ANTAMANIDE AND ANALOGS. STUDIES ON SELECTIVITY AND STABILITY OF COMPLEXES.*)

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Summary: The complexing behaviour of antamanide was studied with various mono- and bivalent cations in different solvents. Complexes of highest stability were found for Na, Ca and Tl(I). Polarity of solvent affects complex stability constants strongly, but the selectivity to a lesser degree. The antamanid-Na complex was isolated as a crystalline perchlorate.
UV and CD spectral changes on addition of water indicate a transition between defined solvation states of antamanide, that were referred to a conformational change previously detected. The biological activity of some analogs of antamanide was related to Na complexation. From the analogs synthesized up to now it is evident, that Na complexation or at least an attribute of the molecule running parallel to this, is a prerequisite, but not the only one, for biological activity.

The interaction of antamanide (AA) (2) with Na and K ions was discovered in Shemyakin's and our laboratories (3). We now describe the selectivity of AA against different ions by solvent extraction experiments as described by Pedersen (4). Here the extraction from aqueous solutions of metal picrates by AA dissolved in CH_2Cl_2 corresponds to complex formation. For alcali ions (% picrate extracted, based on AA) and alcali earth ions (% picrate extracted based on picric acid) we found the values given in table 1:

	r[Å]	% picrate extracted		r[Å]	% picrate extracted
Li	0.60	0.8	Mg	0.65	0.0
Na	0.97	14.0	Ca	0.99	9.7
K	1.33	0.7	Sr	1.13	1.2
Rb	1.48	0.3	Ba	1.35	0.7
Cs	1.67	0.2			
NH_4		0.5			

*) Part XIII of series: Antamanide. Part XII: Ref. (6) of this paper.

Evidently, among the alcali and alcali earth ions those of radii of about 1 Å are preferred by AA. Analogs of AA which were devoid of biological activity, extracted less or non of the Na picrate.

More quantitative assays on complexation capacity, including also other cations, were done by spectroscopic methods. By CD measurement at 224 nm we followed the influence of cations on the $n \rightarrow \pi^*$ transitions of presumably non planar amide groups (6). Here again Na and Ca proved to form the most

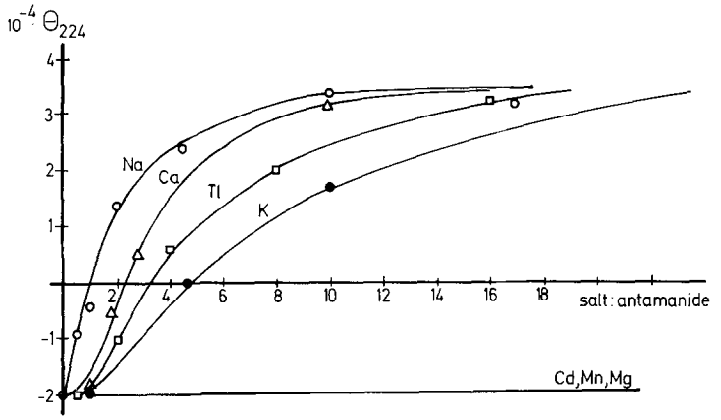

figure 1

stable complexes. Half complexation in CH_3OH was found for $3.4 \cdot 10^{-3}$ M AA solutions at a molar ratio of AA: Na = 1.5 : 1, for Ca correspondingly at 3 : 1, for K at 7 : 1. Surprisingly, a complex was formed with Tl (I) (r = 1.47 Å) with half complexation at a molar ratio of 4.5 : 1. If crystallized, the latter might prove helpful for an X-ray analysis of the complex. No spectral changes were caused by Cd, (r = 0.97 Å), Mn (II) (r = 0.80 Å) and Mg (r = 0.66 Å) ions. Different spectral changes, however, were seen on addition of trivalent rare earth metals. These spectra were clearly distinguishable from the spectrum of the complex, being in all cases identical and independent of the nature of cation and the solvent used. The spectra of Sm(III) (r = 1.04 Å), Gd(III) (r = 0.97 Å), Tb(III) (r = 0.93 Å) and Nd(III) (r = 1.04 Å) indicate an unspecific interaction, perhaps at the surface of the AA molecule. It seems unpromising so far to use paramagnetic shifted NMR signals to distinguish protons in the inner part of the AA-complex from those located on the outside.

