Vol. 83, No. 1, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 14, 1978 Pages 172-179

STEREOSPECIFIC BINDING OF TRIOSTIN A TO NUCLEIC ACID PURINE BASE DERIVATIVES

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Received May 1,1978

Summary: Triostin A exists as two symmetrical conformers in weakly polar solvents like chloroform. Adenine and guanine derivatives were found to specifically interact with one of the two conformers, but uracil and cytosine derivatives did not. From the analysis of nmr and infrared spectra, it was concluded that the purine derivatives form cyclic hydrogen bonds with the alanine residue of triostin A in addition to the stacking interaction between the purine base and the quinoxaline ring.

Triostin A is a member of quinoxaline antibiotics produced by *Streptomyces aureus*. Its chemical structure was proposed by Otsuka and Shoji (1) and confirmed later by the use of ^{1}H and ^{13}C magnetic resonances (2) (Figure 1). In spite of two fold symmetrical chemical structure, its magnetic resonance spectra in $CDCl_3$ exhibited two resonances for each pair of symmetry related protons and carbon atoms. The phenomenon was first interpreted as the presence of an asymmetrical conformation (2), but later revised to the presence of two symmetrical conformers (conformers 1 and 2 by Kawano et al. (3), and n and p by Blake

Abbrebiations used are: Tr, triostin A; A, 9-ethyladenine; U, 1-cyclohexyluracil; C, 2',3'-benzylidine-5'-tritylcytidine; G, 2',3',5'-isobutyl carbonyl ester of guanosine; Qun, quinoxaline caboxylic acid; NMe-Cys, N,N'-dimethylcystine; NMe-Val, N-methylvaline.

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0006-291X/78/0831-0172$01.00/0

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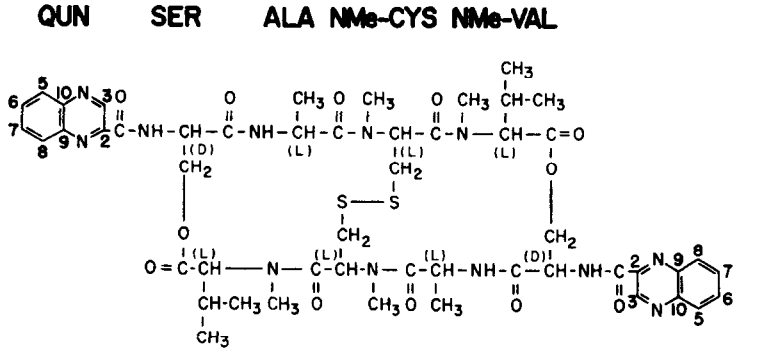

Figure 1. Structure of triostin A

et al. (4)).

Triostin A is known to interfere with nucleic acid synthesis and is thought to bind to double helical DNA with the intercalation of quinoxaline rings between base pairs (5,6). We will report here that adenine and guanine base derivatives in chloroform strongly bind to the alanine residue of triostin A through hydrogen bonding. A more interesting result is that they bind only to conformer 2, and not to conformer 1.

Materials and Methods

Triostin A (Tr) is a gift from Dr. J. Shoji, Shionogi Research Laboratory. As nucleic acid base derivatives, 9-ethyladenine (A), 1-cyclohexyluracil (U), 2',3'-benzylidine-5'-tritylcytidine (C) and 2',3',5'-isobutyl carbonyl ester of guanosine (G) were employed. A, U and C were purchased from Cyclo Chemical Co., Los Angeles, and G was prepared by the reaction of guanosine with isobutylic anhydride in pyridine. They are all soluble in chloroform and the properties of A, U and C were well studied in previous works (7-9).

^{1}H and ^{13}C magnetic resonance spectra were obtained for their $CDCl_3$ solutions with a JEOL FX-100 pulse Fourier transform nmr spectrometer operated at 100 and 25 MHz, respectively. Signals were locked on the D resonance of the solvent, and chemical shifts were relative to internal TMS. Infrared spectra were measured with a Hitachi 225 spectrophotometer. For the measurement of the NH stretching region fused silica cells of thickness ranged 1-10 mm were used.

