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Spectroscopic Study of Hedamycin – DNA Interaction

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Summary: Binding of hedamycin, a member of the antitumour antibiotics pluramycin class, to calf thymus DNA has been studied using UV-Vis absorption spectroscopy. The results have been rationalized in terms of several literature models: Wolfe, Benesi-Hildebrand, Scott and Scatchard.

Keywords: calf thymus DNA; hedamycin; pluramycin antibiotic; UV-Vis absorption spectroscopy

Introduction

Nucleic acids have an important function in the life processes and their study has become an important research field of life sciences. A variety of drugs can bind to DNA and interfere in processes like transcription and replication.

Different classes of anticancer drugs that interact with DNA in different ways have been developed. There are non-covalent interactions by intercalation (e.g. the anthracycline antibiotics[1]: doxorubicin, epirubicin, daunomycin) or minor groove binders (e.g. the aureolic acids[2–4]: mithramycin, chromomycin A_3, olivomycin). Other drugs bind covalently to DNA, including mitomycins, anthramycin and related antibiotics, and some cause backbone cleavages, e.g. bleomycin,[5,6] streptonigrin. Many of these drugs show sequence selectivity, which make them potential targets for DNA from different sources. For example, the minor groove binders preferentially bind to AT-rich sequences whereas the intercalators have been proposed to prefer GC-rich sequences.[1–8]

The interaction of anticancer drugs with DNA is generally highly specific but not necessarily selective. DNA has many specific sites for these interactions, including the polyanionic phosphate, sugar backbone and various hydrogen acceptors and donors of bases from minor and major grooves. For example, the exocyclic N2 atom of guanine in the minor groove and the N7 atoms of guanine and adenine in the major groove are binding sites for alkylating agents. The N3 atoms of both adenine and guanine in the minor groove are also drug-binding sites.[9]

Drugs can be classified into the categories: intercalators, minor or major groove binders and alkylating agents, depending on their mode of the interaction with DNA. In some cases drugs bind via more than one mode (e.g. intercalating alkylators such as the pluramycin antibiotics). The pluramycin antibiotics (altromycin, hedamycin) are a group of the DNA-reactive agents that represent a range of 4H-anthra[1,2]-β-pyran-4,7,12-trione structures with attached carbohydrate and epoxide moieties on the corners of their planar anthrapyrantrione chromophores. Following intercalation of the chromophore into DNA, the epoxide side chain is located in the major groove, allowing selective alkylation of N7 atom of guanine.[10,11]

Hedamycin was originally isolated from fermentation broths of *Streptomyces griseoruber*. Interest in this compound arose from its potent antibacterial action and also its ability to inhibit the growth of HeLa cells in culture and some transplanted rodent tumours.[12] Early studies directed towards elucidating the mechanism of action of hedamycin, revealed that it binds to double-stranded DNA, produces substantial increases

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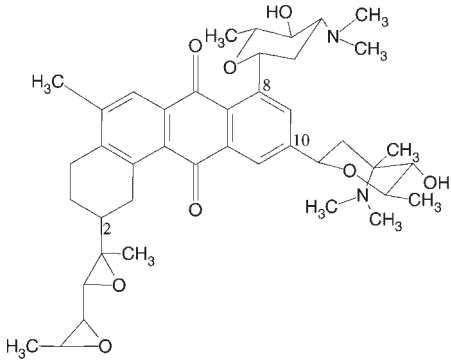

Figure 1.
The chemical structure of hedamycin.

in DNA melting temperature and inhibits DNA and RNA polymerases.[13,14]

Hedamycin (Figure 1) consists of an anthrapyrantrione chromophores to which are attached amino sugar rings at carbons 8 and 10 and a six carbon, bis(epoxide)-containing side chain at carbon 2.

