Vol. 80, No. 2, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 30, 1978 Pages 313-318

BINDING OF ADRIAMYCIN TO SULPHATED MUCOPOLYSACCHARIDES

Milena Menozzi and Federico Arcamone

Farmitalia, Ricerca Chimica, 20146 Milan, Italy

Received November 7, 1977

Summary: The complex of adriamycin with sulphated mucopolysaccharides has been studied by visible spectrophotometry. The stoichiometry of the complex has been found to be 3.5 moles of adriamycin per hexosamine residue of heparin and 2 moles of antibiotic per mole of hexosamine of chondroitin sulphate. Stability of the complex is influenced by the concentration of Na^+ ions. At physiological concentration of Na^+ a 50% displacement of adriamycin from heparin binding sites has been found.

Introduction: Adriamycin is known to form a stable intercalation complex with double-stranded DNA (1) and ionic associations with negatively charged phospholipids (2). The sulphatedmucopolysaccharides represent another well known group of biological important acidic macromolecules, present in a range of different tissues, and generally associated with proteins (3,4). The study of the interaction of adriamycin with biopolymers such as heparin and chondroitin sulphate should provide useful information concerning tissue fixation, pharmacological properties and mechanism of action of this clinically useful antitumour agent, also taking account of the fact that tumours contain larger amounts of sulphated mucopolysaccharides than the corresponding normal tissues and that a role of the sulphated mucopolysaccharides in cell recognition and adhesiveness has been proposed (5). An interest to the evaluation of the interactions of antitumour drugs with sulphated mucopolysaccharides is also related to the extensively used association of these drugs with heparin or heparin-like substances with anticoagulant activity in tumour chemotherapy.

Methods and Material: Pharmaceutical grade adriamycin-hydrochloride was used. Heparin sodium salt from porcine intestinal mucosa was purchased by Fluka A.G., Buchs, Switzerland, and analyzed (found as per cent of the dry matter: S 11.49, N 2.66, Na 11.27, glucosamine

0006-291X/78/0802-0313$01.00/0

25.6) showing hexosamine to S ratio 2.23 (theor. 2.23). Chondroitin sulphate sodium salt grade III from whale and shark cartilage was obtained from Sigma, St. Louis, Mo., U.S.A. and analyzed (found as per cent of dry matter: S 6.53, N 2.70, hexosamine 31.18) showing hexosamine to S ratio 4.78 (theor. for two sulphonic residues per each hexosamine residue 5.58).

Hexosamine was determined as glucosamine following the method of Boas (6). Unbound adriamycin was determined spectrophotometrically (ε = 10,200 at 475 nm and 11,600 at 495 nm in 0.016 M, pH 7.3 phosphate buffer and for drug concentrations in the range 10^{-4}–10^{-5}M). The absorbance difference of completely bound adriamycin (ΔA_{max} at 475 and/or 495 nm) was determined by calculating the asymptotes of the curves obtained plotting ΔA vs. increasing concentration of mucopolysaccharides at different molar adriamycin concentrations (7). A linear relationship was found between ΔA_{max} and the corresponding adriamycin concentrations. The slope of the resulting straight line, representing $\Delta\varepsilon$, has been used for the evaluation of C_B in the equation

$$C_B = \frac{\Delta A}{\Delta \varepsilon}$$

where ΔA is the absorbance difference as deduced by substractin, for each probe, the absorbance measured in the presence of the mucopolysaccharide from the absorbance corresponding to the total concentration C_T of adriamycin in the absence of the mucopolysaccharide, and C_B is the molar concentration of bound adriamycin.

Results: The interaction between adriamycin and sulphated mucopolysaccharides induces important modifications of the electronic spectrum of adriamycin. The spectral changes in the visible consist in a bathochromic shift and hypochromicity as exemplified in Figure 1. Binding curves in which bound adriamycin is plotted as function of heparin concentration at different total drug concentrations are shown in Figure 2.

All binding curves show a linear portion corresponding to the complete saturation of binding sites by the drug. The slope resulting as the weighed mean of the slopes corresponding to all straight lines drawn from the origin to each experimental point for which $\frac{C_T}{[G]} \geq 5$ is equal to 3.3. This value represents approximately the number of binding sites per glucosamine residue in the heparin macromolecule and appears to be roughly equal to the number of anionic groups present in each disaccharide subunit of this mucopolysaccharide.

Figure 3 shows the binding curve for the complex of adriamycin with chondroitin sulphate. Similarly as above, experimental points

