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# INTERACTION OF NUCLEOSOMES WITH ANTHRACYCLINE ANTIBIOTICS: RELEVANCE TO ANTICANCER ACTIVITY

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## INTRODUCTION

The importance of anthracyclines as antitumor drugs and the fact that DNA is believed to participate in their mechanism of action explains the large number of investigations dealing with a characterization of the drug-nucleic acid binding process (1). Since the antibiotics interact *in vivo* with packaged DNA, while most of the physico-chemical studies are performed with protein-free DNA, we examined the binding of a number of second-generation anthracyclines to 175 bp nucleosomes, as suitable subunits of chromatin.

## MATERIALS AND METHODS

175 bp nucleosomes from Calf thymus were prepared according to literature procedures (2) and purified on two successive 5-20% sucrose gradients in TE buffer: 10 mM Tris, 1 mM EDTA, pH 7.5.

Adriamycin (AM), and its 4' deoxy (4' DAM), 9-deoxy (9-DAM), 4' deoxy-4' iodo (4' IAM), analogues, as well as Daunomycin (DM) and its 4-demethoxy analogue (4-DDM) were generously supplied by Prof. F. Arcamone, Farmitalia-Carlo Erba (Italy). Their purity was checked by chromatographic methods.

In all measurements salt concentration was adjusted by addition of the proper amount of NaCl to the TE buffer. Fluorescence titrations were carried out at 25 C using a Perkin Elmer MPF 66 instrumet, equipped with a P.E. 7500 data processor.

## RESULTS AND DISCUSSION

The examined anthracyclines cover a wide range of antitumor activity, 4' IAM being the most active and 9-DAM almost inactive. All of them bind efficiently to DNA in the free and nucleosomal form as shown by the remarkably high binding constants. The results at various salt concentrations are summarized in Table 1.

From the above data it can be inferred that the affinity of the examined anthracyclines for DNA and nucleosomes varies substantially from one drug to the other. In particular Ki varies in the order

AM, DM > 9-DAM > 4-DAM > 4'DAM > 4' IAM

for free DNA, while for nucleosomes the order is

AM > 4' IAM > 4-IAM > DDM > DM > 4' DAM > 9-DAM.

TABLE 1. BINDING CONSTANTS (NEIGHBOR EXCLUSION)[a] OF 4-DDM, 4'DAM, 9-DAM, 4'IAM, DM and AM TO NUCLEOSOMES (NUCL.) AND PROTEIN-FREE DNA at 25 C, pH 7.0 AND VARIOUS $Na^+$ CONCENTRATIONS

| | $Na^+$ Concentration (M) | | | | | |
|---|---|---|---|---|---|---|
| | 0.022 | | 0.104 | | 0.355 | |
| | NUCL. | DNA | NUCL. | DNA | NUCL. | DNA |
| 4-DDM | 3.2 | 25 | 1.5 | 8.5 | 0.8 | 3.2 |
| 4'DAM | 4.2 | 7.2 | 1.2 | 5.4 | 1.0 | 1.3 |
| 9-DAM | 4.5 | n.d. | 1.0 | 11.2 | 0.6 | 2.7 |
| 4'IAM | 6.2 | 1.8 | 3.0 | 1.7 | 2.3 | 1.2 |
| DM[b] | 5.5 | 93 | 1.4 | 17 | 0.5 | 4.4 |
| AM | 17.8 | 120 | 4.4 | 17 | 1.4 | 4.6 |

[a] all values are multiplied by $10^{-5}$

[b] data extrapolated from Ref. 3

On the basis of the results obtained for the system DM-nucleosomes (3) it has been proposed that preferential binding of the drug to linker DNA might be relevant for anticancer activity. Our data show that all examined antibiotics will be distributed between linker and nucleosomal regions in chromatin to a different extent. In particular stronger binding to free DNA does not correspond to higher activity. In fact the inactive compound 9--DAM exhibits considerably higher affinity for the unpackaged polynucleotide, while for the very active compound 4' IAM the reverse is true.

The conclusion follows that anticancer activity does not rest principally on the discrimination of different DNA structures by the anthracyclines.

Even though strong DNA-binding seems to be an essential feature for activity, yet DNA should be somehow involved in the mechanism of action of anthracyclines, it appears that other requirements must be fulfilled for a molecule to be an effective antitumor agent. These possibly include recognition by the DNA-drug complex of enzymes which affect replication and/or covalent modifications of the nucleic acid by the drug.

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