INTERACTION OF ANTHRACYCLINE ANTIBIOTICS WITH BIOPOLYMERS
VIII. BINDING PARAMETERS OF ACLACINOMYCIN A TO DNA

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The binding of aclacinomycin A to DNA was investigated spectrophotometrically under equilibrium conditions. The self-association behaviour of aclacinomycin A was identified as dimerization. Based on a model of overlapping potential binding sites the subsequent results were obtained: equilibrium constant of cooperative binding $K=(7.58\pm2.15)\times10^8$ M^{-1}, size of a binding site $\alpha=3.98\pm0.14$ base pairs, cooperativity parameter $\sigma=0.12\pm0.10$. These parameters were compared with those of adriamycin, daunomycin, and iremycin to draw some conclusions regarding the structural specialities of aclacinomycin A.

The isolation and characterization of aclacinomycin A (ACM, aclarubicin, for chemical structure see Fig. 1) and several aclacinomycin analogues from *Streptomyces galilaeus* MA144-M1 have been carried out by the research groups of UMEZAWA, and of the Sanraku Ocean Co.[1~6]. The mechanism of action of aclacinomycin A was originally investigated in the laboratory of TANAKA[7,8].

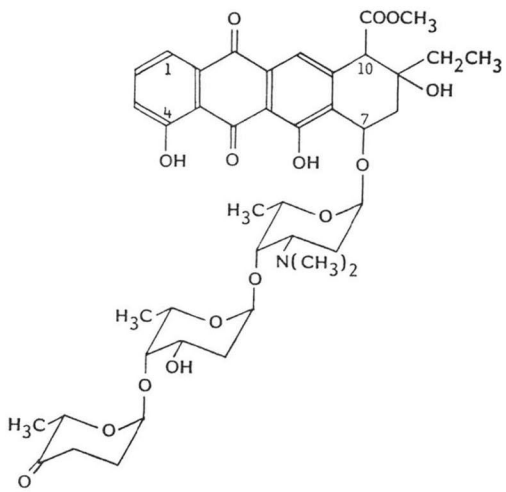
ACM has potent antitumor activity against various experimental tumors coupled with a low cardiotoxicity[3,4] and a fast cellular uptake[9].

From biochemical evidence of the inhibition of DNA-, RNA-, and protein synthesis DI MARCO *et al.*[10] at first concluded that the cancerostatic activity of anthracyclines — having basic sugar residues — is caused by their affinity to form complexes with DNA. Nevertheless other cellular targets are known, *e.g.*, membranes responsible for cytotoxicity[11,12].

Subsequent to the early binding studies[10] on anthracyclines, their interactions and reactions with DNA have been investigated widely from the thermodynamic, kinetic and structural points of view[8,13~22].

Because MATSUZAWA *et al.*[5] have shown that ACM is more effective in the inhibition of DNA- and RNA-synthesis *in vitro* than anthracyclines with monosaccharides, *e.g.*, adriamycin (ADM, doxorubicin) and daunomycin (DAM, daunorubicin), we have investigated the binding of ACM to DNA according to a more appropriate model[15] in order to compare the equilibrium binding parameters with those of ADM, DAM and iremycin (IRM), which were determined recently[15~19].

Fig. 1. Structural formula of aclacinomycin A.



Experimental and Theoretical Details

Substances

ACM was kindly provided by Prof. H. UMEZAWA, Institute of Microbial Chemistry, Tokyo. Because ACM solutions were found to be very light-sensitive (its absorbance at $\nu = 23{,}000\ cm^{-1}$ is diminished to about 50 percent after exposure to day light of different intensities within 15 minutes to several hours) ACM solutions were kept in the dark. Calf-thymus DNA was prepared by SARFERT (Dept. of Mol. Biochemistry of our institute) and extensively dialyzed against McIlvaine buffer (pH 6.0, ionic strength: 0.2 M) prior to the spectrophotometric measurements. All measurements were carried out at 25°C.

Method

For the determination of ACM-DNA binding parameters spectrophotometric measurements were performed with the microcomputer-controlled spectrophotometer Specord M 40 (VEB Carl Zeiss, Jena). The absorbances A were recorded and printed at nine selected wavenumbers. All measurements were performed with the titration technique described by SCHÜTZ *et al.*[15].

Theoretical Analysis

Matrix-rank analysis was applied to determine the number of spectroscopically independent components. Both the parameters of association and binding parameters were estimated with the curve-fitting program ALAU (by H. SCHÜTZ from our institute). The data consistency of the binding measurements was checked by means of a model-free analysis, see GATTI *et al.*[23]. The binding experiments were evaluated according to a single-step-binding-mechanism model of overlapping potential binding sites with nearest neighbour interaction among bound ligands[24], DNA is considered a linear homogeneous and infinite chain. This model was extended taking into account self-association of ACM into dimers. For illustration of the binding data binding isotherms were constructed. The binding isotherm was plotted as r *vs.* c_m (r: number of ligands bound per base pair, c_m: concentration of free monomeric ligands). For further details concerning data analysis see SCHÜTZ *et al.*[15].