Complex stability constants were determined by UV spectrophotometric

titration of AA at 248 nm. The use of other methods such as vapour pressure osmometry and ion-selective glass electrodes have been described earlier (3). The titration was based mainly on the blue shift of the amide $n \rightarrow \pi^*$ transitions caused by interaction of the complexed cation with the carbonyl n-electrons. Some absorption curves of AA-Na between 240 and 280 nm are given in the preceding paper (6). The complex stability constants of AA were determined in solvents of different polarity; they are in good agreement with those found by other methods.

Table 2

Stability constants of AA complexes in different solvents obtained by spectrophotometric titration (SPT), vapor pressure osmometry (VPO) and ion selective glass electrodes (ISG).
Accuracy $\pm$ 20 % for $10^2 < K < 2 \cdot 10^3$ and $\pm$ 100 % for $10^2 > K > 10^4$.

cation	anion	solvent	complex stability constants K[l/mole]	method
Na	ClO_4	CH_3CN	$3.0 \cdot 10^4$	VPO, SPT
K	SCN	"	$2.9 \cdot 10^2$	VPO
Ca	ClO_4	"	$1.0 \cdot 10^5$	SPT
Li	Br	CH_3CN-H_2O (96:4)	$1.3 \cdot 10^2$	VPO
Na	ClO_4	"	$2.6 \cdot 10^3$	VPO
K	Br	"	20	VPO
Na	ClO_4	CH_3CN-H_2O (92:8)	$1.2 \cdot 10^3$	VPO, SPT
K	SCN	"	$2.8 \cdot 10^2$	VPO
Na	Br	$C_2H_5-OH-H_2O$ (96:4)	$2.0 \cdot 10^3$	ISG, VPO
K	Br	"	$1.8 \cdot 10^2$	ISG, VPO
Na	Br	$C_2H_5OH-H_2O$ (30:70)	0	ISG
Li	Cl	CH_3OH	< 10	SPT
Na	ClO_4	"	$5.0 \cdot 10^2$	VPO, SPT
K	Br	"	10	SPT
Ca	Cl	"	30	SPT
Tl(I)	NO_3	"	$1.9 \cdot 10^2$	VPO

The results may be summarized as follows: The stability constants depend strongly on polarity of solvent. Large values are found in the more lipophilic solvents CH_3CN and C_2H_5OH. In CH_3OH, $C_2H_5OH-H_2O$ or CH_3CN-H_2O the

values are lower by 1 - 2 orders of magnitude. This was observed likewise for cyclodepsipeptides by Shemyakin et al.(8) and may be explained by the influence of solvation energies on complex stability. The cation as well as the chelating carbonyls must be desolvated during complexation. So addition of water, which increases solvation energies, consequently diminishes the complex stabilities. In C_2H_5OH-H_2O (30 : 70) e.g. no complexation at all was observed (table 2). Correspondingly, attempts to recrystallize the Na complex of AA from CH_3OH-H_2O mixtures (1 : 1) yielded pure AA. Even by flame photometry no Na was detected. On the other hand the (AA-Na)ClO_4 complex was obtained in a crystalline state from organic solvents such as methanol or acetone. Details and analyses are given in the experimental part.

The selectivity of AA for Na > Li, K, which is essentially the consequence of a geometrical adaption of the cyclopeptide to a certain ionic size, is maintained throughout the different solvents, but changes quantitatively due to the individual solvation energies. Values of $K_{Na}/K_K \approx 100$ were observed in C_2H_5OH, CH_3CN, but also in a CH_3CN-H_2O mixture (96 : 4). Decreased selectivity was found in CH_3OH ($K_{Na}/K_K \approx 50$) and in C_2H_5OH-H_2O (96 : 4) ($K_{Na}/K_K \approx 10$).

