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SEQUENCE SPECIFICITY IN THE INTERACTION OF DAUNOMYCIN WITH ALTERNATING POLYDEOXYNUCLEOTIDES

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The interaction of daunomycin, a widely used chemotherapeutic agent [1], with natural and synthetic DNAs has received considerable attention by several research groups [2-8]. All the experimental evidences are clearly consistent with the intercalation of the chromophore daunomycinone between adjacent base pairs of the double helix. As to the sequence specificity of this interaction, the results are not so clearcut and appear even partly controversial: for instance, a higher affinity of the drug for poly(dAdT) than for poly(dGdC) has been demonstrated [8], whereas the X-ray diffraction study of the single crystal of the complex between daunomycin and the double helix of the hexamer d(CGTACG) has shown intercalation between two dC:dG base pairs [9,10]. With the aim to contribute to a better understanding of this aspect we have performed a series of UV-visible absorption and fluorescence titrations of daunomycin with a number of synthetic purine-pyrimidine alternating polydeoxynucleotides: poly(dAdT), poly(dGdC), poly(dGdT):poly(dAdC), poly(dGdm5C), poly(dIdC) and poly(dAdU). Increasing amounts of each polynucleotide were added to cuvettes containing daunomycin (in 150 mM NaCl, 10 mM phosphate buffer, pH 7.4, 1 mM EDTA) at a concentration of 10 μM ca. (for absorption titrations, Cary 219) and of 1 μM ca. (for fluorescence titrations, Perkin-Elmer MPF-3L), at 25°C. As a consequence of the interaction with the polynucleotides the visible absorption band of daunomycin undergoes a batho- and hypochromic effect and its fluorescence quantum yield experiences a marked decrease. Once determined the limit spectra and the relative quantum yields in large excess of polymer (practically all drug bound), the amounts of bound and free daunomycin have been evaluated in each case as a function of polymer concentration, according to standard methods [11]; drug dimerization equilibrium [12] was accounted for in the analysis of the absorption data. The resulting Scatchard plots are concave upward, clearly suggesting anticooperativity in the interaction, whose origin can be entropic, due to site exclusion effects [13], and/or energetic, mainly due to polyelectrolytic effects [14]. The data have been analyzed according to the formalism of Friedman and Manning [14], which includes both effects. Imposing the usual value of ξ=4.2 for DNA, best fit values of K (intrinsic affinity constant) and n (exclusion parameter, base pairs/drug) have been obtained through the algorithm of Marquardt and are reported in Table I. The table shows nearly constant values of n, which results largely independent of the sequence of the polydeoxynucleotides considered, contrary to previous evidences [8], and not significantly different from 2. The higher and sequence-depending values reported previously could be a consequence of neglecting the electrostatic component of the interaction energy in the treatment of the experimental data, rather than a consequence of major conformational alterations of the double helix induced by intercalation beyond the nearest neighbour base pairs. The affinity constants appear remarkably, if not dramatically, different in the various cases, confirming the previous evidences [8] of a clear influence of the base sequence on the strength of the interaction. In particular the higher affinity of daunomycin for poly(dAdT) than for poly(dGdC) already reported [8] is confirmed quantitatively if the differences in ionic strength are considered.

Rather surprisingly, the highest affinity is exhibited for poly(dGdm5C): this finding suggests that the most important feature responsible for the difference in affinity between poly(dAdT) and poly(dGdC) could be the favourable presence of the methyl group at position 5 in thymine in poly(dAdT) rather than the unfavourable presence of the amino group at position 2 in guanine immediately upstream to the binding site in poly(dGdC) as previously suggested [15]. A support to this deduction comes from the results obtained with poly(dIdC) and poly(dAdU): in these polymers both the purine amino and the pyrimidine methyl groups are lacking: their affinity for daunomycin is the same and is only slightly higher than that of poly(dGdC). Thus the presence or absence of the methyl at position 5 of the pyrimidine ring appears to be the dominant factor in determining the specificity of daunomycin for the various alternating sequences in DNA. A final remark comes from the fluorescence experiments: it has already been observed [8] that the interaction of daunomycin with poly(dGdC) and poly(dG):poly(dC) induces a strong quenching of the drug fluorescence, whereas upon interaction with poly(dAdT) and poly(dA):poly(dT) the quenching is much more limited. The extension of these experiments to other sequences clearly shows (Table I, last column) that this difference in the fluorescence behaviour of the drug is correlated to the presence of the purine 2-amino group poly(dGdC), poly(dGdT):poly(dAdC) and poly(dGdm5C): nearly total quenching, a few percent residual quantum yield) or its absence poly(dAdT), poly(dIdC) and poly(dAdU): partial quenching, more than half residual quantum yield .

Table I.

Binding parameters for the interaction of daunomycin with alternating polydeoxynucleotides in aqueous solution (150mM NaCl, 10 mM phosphate buffer, pH 7.4, 1 mM EDTA), 25°C. K and n have been obtained from a best fit of the experimental data using eq.77 of ref.14. R.q.y. is the ratio of the fluorescence quantum yield of daunomycin in the presence of a large excess of polymer and in its absence.

polymer	K (μM^{-1})	n (bp/d)	R.q.y.
poly(dGdm5C)	8.0	1.6	0.02
poly(dAdT)	5.0	1.7	0.58
poly(dGdT):poly(dAdC)	4.0	1.8	0.05
poly(dIdC)	2.0	1.7	0.60
poly(dAdU)	1.0	1.8	0.52
poly(dGdC)	1.2	1.8	0.05

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