Results

In the proton magnetic resonance spectrum of the chloroform

solution of triostin A, two resonances were observed for each pair of symmetry related protons. The relative intensities varied by changing temperature and by mixing another solvent. The phenomenon was interpreted by the presence of two symmetrical conformers, and their populations change depending on the polarity of the solvent (3,4). The assignment of signals was performed by N deuteration and spin decoupling (2-4).

On the addition of equimolar adenine compound to the triostin A solution, remarkable changes were observed in the resonance positions and in peak intensity (Fig. 2). The alanine NH signal at 7.25 ppm and the adenine NH_2 signal at 5.85 ppm move to downfield. In contrast, serine NH signal at 8.84 ppm, quinoxaline C(3)-H resonance at 9.65 ppm and the adenine CH signals shift to upfield. These signals of triostin A increased in relative intensity and they all come from conformer 2, while the resonance of conformer 1 remained unchanged in peak positions and decreased in intensity. The temperature dependence of the resonance position is shown in Figure 3. The signals are classified into two groups which shift to up and downfield by raising temperature.

Similar and more remarkable changes were observed for the addition of the guanosine compound. The guanine NH signal at 11.76 ppm, which showed a remarkable downfield shift by 3 ppm on complexation, becomes broad by exchange at higher temperatures. On the addition of uracil and cytosine derivatives, however, no changes were observed for the positions and intensity of triostin signals. Interaction seems specific to the purine bases among four nucleic acid bases.

Specificity in interaction was confirmed by taking infrared spectra. The spectra of the equimolar mixtures of Tr-A, Tr-U,

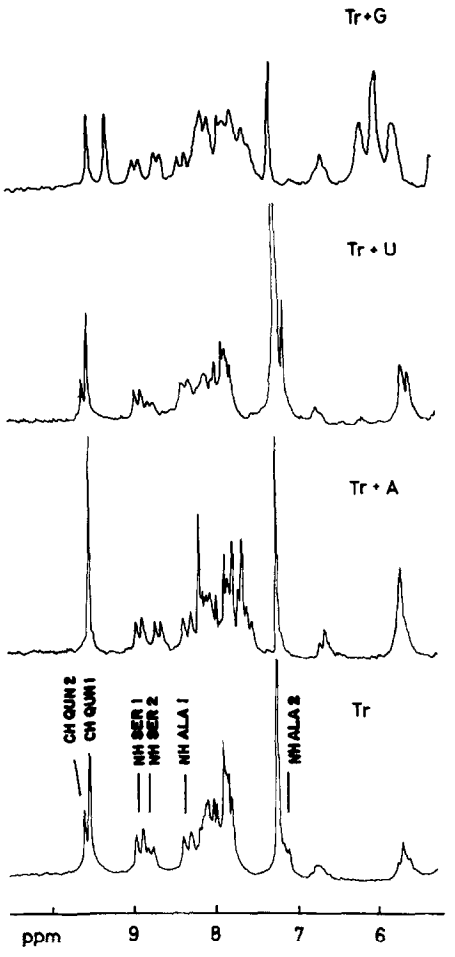

Figure 2. Low field region of ^{1}H magnetic resonance spectra of the equimolar mixtures of triostin A and nucleic acid base derivatives. Tr: triostin A, A: 9-ethyladenine, U: 1-cyclohexyluracil, G: 2',3',5'-isobutyl carbonyl ester of guanosine. Each solute was dissolved in $CDCl_3$ at 0.05 M.

Tr-C and Tr-G were compared with the sums of each component spectrum. Except for the cases of Tr-A and Tr-G the observed spectra were virtually identical with the sums. In the spectrum of A itself at 0.05 M, there are two strong bands at 3525 and 3414 cm^{-1} which are assiged to the antisymmetic and symmetric stretching vibrations of the non-bonded amino group (7). On the addition of Tr, the intensity of the non-bonded bands decreased and new association bands appeared at 3480, 3330 and 3200 cm^{-1}.