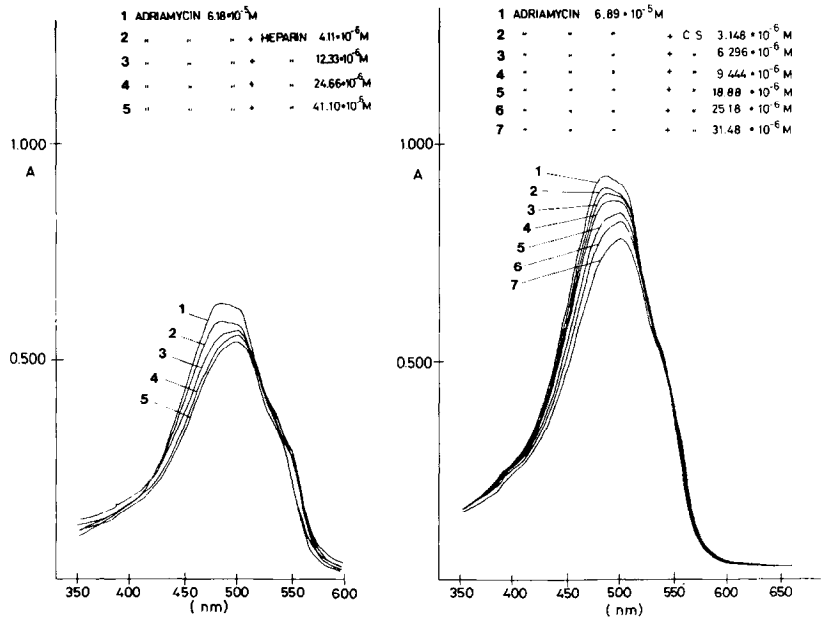

Figure 1 : Visible spectral changes induced by the addition of increasing amounts of heparin and of chondroitin sulphate (CS) to adriamycin in 0.016 M, pH 7.3 phosphate buffer. Molar concentrations of mucopolysaccharides are expressed as moles/litre of hexosamine residue.

obtained at $\frac{C_T}{[G]} \geq 4.5$ were used to determine n max, the number of binding sites per hexosamine residue in the chondroitin sulphate macromolecule. This value was found to be 1.89 indicating that the number of binding sites on each disaccharide subunit approaches to two, which is the average number of anionic group present in each subunit of chondroitin sulphate. As inorganic cations are known to be bound by mucopolysaccharides, and in particular heparin exhibits high binding capacity for different i ns, including Na^+ (8), the effect of Na^+ concentration on the formation of the complex between adriamycin and heparin was investigated. Figure 4 shows the effect of NaCl on the formation of the complex between adriamycin and heparin. It appears that

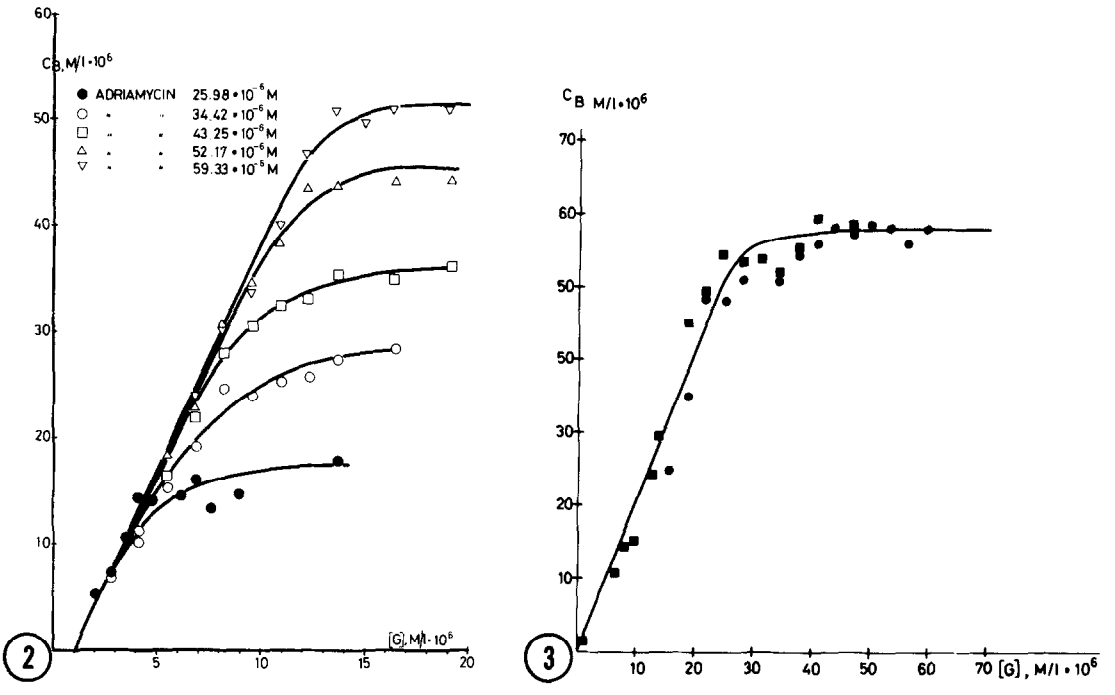

Figure 2: Binding curves representing the concentration of bound drug as a function of the concentration of heparin (expressed as glucosamine) for different total concentrations of adriamycin. Buffer as in Figure 1.

Figure 3: Binding curve representing the concentration of bound drug as a function of the concentration of chondroitin sulphate (expressed as moles/litre of hexosamine residues). Different symbols correspond to different experiments. In all cases the total concentration of adriamycin was 6.57×10^{-5}M. Buffer as in Figure 1.

the binding of adriamycin to heparin is inversely related to the concentration of Na^+, which indicates a competition between adriamycin and this cation for the negatively charged binding sites of the biopolymer.