In this paper, we present the results of the interaction of hedamycin with calf thymus DNA, obtained by the UV-Vis absorption spectroscopy. The results have been rationalised in terms of several literature methods: Wolfe,[15] Benesi-Hildebrand,[16] Scott[17] and Scatchard.[18] We have determined the binding constant (K) and the number of binding sites per DNA segment (n).

Experimental Part

Calf thymus DNA was obtained from Sigma-Aldrich, USA. Hedamycin supplied by the National Cancer Institute (NCI), National Institutes of Health (NIH), USA, was generously donated by the Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis. The stock solutions were prepared by dissolving the reagents in doubly distilled water. The concentrations of the stock solutions of reagents were determined by the molar absorption coefficients $\varepsilon_{260nm}=6600 M^{-1}cm^{-1}$ for DNA[1–6] and $\varepsilon_{428nm}=10000 M^{-1}cm^{-1}$ for hedamycin.[19]

The absorption measurements were performed on a Perkin-Elmer Lambda 25 UV-Vis spectrophotometer using a 1cm optical path length quartz cell, at room temperature.

Results and Discussion

Figure 2a presents three absorption spectra of hedamycin, which shows a major band centred at 428 nm and a shoulder at 340 nm.

The influence of DNA on hedamycin is presented in Figure 2b by a family of curves obtained at the titration of hedamycin solutions of concentrations in the range 10^{-6}–10^{-5}M with calf thymus DNA. It may be observed that at small polymer to drug ratios ($\frac{P}{D}$) the changes of the drug absorption spectrum are similar to those observed at increasing concentration of drug.

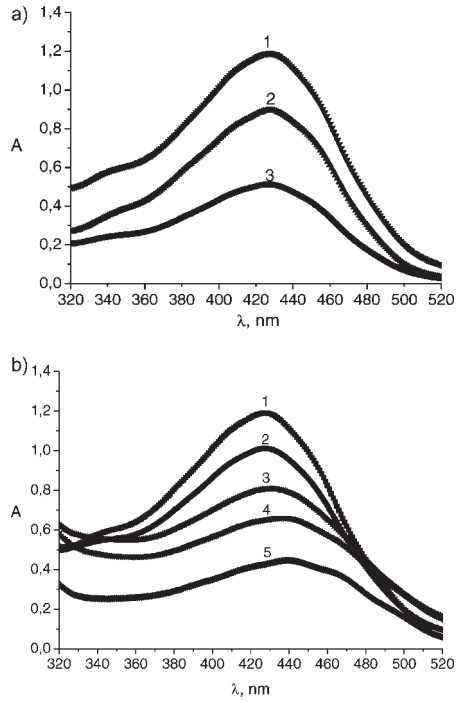

Figure 2.
(a) The absorption spectra of hedamycin at concentrations: (1) $11{,}89 \cdot 10^{-5}$ *M*, (2) $8{,}95 \cdot 10^{-5}$ *M*, (3) $5{,}12 \cdot 10^{-5}$ *M*. (b) Absorption spectra of hedamycin - DNA system. The polymer to drug ratios are: (1) 0; (2) 0,39; (3) 0,94; (4) 1,44; (5) 2,22.

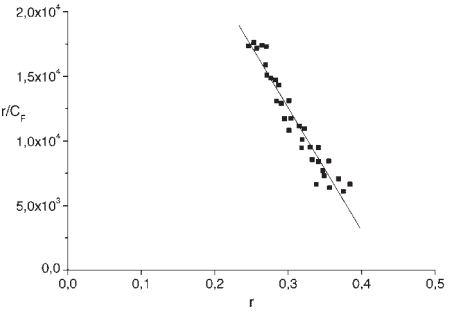

Figure 3.
The plot of $\frac{r}{C_F}$ versus the binding ratio *r* for hedamycin - DNA interaction.