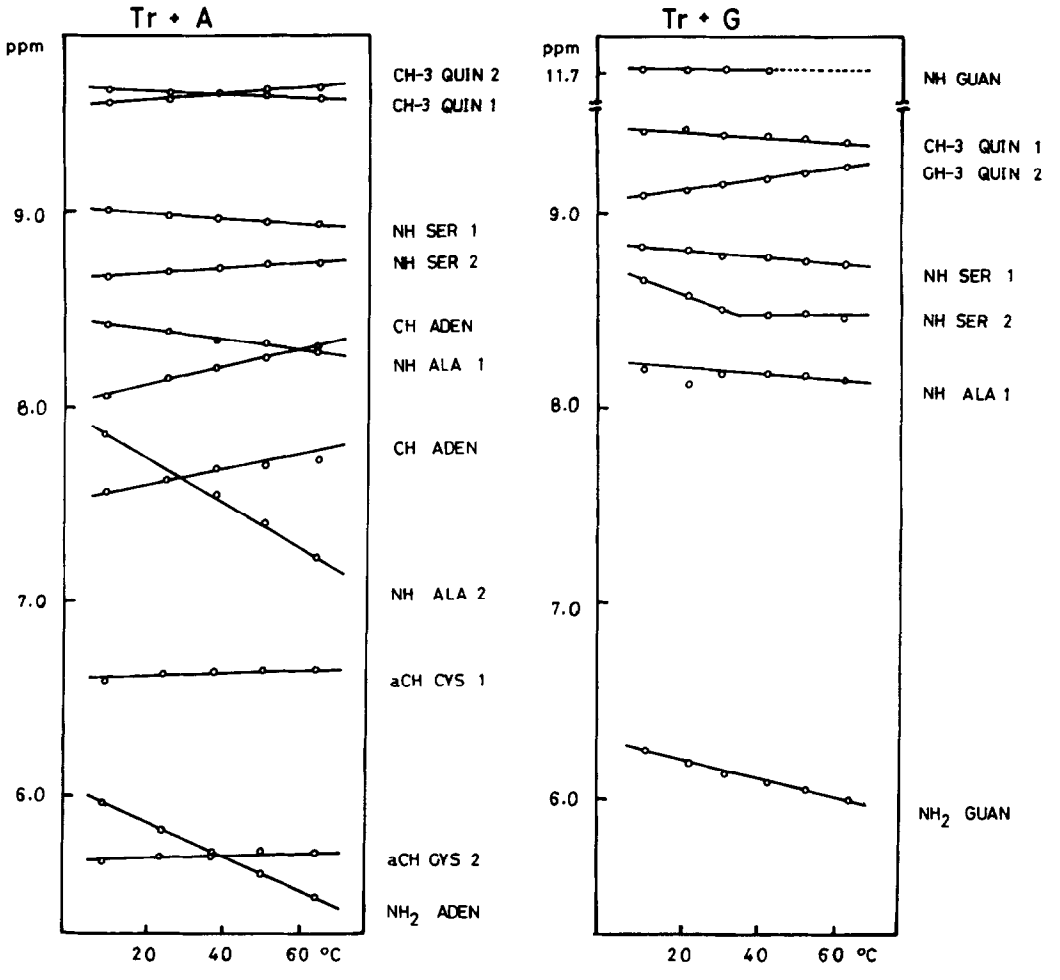

Figure 3. Temperature dependence of the ^{1}H magnetic resonance of the equimolar mixture of triostin A and adenine or guanosine derivative.

Thus it is clear that ethyladenine associates with triostin A by the hydrogen bonds of the NH_2 group.

To determine the proton acceptor sites of the hydrogen bonding, the ^{13}C magnetic resonance spectrum of triostin A was compared with those of the mixtures with A and G (Table 1). There are no shifts of the signals in the upper field than 150 ppm, but some peaks of conformer 2 in the carbonyl carbon region (160∿175 ppm) shift in slight but definite amount. The magnitude

Table 1. Assignments of the ^{13}C magnetic resonances of carbonyl carbons in $CDCl_3$ and the induced shifts on the addition of equimolar purine base derivatives.

