The squares and circles indicate signals of the spin coupled protons as determined by double resonance experiments

Ala-Phe-Phe and Phe-Phe-Val, it could be conjectured that the above mentioned properties of the spectra reflect the existence of a pseudo 2-fold axis and that the above pairs of signals stem from conformationally equivalent amino acid residues situated on diametrically opposite sides of the ring, i. e. ^{2}Ala and ^{7}Phe, ^{3}Phe and ^{8}Phe, ^{4}Phe and ^{9}Val. As one can see from Fig. 4 and Table 1, this assumption is in agreement with the signal assignments by double resonance.

The considerable differences between the IR amide I region of the Na^{+} complex and the free AA (Fig. 3) are apparently due to participation of the amide CO groups in ion-dipole interaction with the central cation. The IR spectra also indicate that

Table 1. NMR Data on the NH Signals of the Antamanide·Na^+ Complex*

Solvent	Spectral parameter	Amino acid residue						MeCO--NHMe
		^{2}Ala	^{7}Phe	^{4}Phe	^{9}Val	3 or ^{8}Phe	8 or ^{3}Phe	
$CDCl_3$-CH_3OH, 1:1	δ, ppm	8.89	8.57	-	-	6.54	6.21	-
	$\Delta\delta/\Delta T\cdot10^3$, ppm/deg.	6.3	6.0	-	0.6	4.0	-2.9	8.7
	$^3J_{NH-CH}$, Hz**	<2.9	<3.0	-	-	7.1	8.2	-
$CDCl_3$-CD_3OD, 1:1	$t_{1/2}$***	~30 min	~40 min	-	-	~3.5 hrs	~3 hrs	-
CD_3CN	δ, ppm	8.49	8.11	7.26	7.03	6.50	6.43	-
	$\Delta\delta/\Delta T\cdot10^3$, ppm/deg.	4.4	4.4	1.5	1.7	2.3	-0.3	5.0
	$^3J_{NH-CH}$, Hz**	3.0	3.9	9.8	10.0	7.1	7.8	-
CH_3CN-CH_3OH, 2:1	δ, ppm	8.56	8.20	-	-	6.56	6.42	-
	$\Delta\delta/\Delta T\cdot10^3$, ppm/deg.	3.7	4.4	-	1.5	4.4	0.7	7.2
	$^3J_{NH-CH}$, Hz**	3.0	3.8	-	-	7.3	8.3	-
CH_3CN-CD_3OD, 2:1	$t_{1/2}$***	5-10min	5-10min	>24hrs****	-	~1.5 hr	~45 min	-
CH_3OH	δ, ppm	8.98	8.64	-	-	6.79	6.47	-
	$\Delta\delta/\Delta T\cdot10^3$, ppm/deg.	4.1	4.6	-	2.0	6.7	1.4	7.5
	$^3J_{NH-CH}$, Hz**	<2.6	3.0	-	-	6.9	8.1	-
CD_3OD	$t_{1/2}$***	<5 min	<5 min	-	-	~35 min	~15 min	-

*The complex was prepared by adding 4-10 fold excess of NaNCS to the solution

**Corrected for electronegativity of the substituents (+0.6 Hz[15])

***$t_{1/2}$ - half lifetime of the NH signals

****Deuterium exchange rate estimated from the shape of the $C^{\alpha}H$ signal

the complex contains both free (ν_{NH}=3414 cm^{-1}) and bound (ν_{NH}= =3354 cm^{-1}) NH, the ratio being 2:4 as shown from the integral intensities A, making use of a specially plotted A - ν_{NH} correlation curve[6]. The NH groups were located from the NMR determined rates of their deutero-exchange in different media and the temperature dependence of their proton chemical shifts (cf.[7,14]). Thus it can be seen (Table 1) that the ^{3}NH and ^{8}NH groups have lower exchange rates in all solvents than the ^{2}NH and ^{7}NH groups, and that the ^{4}Phe and ^{9}Val NH have only slight temperature dependences of the chemical shifts while the ^{9}Val NH exchange rate is the slowest of all in CH_3CN-CD_3OD (2:1). Hence, despite the considerable barriers presented to the straightforward interpretation of the NMR spectra by the presence of the phenyl groups it can be quite reliably concluded that the four NH groups participating in IMHB are ^{3}NH, ^{4}NH, ^{8}NH and ^{9}NH, whereas the ^{2}NH and ^{7}NH groups are free and are the ones responsible for the 3414 cm^{-1} band in the IR spectra.

