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FLUORESCENCE STOPPED-FLOW KINETIC ANALYSIS OF ANTHRACYCLINES-DNA INTERACTION

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Anti-proliferative activity of anthracyclines is thought to be mediated by their interaction with DNA. Site specificity, a much controversial issue in the case of anthracyclines, may be one further discriminating factor because of the possible existence of biologically competent and non-competent sites. An investigation of anthracycline association kinetics with DNA has been started in our laboratory with the aim of detecting site heterogeneity and specificity. Here we present data obtained on four compounds (Table 1) having different cytotoxicity but comparable DNA affinity. The study was carried out by rapidly mixing an anthracycline solution with a calf thymus DNA solution in a stopped-flow at 20°C and monitoring fluorescence quenching during the reaction. Anthracycline concentration was kept below 1 μM, thus excluding any interference from dimerization [1,2]. DNA concentration was varied from 3 to 100 μM (base-pair) with keeping the molar ratio drug/base-pair always below 0.1, where site exclusion can be taken into account with an appropriate correction of DNA concentration [3]. In all cases three exponentials ware required to fit the time dependence of the fluorescence signal, in good agreement with an absorption stopped-flow study of daunomycin (**1**)-DNA interaction [4]. In this latter study two possible mechanisms have been proposed :

$$D + DNA \longleftrightarrow B_1 \longleftrightarrow B_2 \longleftrightarrow B_3 \qquad D + DNA \longleftrightarrow B_1 \begin{cases} \nearrow B_2 \\ \searrow B_3 \end{cases}$$

Table 1

Biological properties of investigated anthracyclines

		K	ID_{50}
1	Daunorubicin	$5.5\ 10^5$	9
2	Doxorubicin	$2.4\ 10^6$	9
3	4-Demethoxydaunorubicin	$8.3\ 10^5$	4.2
4	9-Deoxy-doxorubicin	$5.0\ 10^5$	230

Values of DNA binding constants, K [M^{-1}], evaluated according to Ref. 5 are courtesy of Dr. M. Menozzi. Growth inhibitory dosis, ID_{50} [ng/ml], for Hela cells were provided by Dr. M. Grandi.

Table 2

Kinetic parameters of anthracyclines-DNA association

Compound	k_1 [$M^{-1}s^{-1}$]	k_{-1} [s^{-1}]	K_1 [M^{-1}]	$\underline{K}_1$ [M^{-1}]	k_2 [s^{-1}]	k_{-2} [s^{-1}]
1	$3.8 \cdot 10^6$	206	$1.8 \cdot 10^4$	$6.0 \cdot 10^4$	52	8.4
2	$4.4 \cdot 10^6$	168	$2.6 \cdot 10^4$	$2.0 \cdot 10^5$	45	3.6
3	$6.1 \cdot 10^6$	220	$2.8 \cdot 10^4$	$1.4 \cdot 10^5$	47	9.2
4	$6.0 \cdot 10^6$	188	$3.2 \cdot 10^4$	$8.3 \cdot 10^4$	60	9.9

Both mechanisms predict one reciprocal relaxation time which is linearly dependent on the corrected DNA concentration, C_c, and two reciprocal relaxation times which plateau at high DNA concentration. This behavior was observed in all four cases. Results of relaxation analysis for the first two phases are reported in Table 2. Rate constants k_1, k_{-1} and the corresponding equilibrium constant of the first step, K_1, were obtained from linear regression on $1/\tau_1$ data vs. C_c. Similarly k_2, k_{-2} are rate constants of the second step as obtained from non-linear regression of $1/\tau_2$ data with the expression:

$$1/\tau_2 = k_2 \frac{\underline{K}_1 C_c}{1 + \underline{K}_1 C_c} + k_{-2}$$

where $\underline{K}_1$ should coincide with K_1. However, as shown in Table 2, $\underline{K}_1$ values are generally found to be higher than those of K_1. This discrepancy is solved by postulating a more complex mechanism including at least two bimolecular steps:

$$D + DNA \begin{cases} \nearrow B_1 \leftrightarrow B_2 \leftrightarrow B_3 \\ \searrow B_1^* \leftrightarrow B_2^* \leftrightarrow B_3^* \end{cases}$$

Each phase is then a complex average of two steps. Numerical simulation indicates that such a reaction mechanism is compatible with experimental data. We are then lead to conclude that heterogeneous binding sites exist. Further studies with synthetic poly- and oligo-nucleotides are in progress for their characterization.

References

1. J.B.Chaires, N.Dattagupta and D.M.Crothers, *Biochemistry* **21**, 3927 (1982).
2. M.Menozzi, L.Valentini, E.Vannini and F.Arcamone, *J.Pharm.Sci.* **73**, 766 (1984).
3. T.M.Jovin and G.Stricker, in *Chemical Relaxation in Molecular Biology* (Eds. I.Pecht and R.Rigler) pp. 245-282. Springer Verlag, New York (1977).
4. J.B.Chaires, N.Dattagupta and D.M.Crothers, *Biochemistry* **24**, 260 (1985).
5. J.D.McGhee and P.H.von Hippel, *J.Mol.Biol.* **86**, 469 (1974).