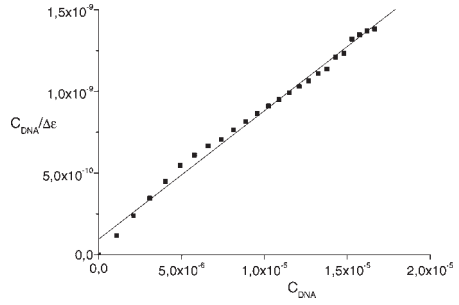

Figure 4.
Wolfe plot of hedamycin - DNA interaction.

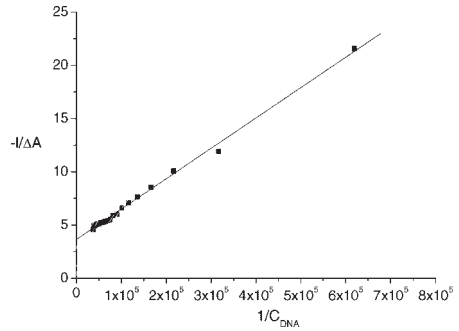

Figure 5.
Benesi-Hildebrand plot of hedamycin - DNA interaction.

On the basis of the equilibrium between hedamycin, DNA and hedamycin - DNA complex:

$$\text{Hedamycin} + \text{DNA} \rightleftharpoons \text{complex}$$

the absorbance is assumed to be the sum of the absorbance of the free and bound species, weighted by their respective concentrations:

$$A = f_0 \cdot (C_D^0 - C_B) + f_B \cdot C_B$$

$$A_0 = f_F \cdot C_D^0$$

where A_0 is the absorbance of free drug, A - the absorbance of drug measured at each DNA concentration, C_D^0 - the total drug concentration, C_B - the bound drug concentration. On the assumption of the absorption is due only to the free form of drug ($f_B = 0$), the concentrations of free and bound drug are given by:

$$C_B = C_D^0 \cdot \frac{A - A_0}{A_0}$$

$$C_F = C_D^0 - C_B$$

Thus, the experimental data (Figure 3) may be fitted on the basis of Scatchard

Table 1.
Results of the hedamycin - DNA interaction.

Method	Equations	K, M^{-1}
Wolfe	$\frac{C_{DNA}}{\Delta\varepsilon_{app}} = \frac{C_{DNA}}{\Delta\varepsilon} + \frac{1}{K \cdot \Delta\varepsilon}$	$3{,}36 \cdot 10^5\ M^{-1}$
Benesi-Hildebrand	$\frac{l}{\Delta A} = \frac{1}{C_D^0 \cdot K \cdot \Delta\varepsilon} \cdot \frac{1}{C_{DNA}} + \frac{1}{C_D^0 \cdot \Delta\varepsilon}$	$1{,}28 \cdot 10^5\ M^{-1}$
Scott	$\frac{l \cdot C_{DNA}}{\Delta A} = \frac{1}{C_D^0 \cdot \Delta\varepsilon} \cdot C_{DNA} + \frac{1}{C_D^0 \cdot K \cdot \Delta\varepsilon}$	$1{,}36 \cdot 10^5\ M^{-1}$
Scatchard	$\frac{\Delta A}{l \cdot C_{DNA}} = -\frac{K}{l} \cdot \Delta A + C_D^0 \cdot K \cdot \Delta\varepsilon$	$1{,}29 \cdot 10^5\ M^{-1}$
	$\frac{r}{C_F} = (n - r) \cdot K$	$1{,}07 \cdot 10^5\ M^{-1}$

where ε_{app}, ε_F and ε_B are the apparent, free and bound drug absorption coefficients, l is path length, ΔA is the change in absorbance at a given wavelength, C_D^0 is the total concentration of drug, C_{DNA} is the concentration of calf thymus DNA and $\Delta\varepsilon$ is the molar absorptive difference.