Discussion: The present study shows that adriamycin, a positively charged molecule at physiological pH, binds both to heparin and to chondroitin sulphate and that the binding sites on these biopolymers can be identified with the anionic groups of the sulphonic and uronic acid residues. These results are further supported by competitivity displayed by Na^+ ions in the binding process.

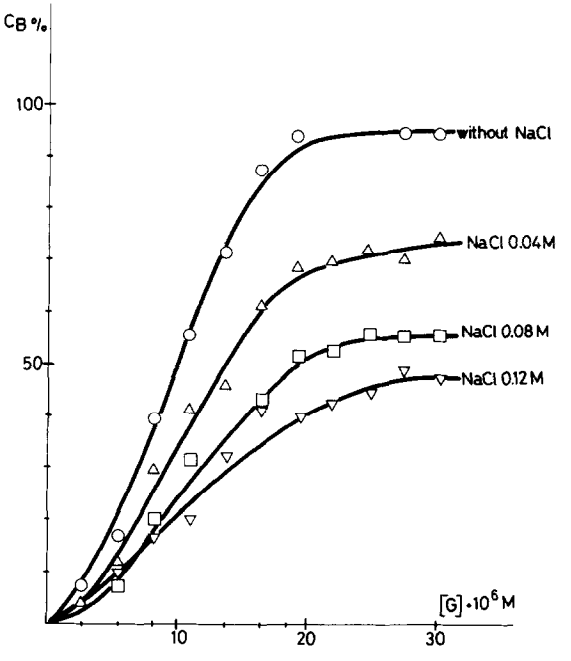

Figure 4: Binding curves representing the concentration of the bound drug expressed as per cent of the total adriamycin concentration (8.19×10^{-5}M) as a function of heparin concentration for different levels of NaCl in the medium. Buffer as in Figure 1.

The binding of heparin and other sulphated polysaccharides to cationic dyes is a well known property of these compounds and also utilized for analytical purposes (9). However spectral changes induced on adriamycin as consequence of binding are different from those exibited by the above mentioned complexes. As shown in Table 1 heparin complexes with cationic dyes display a shift of the maximum of absorption towards shorter wavelenghts accompanied by substantial reduction of molar specific extinction. On the other hand in the case of adriamycin the shift is toward longer wavelenghts and the quencing of adsorption although noticiable appears to be less marked. Spectral changes shown by adryamycin recall the behaviour of the adriamycin-DNA complex (1). Thise rises the possibility of a similarity in the spatial arrangement of the antibiotic molecules in the two complexes, and consequently of a parallel orientation of the quinone-chromophores along the tertiary structure of the mucopolysaccharides. A parallel alignement of the chromophores is also exibited by the free anthracycline antibiotics in concentrated solutions (11) and may contribute to the stabilization of the complex of adriamycin with the mucopolysaccharides in addition to the stabilization derived from the electrostatic binding between basic group of the aminosugar moiety and the polyanionic macromolecule (12).

TABLE 1

COMPARISON OF SPECTRAL CHANGES FOLLOWING THE COMPLEXATION OF CATIONIC DYES AND OF ADRIAMYCIN WITH HEPARIN

Compound or Complex	λ max (nm)	$\epsilon \times 10^{-2}$
Proflavine [a]	445	434
Proflavine + Heparin [a]	435	257
Acridin Orange + Heparin	450	252
Acridin Orange [a]	492	516
Neutral Red [a]	530	482
Neutral Red + Heparin [a]	480	239
Adriamycin	485	102
Adriamycin + Heparin	505	83

(a) Data from ref. (10)

REFERENCES

1. Di Marco, A. and Arcamone, F. (1975) Arzneim. Forsch., 25, 368
2. Duarte-Karim, M., Ruysschaert, J.M. and Hildebrand, J. (1976) Biochem. Biophys. Res. Commun., 71, 658-663.
3. Sharon, N. (1971) Complex Carbohydrates, 258-302. Addison - Wesley Publishing Co., Reading, Mass. USA.
4. Ehrlich, J., Stivala, S.S. (1973) J. Pharmac. Sci, 62, 517-544.
5. Dietrich, C.P., Sampaio, L.O., Toledo, O.M.S. and Cassano, C.M.F. (1977) Biochem. Biophys. Res. Commun., 75, 329-336.
6. Boas, M.F. (1953) J. Biol. Chem., 204, 553-563.
7. Zia, H., Price, J.C. (1975) J. Pharmac. Sci., 64, 1177-1171.
8. Dunstone, J.R. (1962) Biochem. J., 85, 336.
9. Young, M.D., Phillips, G.O. and Balazs, E.A. (1967) Biochim. Biophys. Acta, 141, 374-381.
10. Stone, A.L., Bradley, D.F. (1967) Biochim. Biophys. Acta, 148, 172-192.
11. Barthelemy-Clavey, V., Maurizot, J.C., Dimicoli, J.L. and Sicard, P. (1974) FEBS Letters, 40, 5-10.
12. Edstrom, R.D. (1969) Anal. Biochem., 29, 421-432.