In the studies of conformational transition (6) we observed that there must be a rather specific interaction of AA with H_2O, which likewise could be followed by spectrophotometric methods. From ultrasonic absorption measurements we knew that even in non polar solvents there is an equilibrium of conformers (12). Addition of H_2O or CH_3OH to AA in non polar

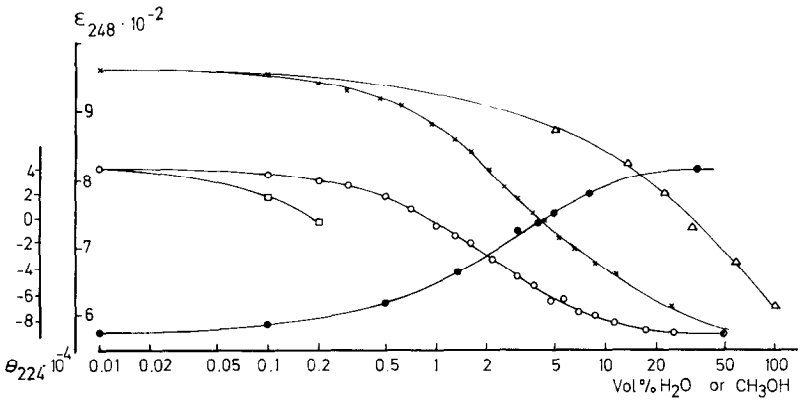

figure 2

solvents caused spectral changes that were quite similar to those caused by complexation. The blue shift of carbonyl absorption observed here can be ascribed to formation of hydrogen bonds to AA by these polar solvents. The titration curves in fig. 2 show the transition of probably two defined solvation states of AA. In CH_2Cl_2 the addition of only 0.2 % of water caused a distinct shift in equilibrium. In CH_3CN 1 % of water was necessary for a similar shift. A final solvation state was reached on addition of H_2O to CH_3CN or 1.4-dioxane, but not on addition of CH_3OH. (Even in pure CH_3OH there is still an equilibrium between different solvation states.) Parallel to this solvation equilibrium a conformational equilibrium of only two species has been discovered by ultrasonic absorption measurements (12) and by the existence of an isobestic point in the ORD spectra (5). As both the solvation and the conformation equilibria depend strongly and similarily on polarity of solvent, it is highly probable that the different solvation states discussed here are identical with the two conformations described earlier (6). In this conformational transition at least two more carbonyls of AA are solvated by hydrogen bridges at the cost of two intramolecular hydrogen bonds.

A specific interaction of H_2O with AA is also suggested by the X-ray studies of Littke (7), who calculated that in the elementary cell of AA crystals, 12 H_2O molecules adjoin one AA molecule.

Structural analogs of AA have been synthesized in order to elucidate relations between constitution and biological activity (9, 10). We have determined Na-complex stability constants for some of these AA derivatives (table 3).

In all analogs investigated so far complexation capacity and biological activity are two attributes of the molecules, which run parallel to each other. This argues for an ionophoric mechanism of AA action against Amanita toxins. From perhydrogenated AA (HAA), however, independently prepared by Ovchinnikov's group and in our laboratories, we learned that Na complexation could not be the only prerequisite of biological activity. HAA formed Na complexes as stable as AA itself, but did not protect against Amanita toxins. Following this observation we compared more extensively the complexing properties of HAA and Gly^1-Gly^4-AA (GGAA), - as the two prototypes of biologically inactive compounds of high and low complexing capacity, respec-

Table 3

Na-complex stability constants in ethanol-water (96:4) related to protective dose of AA and some analogs against 5 mg phalloidine per kg white mouse (LD_{100}).
[AA = cyclo(Val^1-Pro^2-Pro^3-Ala^4-Phe^5-Phe^6-Pro^7-Pro^8-Phe^9-Phe^{10})]

AA analogs	Na-complex stability constants K_{Na}[l/mol]	protective dose PD_{100}[mg/kg]
- - AA	2000	0,5
Leu^1 - -AA	1000	0,5
Ile^1 - -AA	2300	0,5
Ala^1 - -AA	150	15
Gly^1 - -AA	180	10
Gly^1,Gly^4 -AA	100	> 20
Ala^1,Gly^4 -AA	120	15
Pro^6,Phe^7 -AA	50	> 20
Tyr^6 - -AA	2000	0,5
(O-glucosido) Tyr^6 - -AA	1700	1
(O-dodecyl) Tyr^6 - -AA	< 10	> 20
Gly^7 - -AA	60	> 20
(des-Pro^8) -AA	< 10	> 20
Abu^1 - -AA	1000 - 2000	2,5
$(Br_2)Tyr^6$- -AA	3500	2,5