Assignments	Resonance positions* (ppm)	Induced shifts, ppm +A	+G
Quinoxaline, 2	163.5	0	-0.1
Quinoxaline, 1	163.8	+0.1	+0.1
Serine, 2	167.9	+0.4	+0.3
Serine, 1	168.3	0	+0.1
N,N'-Dimethylcystine, 2	169.3	-0.2	-0.1
N-Methylvaline, 2	170.1	-0.2	-0.2
N,N'-Dimethylcystine, 1	170.4	0	0
N-Methylvaline, 1	170.5	0	+0.1
Alanine, 2	172.6	+0.5	+0.5
Alanine, 1	172.8	0	0

* relative to internal TMS.

of the induced shift (0.5 ppm to downfield) is reasonably ascribed to hydrogen bonding, judged from the shift in the ^{13}C spectrum of the A-U pair system (9). By selective decoupling at the C_α protons and nuclear Overhauser enhancement, all of the carbonyl carbon resonances could be assigned as given in Table 1. It is obvious that the carbonyl group of the alanine residue of conformer 2 is utilized as the proton acceptor site in the complexes with A and G. The downfield shift of the serine carbonyl may arise indirectly from effects of the strong hydrogen bonding of the alanine NH group adjacent to the carbonyl group.

For the purpose of determining the stoichiometry of the complex, the induced shifts on the addition of A were plotted against the concentration of conformer 2 calculated from the relative peak intensity of the spectra. The Ser 2 resonance shows an inflection point at approximately 1/1.5 of the ratio

(conformer 2 of Tr/A), but the other resonance signals display only monotonic curves.

Discussion

Based on the above evidences we can postulate the mode of interaction as shown in Figure 4. The adenine ring is stacked over the quinoxaline ring and serine NH, and is supported by cyclic hydrogen bonds to the alanine residue. In a previous paper (2), we proposed the conformation of isomers 1 and 2. In conformer 2 all the peptide bonds are in *trans* and two β type peptide chains of Ser-Ala-NMeCys-NMeVal are connected by carboxy ester linkages between Ser and NMeVal. Quinoxaline rings stick out to the same side of the molecule between the two β chains. When one adenine molecule occupies one binding site, it may partly disturb the binding of another adenine molecule to the other corresponding binding site by partial overlapping or distortion of conformation. This may be the reason why the ratio is not exactly 1/2. While we believe the complex of Fig. 4 predominates, others may coexist, producing mixing curves of the variety observed.

In conformer 1, we assumed the presence of *cis* peptide bonds at either Ala-NMeCys or NMeCys-NMeVal bond (3). If the Ala-NMeCys bond takes the *cis* form, the carbonyl group of alanine directs to the other side of the alanine NH bond. In this manner adenine or guanine bases would be unable to form cyclic hydrogen bonds to the alanine residue.

The present experiment was performed for the monomers of base derivatives in chloroform. These results, therefore, cannot be directly correlated with interaction mechanisms of triostin A with polynucleotides in aqueous solution. However the proposed

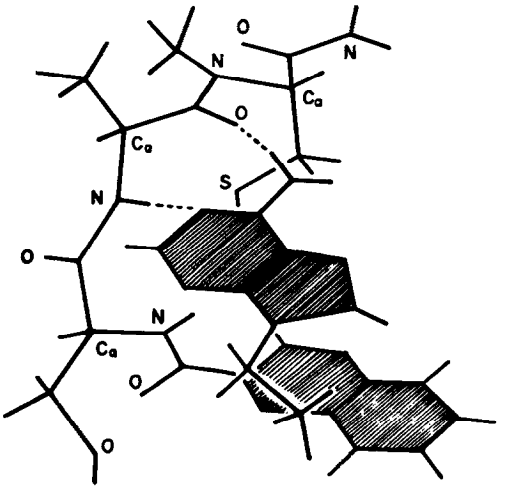

Figure 4. Possible scheme of binding of 9-ethyladenine to triostin A.

mechanism seems reasonable since it is similar to that of actinomycin D - G_pC complex (10). Thus, it seems probable that such interactions take place in aqueous polynucleotide-triostin system.

Acknowledgement: This work was partly supported by a grant from the Naito Foundation.

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