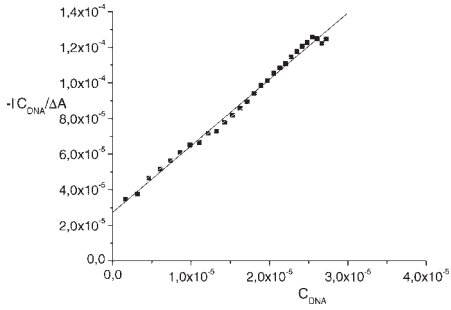

Figure 6.
Scott plot of hedamycin - DNA interaction.

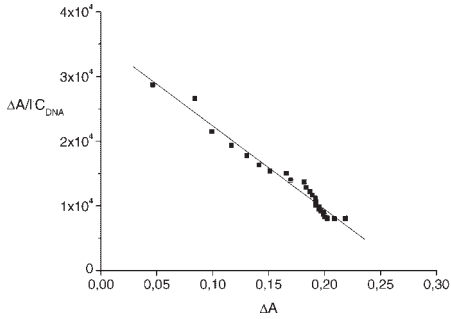

Figure 7.
Scatchard plot of hedamycin - DNA interaction.

equation:[18]

$$\frac{r}{C_F} = (n - r) \cdot K$$

corresponding to a single class of the binding sites. In this relationship, C_F is the free drug concentration, n - the number of binding sites and r is the binding ratio:

$$r = \frac{C_B}{C_{DNA}}.$$

Therefore, the binding constant $K = 1{,}07 \cdot 10^5\ M^{-1}$ and the number of binding sites $n = \sim 0{,}4$ were obtained.

The binding constant may be also evaluated by the methods proposed by Wolfe,[15] Benesi-Hildebrand,[16] Scott[17] and Scatchard.[18] The equations utilized and the results obtained are summarized in Table 1. In the Figures 4–7 are presented the used plots.

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[1] E. Volanschi, L. E. Vîjan, *Revue Roumaine de Chimie* **2001**, *46*, 163.
[2] L. E. Vîjan, *Rev. Chim. - Bucharest* **2005**, *56*, 527.
[3] L. E. Vîjan, *Rev. Chim. - Bucharest* **2005**, *56*, 655.
[4] L. E. Vîjan, *Rev. Chim. - Bucharest* **2005**, *56*, 735.
[5] L. E. Vîjan, A. Tase, *Rev. Chim. - Bucharest* **2007**, *58*, 199.
[6] L. E. Vîjan, A. Tase, *Rev. Chim. - Bucharest* **2007**, *58*, 628.
[7] L. E. Vîjan, E. Volanschi, M. Hillebrand, *Progr Colloid Polym. Sci.* **2003**, *122*, 67.
[8] M. J. Waring, *Ann. Rev. Biochem.* **1981**, *50*, 159.
[9] X. L. Yang, A. H. J. Wang, *Pharmacology & Therapeutics* **1999**, *83*, 181.
[10] F. Charmantray, A. Duflos, J. Lhomme, M. Demeunynck, *J. Chem. Soc., Perkin Trans.* **2001**, *1*, 2962.
[11] S. J. Lee, L. H. Hurley, *J. Am. Chem. Soc.* **1999**, *121*, 8971.
[12] W. T. Bradner, B. Heinemann, A. Gourevitch, *Antimicrobial Agents Chemother.* **1966**, *6*, 613.
[13] H. L. White, J. R. White, *Biochemistry* **1969**, *8*, 1020.
[14] P. B. Joel, I. H. Goldberg, *Biochim. Biophys. Acta* **1970**, *224*, 361.
[15] A. Wolfe, G. H. Shimer, T. Meehan, *Biochemistry* **1987**, *26*, 6392.
[16] H. Benesi, J. H. Hildebrand, *J. Am. Chem. Soc.* **1949**, *71*, 2703.
[17] R. L. Scott, *Rec. Trav. Chim.* **1956**, *75*, 787.
[18] G. Scatchard, *Ann. N. Y. Acad. Sci.* **1949**, *51*, 660.
[19] G. N. Bennett, *Nucleic acids Research* **1982**, *10*, 4581.