tively, - with those of AA (table 4). It became evident that Na complex stability constants of GGAA in all solvents used are one order of magnitude lower than those of HAA and AA. Further, however, there is one striking anomality that the two biologically inactive analogs of AA have in common: the decreased ability to discriminate between Na and K. As HAA forms stronger complexes with K than does AA, Na selectivity of HAA is only 10 % of that of AA. The Na selectivity of GGAA is even further decreased to only 4 % of the AA value. For HAA, we must consider another dissimilarity, as compared to AA: the four cyclohexyl residues of the former compound decrease solubility in H_2O-containing solvents to such an extent that HAA perhaps does not arrive at the site of AA action in biological systems and consequently is devoid of activity against Amanita toxins.

Table 4

Complex stability constants of AA, perhydro-AA (HAA) and Gly^1Gly^4-AA (GGAA) in different solvents with alcali cations determined by vapour pressure osmometry.

complexing peptide	cation	anion	solvent	complex stability constant K [l/mole]	selectivity $K_{Na}:K_K$
AA	Na	ClO_4	CH_3CN	30 000	
HAA				15 000	
GGAA				1 900	
AA	Li	Br	CH_3CN-H_2O (96:4)	130	
HAA				380	
GGAA				200	
AA	Na	ClO_4	CH_3CN-H_2O (96:4)	2600	~ 100
HAA				3600	~ 10
GGAA				250	~ 4
AA	K	Br	CH_3CN-H_2O (96:4)	20	
HAA				230	
GGAA				70	
AA	Na	ClO_4	CH_3OH	500	
HAA				500	
GGAA				40	

Whatever the reasons may be for the loss of the antitoxic properties of HAA, complexation capacity seems to be at least one prerequisite for biological activity of AA. As known so far, the analogs of AA behave like those of valinomycins and enniatine B, where, for the large number of derivatives synthesized, few complexed K ions, and were devoid of biological activity; in all cases of biological activity, however, the derivative also complexed K ions.

EXPERIMENTAL

Solvent extraction: Alcali picrates were extracted from an aqueous solution

of $5 \cdot 10^{-2}$M metal(I) hydroxide and $5 \cdot 10^{-3}$M picric acid by an 10^{-3}M solution of AA in CH_2Cl_2. Alcali earth picrates were extracted from an aqueous solution of $5 \cdot 10^{-3}$M metal(II) hydroxide, $4.5 \cdot 10^{-2}$M metall(II) chloride and $5 \cdot 10^{-4}$M picric acid by a 10^{-3}M solution of AA in CH_2Cl_2. The picrate concentration in the organic solvent was measured spectophotometrically at 357 nm.

CD measurements were done with a Dichrograph II (Roussel Jouan).

Spectrophotometric titration was carried out in the cuvette with concentrated solutions of salts added by precision syringes. After each addition absorbance was followed with a Cary 14 spectrophotometer at 248 nm. Dilution was corrected for by calculation. K-values were calculated (a) from the half-value points in plots A_{248} against $1/C_{salt}$; (b) from a Hildebrand-Benesi plot (13): $C_{salt}/(A-A_o)$ against $1/(A_\infty - A)$, which yielded straight lines with slope K^{-1}, where A_o, A, A_∞ are the absorbances at 248 nm before, during and after titration, respectively.

Isolation of $[Na \cdot AA]ClO_4$: To a $5 \cdot 10^{-2}$M solution of AA in dry CH_3OH an equimolar quantity of $NaClO_4$ was added under anhydrous conditions. The salt dissolved readily and after a short time the complex crystallized. The liquid was decanted and the solid was recrystallized from a little warm CH_3OH. The crystals were dried and stored in a desiccator. Crystallisation was also successful from acetone solutions.

M.P. : $215-220^{\circ}$ (AA: 172°)

	C	H	N	Na
AA (calc.)	67.00	6.85	12.21	
$[Na \cdot AA]ClO_4$ (calc.)	59.70	6.11	10.87	1.79
(found)	59.60	6.89	10.68	1.88

Na was determined by atomic absorption in a Perkin Elmer spectrophotometer.

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