Results

Self-association

Recently the tendency of several anthracyclines to form aggregates in solution[15~17,25,26] has been shown. Therefore the concentration dependence of absorption spectra of ACM-buffer solutions was investigated in the range of 3×10^{-7} M to 6×10^{-4} M. In Fig. 2 the apparent extinction coefficient ε_{app} *vs.* the logarithm of the total ACM concentration c_t is shown. The apparent extinction coefficient ε_{app} decreases at higher concentrations, *i.e.*, ACM also shows self-association. The results of the matrix-rank analysis indicate the occurrence of a pure monomer-dimer equilibrium. The dimerization parameters ε_m, ε_d (extinction coefficients of monomers and dimers) and K_d (equilibrium constant of dimerization) are listed in Table 1. The extinction coefficients of monomers and dimers are comparable with those of IRM[17], ADM[16], and DAM[15], whereas K_d is significantly smaller. With the parameters from Table 1 the full lines in Fig. 2 were calculated. At low concentrations hints for cell-wall adsorption of the drug exist. However, the corresponding effects[15] were neglected in the further evaluations.

Binding Parameters

The binding parameters K (cooperative equilibrium constant in the single contiguous case, corresponds to $K\omega$ in the nomenclature of MCGHEE and VON HIPPEL[27]), σ (cooperativity parameter after ZIMM and BRAGG[28]), α (number of base pairs per bound ligand) and ε_b (extinction coefficient of the complex) were obtained from titrations according to the description in section 2 and are summarized in Table 2. K and α of ACM are significantly higher than the corresponding values of IRM, DAM, and ADM.

Fig. 2. Apparent extinction coefficient ε_{app} *versus* the logarithm of the total aclacinomycin concentration c_t at two different wave numbers (points: experimental values obtained by measurements in cells with a length d between 0.2~5 cm, full lines: calculated with parameters of Table 1).
ε_{app} is defined by $\varepsilon_{app} \equiv A/(d\ c_t)$.

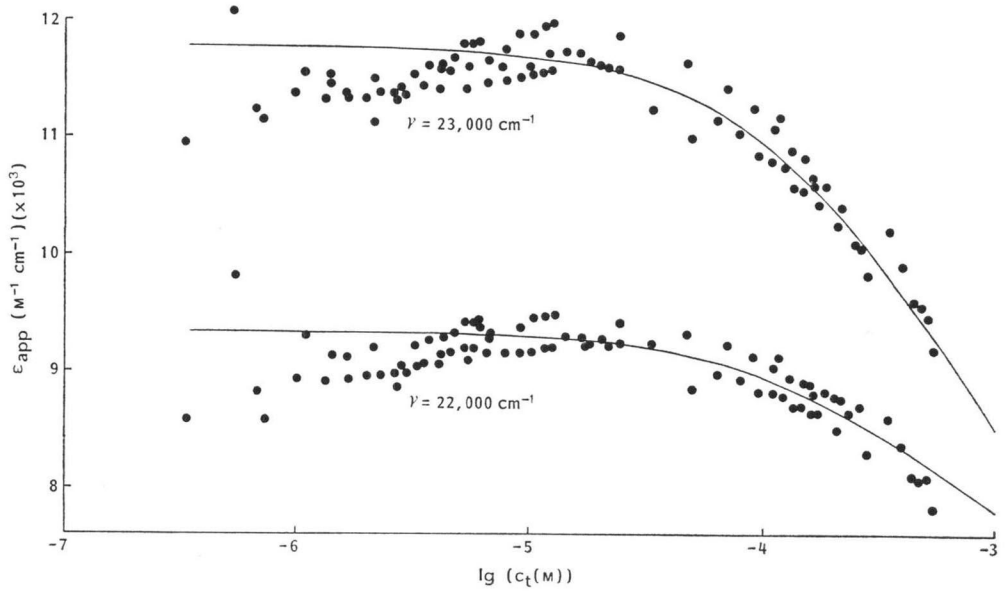


Table 1. Dimerization parameters of aclacinomycin A.

Wave number (cm^{-1})	ε_m (M^{-1} cm^{-1})	ε_d (M^{-1} cm^{-1})	K_d (M^{-1})
22,000	9,328±19	5,859±387	720±122
23,000	11,769±21	4,326±789	

Table 2. Equilibrium binding parameters of aclacinomycin A to DNA.

