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INTERACTION OF NOGALAMYCIN WITH POLYADENYLIC AND POLYURIDYLIC ACID

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The mechanism of interaction of the anthracycline antitumour antibiotic, nogalamycin with DNA has been a subject of investigation in recent time (1-7). The detailed model for interaction was not available due to the lack of a full structure of the drug, which has now been elucidated (8). Evidences point to a strong intercalative mode of binding associated with weak electrostatic interactions. However, the reports regarding the DNA base specificity required for nogalamycin binding were confusing. Inhibition of DNA dependent RNA synthesis was correlated with presence of adenine and thymine in DNA (9-11). Nogalamycin induced decrease in buoyant density of DNA could be correlated with increasing G+C content of DNA (12), but the binding parameters (n and k) calculated from spectral titration could not be correlated with G:C content of DNA (6). Moreover, nogalamycin undergoes typical spectral changes in presence of $dG_n:dC_n$ (9) as also polyuridylic (poly U) and polyadenylic (poly A) acids (6). Although detailed models for interaction can not yet be formulated, an analysis of binding parameters for interaction of nogalamycin with homopolymers would lead to a better understanding of the binding mechanisms.

The equilibrium of drug with synthetic homopolymers was studied by spectral titration (2). Nogalamycin was a kind gift from Upjohn Company, USA, poly U and poly A were products of Sigma Chemical Company, USA.

The spectral changes associated with the binding of nogalamycin with poly U and poly A are similar to that obtained for binding with DNA (2). Interaction of the drug with poly U and poly A cause hypochromicity and bathochromic shift in drug absorption of 10nm and 5nm respectively A binding curve calculated according to Scatchard equation (13) is shown in Figure-I. The r/c versus r plots for binding of nogalamycin with both the polynucleotides are nonlinear. Scatchard equation, considering the presence of a single class of binding site, predicts a straight line. However, the curvature of the plot at higher values of 'r' is indicative of the presence of more than one class of binding sites with different affinities or the presence of single type of binding site with negative cooperativity. Presuming the presence of two classes of binding sites, it is possible to express the binding constants from the four intercepts in the Scatchard plot (6). After proper algebric manipulations, the equation given by Fletcher (14) may be written according to Hunston (15).

$$k_1 = (B+R)/2C \; ; \; n_1 = \theta_2/2 + (2\theta_1 C - \theta_2 B)/2R.$$

$$k_2 = (B-R)/2C \; ; \; n_2 = \theta_2/2 - (2\theta_1 C - \theta_2 B)/2R.$$

where $B = \theta_1\theta_2(\theta_1\theta_2 - \theta_3\theta_4)$; $C = (\theta_2)^2.\theta_3.(\theta_1 - \theta_4)$;

$D = (\theta_1)^2.\theta_4.(\theta_2 - \theta_3)$; $R = (B^2 - 4CD)^{\frac{1}{2}}$;

$n = n_1 + n_2 = \theta_2$ and $k = \theta_1/\theta_2$.

$\theta_1, \theta_2, \theta_3$ and θ_4 designate the four intercepts, k_1 and k_2 are site binding constants for two classes of sites 1 and 2 respectively, n_1 and n_2 represent respective number of available binding sites,'n' is the total number of binding sites, and k is the intrinsic binding constant.

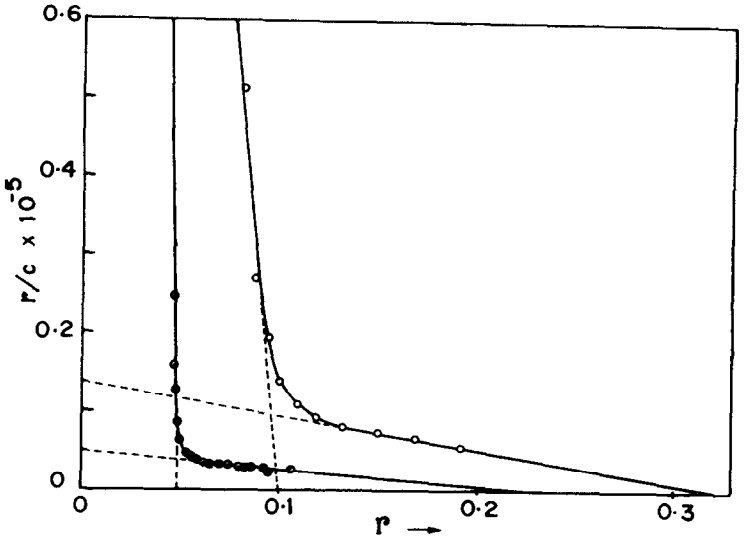

Figure-I. Scatchard plots for binding between nogalamycin and poly U (O---O) and poly A (Θ---Θ) in 1mM Tris-HCl buffer (pH-7.4).

Nevertheless this simple treatment sets up a theoretical framework for the discussion of the complexity arising from fitting of binding data to the stepwise equilibrium model (14). The Scatchard model obtained by relatively crude method of graphical estimation may adequately fit data inspite of the fact that the model may have no true interpretation as a binding model (16). Therefore,binding process need not necessarily satisfy Scatchard's assumptions even though the data are adequately fitted by Scatchard model.

Calculated values of binding parameters obtained from these plots are shown in Table-I. It indicates that nogalamycin could strongly bind to both poly U and poly A. Though the drug has greater affinity for poly A,the available drug binding sites in poly A is less than that present in poly U.

The binding parameters are apparently related to the conformation of the polymer which governs the spatial arrangements of binding sites. In a very compact structure,in which average spatial distance among

Table-I. Parameters of binding of nogalamycin with poly U and poly A.

Polymer	n_1	n_2	n	$k_1(M^{-1})$	$k_2(M^{-1})$	$k(M^{-1})$
poly U	0'095	0'225	0'32	$2'57.10^6$	$3'06.10^4$	$7'81.10^5$
poly A	0'058	0'166	0'224	$1'11.10^7$	$1'14.10^4$	$2'41.10^6$

all the binding sites is short,large intrinsic binding constants should be expected. It is known that poly A forms single-stranded stack in solution (17),it has a more ordered conformation than poly U (18) owing to a greater hydrophobic stacking interaction. It is not surprising that the average spatial distance among all the phosphate groups is shorter in poly A than that in poly U. Therefore,the number of neighbouring drug binding sites excluded due to the binding of one nogalamycin molecule are more in poly A than poly U,resulting the less number of available binding sites in poly A.

The intercalation model of Lerman (19),has been accepted for strong binding process between native DNA and nogalamycin,but to explain the binding of drug with these single-stranded polynucleotides 'modified intercalation model' has been postulated by Pritchard (20). According to this model,the drug molecules are inserted between the neighbouring bases of the same strand. The plane of the drug molecules remain parallel to the bases. This model does not require the double helix for the strong binding. Therefore,the primary mode of interaction between nogalamycin and these homopolymers could reasonably be explained by the modified intercalation model of Pritchard. The secondary binding probably involves weak electrostatic interaction between the amino group of the drug and nucleotide phosphate.

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