Following this we carried out a conformational analysis of the AA molecule, the aim being to select structures fulfilling the conditions: a) the existence of a pseudo C_2 axis; b) trans configuration of the secondary amide groups, following from the presence of an intense amide II band in the IR spectra of the Na^+ complex (~1530 cm^{-1}, Fig. 3); c) participation of the NH groups of the ^{3}Phe, ^{4}Phe, ^{8}Phe and ^{9}Val residues in IMHB with the amide carbonyls; d) gauche orientation of the protons in the NH-C^{α}H fragments of the ^{2}Ala and ^{7}Phe residues (Φ 0-10, 110-200 or 280-360), as follows from the $^{3}J_{NH-CH}$ constants ($\leq$3.9 Hz), taking into account their stereochemical dependence[15]; e) cis or distorted trans orientation of the NH-C^{α}H protons in ^{3}Phe and ^{8}Phe residues (Φ 220-260,~30 or ~90), from $^{3}J_{NH-CH}$=6.9-8.3 Hz;

f) trans orientation of the NH-C^{α}H protons in ^{4}Phe and ^{9}Val residues (Φ 40-80), from $^{3}J_{NH-CH}$=9.8-10.0 Hz.

In the course of the analysis consideration was given to all possible combinations of IMHB corresponding to trans-trans--trans-trans, trans-cis-trans-cis, cis-trans-cis-trans and cis--cis-cis-cis configurations of the proline tertiary amide bonds (in all over 100 structures). Bearing in mind that for the formation of a stable complex the presence of 4-8 interiorly oriented CO groups (by analogy with other macrocyclic complexones) is necessary, the above cited conditions are satisfied by only one type of conformations with all amide bonds trans, the ^{2}Ala and ^{7}Phe carbonyls in type 3→1 IMHB with ^{4}NH and ^{9}NH and the CO of ^{5}Pro and ^{10}Pro in type 4→1 IMHB with ^{8}NH and ^{3}NH; the ^{1}Pro, ^{3}Phe, ^{4}Phe, ^{6}Pro, ^{8}Phe and ^{9}Val carbonyls being oriented inwardly in the molecule and participating in ion-dipole interaction with the central Na^{+} (Fig.5). The following mean conformational parameters of the AA·Na^{+} complex were determined with

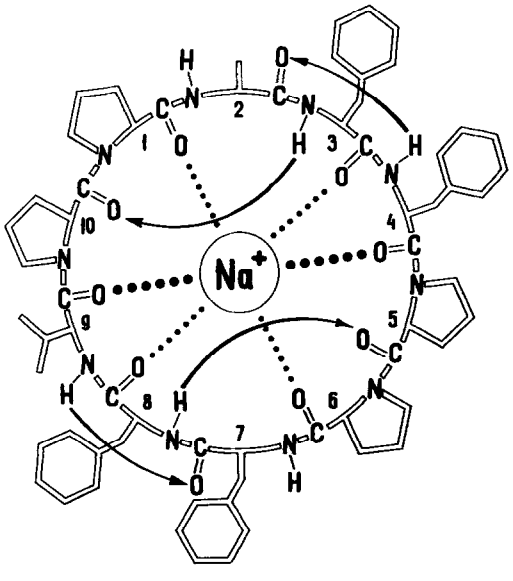

Fig. 5. Hydrogen and ion-dipole bonding in the Na^{+}·AA complex

account of the theoretical data on proline-containing peptides [12,13], of the type $3 \rightarrow 1^{13}$ and $4 \rightarrow 1^{16}$ IMHB and of the restrictions imposed by the cyclic structure:

	$^{1}Pro, ^{6}Pro$	$^{2}Ala, ^{7}Phe$	$^{3}Phe, ^{8}Phe$	$^{4}Phe, ^{9}Val$	$^{5}Pro, ^{10}Pro$
Φ	120	330	250	80	120
Ψ	270	150	110	20	130

Examination of the deduced conformation (Fig. 6) reveals striking similarities to the bracelet form of valinomycin[9], namely the presence of a IMHB system of condensed rings and participation of six carbonyls in the ion-dipole interaction with the cation. There are, however, essential differences between the structures of the two complexes. Thus, the smaller inner cavity of AA as determined by the distance between ^{4}O and ^{9}O atoms is 2.5 Å (compared with ~3.0 Å for the diameter of the valinomycin cavity) which, taking into account the conformational rigidity of the IMHB system, explains the Na^{+} specificity of the AA complexing reaction. Moreover, whereas all six carbonyl groups of

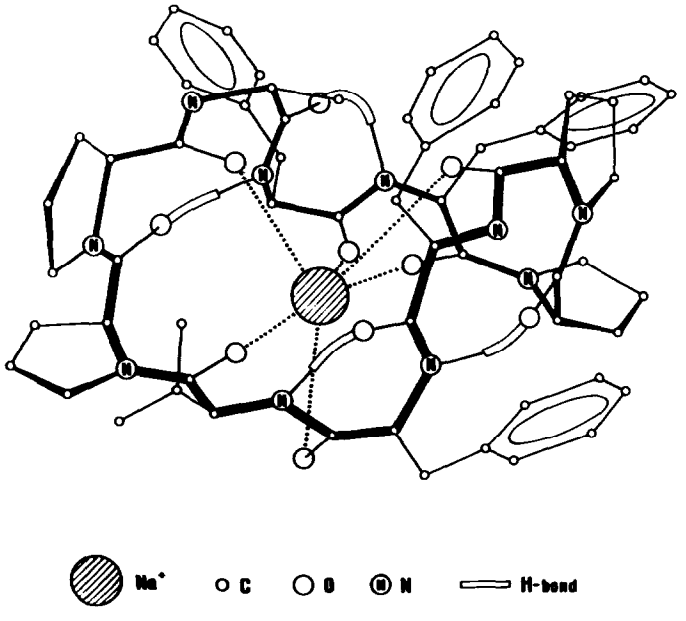

Fig. 6. Conformation of the Na^{+}·AA complex in solution

valinomycin participating in the ion-dipole interaction are oriented almost exactly in the direction of the central cation, similarly efficient interaction in AA is possible only for the ^{4}CO and ^{9}CO groups; the ^{1}CO, ^{3}CO, ^{6}CO and ^{8}CO bonds forming a considerable angle with the $Na^{+}\cdots O$ direction, resembling in that respect the enniatin complexes[3]. Furthermore, the ^{1}O, ^{3}O, ^{6}O and ^{8}O atoms are more remote from the center (3.4–4.0 Å) than the ^{4}O and ^{9}O atoms (~2.65 Å), which also lowers the efficiency of their interaction with the cation. Apparently these circumstances are responsible for the considerably lower stability constant of the $Na^{+}\cdot AA$ than the $K^{+}\cdot$valinomycin complex and for the close values of these constants in the Na^{+} complexes of AA and the enniatins.

The data obtained may serve as basis for analyzing the dependence between primary structure and complexing ability and also biological activity in the series of AA and its analogs. In addition, elucidation of the conformation of the $Na^{+}\cdot AA$ complex should lay a more rational basis for the search for AA analogous Na^{+} complexones that could selectively induce sodium permeability in artificial and biological membranes. Such work is at present in progress in our laboratory.

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