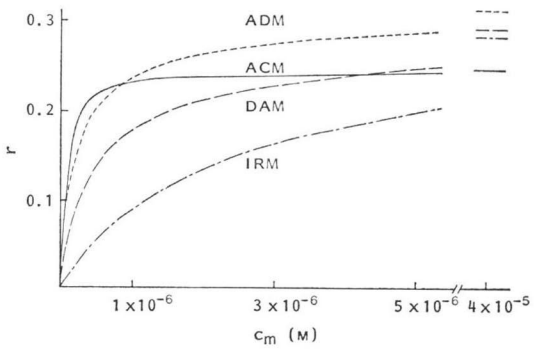
K (M^{-1})	$(7.58\pm2.15)\times10^6$
σ	0.12±0.10
α (Base pairs)	3.98±0.14
ε_b (M^{-1} cm^{-1}) at ν=23,000 cm^{-1}	6,214±46

WALTER *et al.*[17] found, that in the region of drug concentrations lower than $1/(200\ K_d)$ the influence of the dimerization on the binding values is negligible. Because of the high binding constant K and the low dimerization constant K_d compared with the corresponding constants of the other anthracyclines this condition was fulfilled for most of the test points. Thus, in contrast to other anthracyclines[15~17] the binding

Fig. 3. Binding isotherm of aclacinomycin A in comparison to the isotherms of adriamycin[18], daunomycin[15], and iremycin[17].

For the computation of the binding isotherms the following binding parameters are used (presumed that α is an integer[22]):

	K (M^{-1})	σ	α (Base pairs)
Adriamycin	$(2.3\pm0.2)\times10^6$	0.64±0.13	3
Daunomycin	$(7.1\pm0.5)\times10^5$	0.98±0.04	3
Iremycin	$(2.3\pm0.5)\times10^5$	0.58±0.07	3
Aclacinomycin A (see Table 2)	$(7.5\pm2.0)\times10^6$	0.11±0.04	4



parameters are changed by only ≤ 10 percent if the dimerization is disregarded. The cooperativity parameter σ is markedly smaller than unity, *i.e.*, related to other anthracyclines (see the legend from Fig. 3) the formation of bound ligand clusters is more probable than the binding of isolated ligands.

Thus the trisaccharide ACM shows several new aspects regarding K, α, and σ.

Discussion

To compare the results from Table 2 with the binding parameters of IRM, DAM, and ADM the computed binding isotherms of the four anthracyclines are used (Fig. 3). OKI *et al.*[4], CROOKE *et al.*[20], and MISUMI *et al.*[8] published, that the size of an ACM binding site runs to about three base pairs as in case of IRM, DAM, and ADM. In contrast to this we have found a size of a binding site of four base pairs for ACM as can be seen also from Fig. 3 ($r_{max}=1/\alpha$). This may be due to the fact, that the sugar chain of ACM is longer (trisaccharide) than those of the other three anthracyclines (monosaccharides). It is commonly accepted that their chromophores are intercalated between adjacent nucleotide bases of the DNA. On the other hand the sugar chain of the intercalated ACM is relatively flexible and possibly covers several potential intercalation sites. From the standpoint of mechanical inhibition mechanism[10] of DNA- and RNA-synthesis a larger binding site may be more favourable than a smaller one since a certain degree of DNA-coverage requires a smaller number of drug molecules.

The relation between the magnitudes of the cooperative binding constants of IRM, DAM, and ADM is given by $K_{IRM}<K_{DAM}<K_{ADM}$. However, the new antibiotic ACM binds even more strongly than ADM to DNA in the series as mentioned above. In this connection DUVERNAY *et al.*[20] and BERG *et al.*[14] found that the longer the glycosidic side chain the higher the DNA binding affinity. Responsible for strong binding are at least the combination of three types of interaction: the electrostatic forces between the basic sugar and negative phosphate groups, the stacking forces[14,20] for the intercalated part of the chromophore, and the hydrogen bonds between sugar residues and bases. Consequently steffimycin with one "neutral" sugar forms a weaker complex whereas the aglycones show the weakest interactions[14,80] and also no longer have biological activity.

The sequence of the binding constants $K_{IRM}<K_{DAM}<K_{ACM}$ is valid also in presence of proteins as shown from FRITZSCHE *et al.*[81] in case of the binding to 145 base-pair nucleosomes. Moreover, it should be noted that ACM inhibits DNA-synthesis in an *in vitro* polymerase system more strongly than the other three anthracyclines[82], as well as the growth, DNA- and RNA- synthesis of L1210 leukemia cells[5].

Moreover σ of ACM is markedly smaller than that for IRM, DAM and ADM. All these differences must be due to structural changes at positions C-10, C-11, C-13 of the chromophore and to the two additional neutral sugars. These peculiarities are responsible also for other metabolic pathways[3,4] and chemical behavior[2,6,83].

In conclusion, ACM is an anthracycline ligand type which exhibits in relation to IRM, DAM, ADM a smaller dimerization constant, a greater constant of cooperative binding to DNA, a smaller cooperativity parameter and a greater size of a binding site. The synergistic cooperation of these results plays an attractive role in the investigation and explanation of the molecular mechanisms of drug-DNA interaction. Our interaction results give more insight into the molecular properties of ACM and consequently to its biological behavior.

Acknowledgments

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