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Effect of a kosmotropic ion on doxorubicin self-assembly and interaction with biomimetic systems

Abstract Ions of the Hofmeister series alter water structure. Kosmotropes (e.g. sulfate) increase, while chaotropes decrease water structure. Hofmeister ions modulate various properties, such as the solubility and the partitioning of solutes between water and organic solvents and into model membranes. The effect of the kosmotropic ion sulfate on the self-assembly and interaction of the anticancer drug doxorubicin (DOXO) with lipid bilayers was studied by employing fluorescence spectroscopy. As expected, sulfate decreased DOXO's water solubility. In the absence of sulfate, DOXO binds more strongly to large unilamellar vesicles of the anionic phospholipid dioleoyl phosphatidylglycerol (DOPG) than to large unilamellar vesicles of the

zwitterionic phospholipid dioleoyl phosphatidylcholine (DOPC). While increasing sulfate concentrations promoted increased DOXO binding to DOPC, the addition of salt caused an initial decrease in drug binding to DOPG, followed by an increase at higher sulfate concentrations. The results indicate that water structure effects prevail in the effect of sulfate upon DOXO solubility and partitioning into DOPC bilayers, whereas a more complex series of events contributes to the observed effect of the ion on the partitioning of the drug into DOPG bilayers.

Keywords Doxorubicin · Large unilamellar vesicles · Kosmotropic ion · Fluorescence spectroscopy · Molecular self-assembly

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Introduction

Many pharmacologically active compounds are amphiphilic or hydrophobic molecules. As a result, they tend to self-associate and to interact with biological membranes. The modulation of the physical properties of drugs by self-assembly and membrane binding is of fundamental importance with regard to both academic research and bionanotechnological applications [1].

The anthracycline doxorubicin (DOXO) (Fig. 1) is a potent anticancer drug employed in chemotherapy [2]. However, multidrug resistance mediated by a membrane P-glycoprotein causes decreased drug intracellular accumulation, jeopardising its effectiveness [3]. The ability of anthracyclines to overcome multidrug resistance depends

largely on the kinetics of their passive influx. The creation of new strategies to improve a drug's passive influx is a crucial task. The incorporation of DOXO into phospholipid vesicles has been extensively studied, both from the basic and from the applied points of view [4, 5].

Ions of the Hofmeister series alter water structure. Kosmotropes (sulfate, phosphate, citrate) increase, while chaotropes (thiocyanate, perchlorate, trichloroacetate) decrease water structure. For this reason, Hofmeister ions modulate various phenomena in aqueous solutions, playing a role in fundamental properties, such as the solubility of relatively nonpolar compounds, molecular and mesoscopic organization of aggregates, and partitioning of solutes between water and organic solvents [6] and into model membranes (detergent micelles and

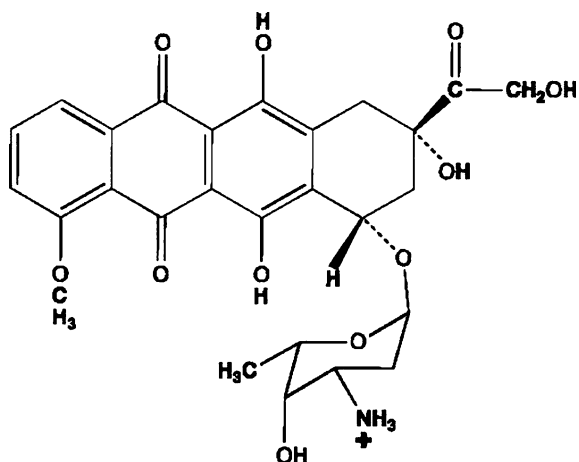


Fig. 1 Chemical structure of doxorubicin (*DOXO*). The pK of the charged group is 8.3

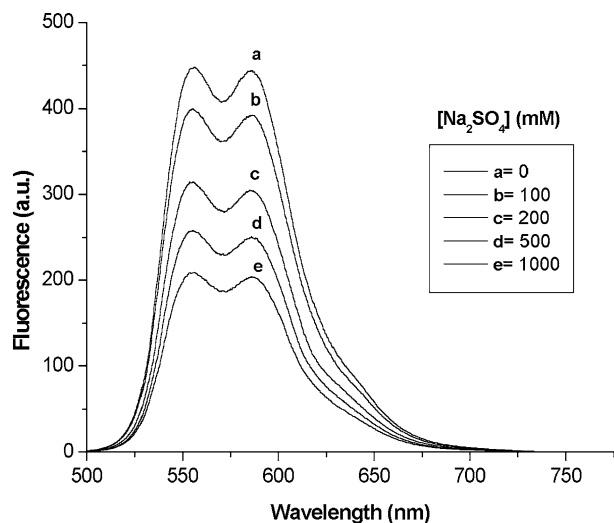


Fig. 2 Fluorescence spectra of 25 μM DOXO in phosphate-borate-citrate buffer, pH 7.2, at different Na_2SO_4 concentrations (M): 0 (*a*), 0.1 (*b*), 0.2 (*c*), 0.5 (*d*), 1.0 (*e*)

phospholipid bilayers) [7]. Kosmotropes decrease solute solubility and favor partitioning of solutes into hydrophobic phases, while chaotropes exert opposite effects. In this work we investigated the effect of the kosmotropic ion sulfate on the self-association of DOXO and on its interaction with model membranes by means of fluorescence spectroscopy.

Materials and methods

Pure DOXO was a gift from Pharmacia-Upjohn, Italy. DOXO stock solutions (25 μM) were freshly prepared in 5 mM

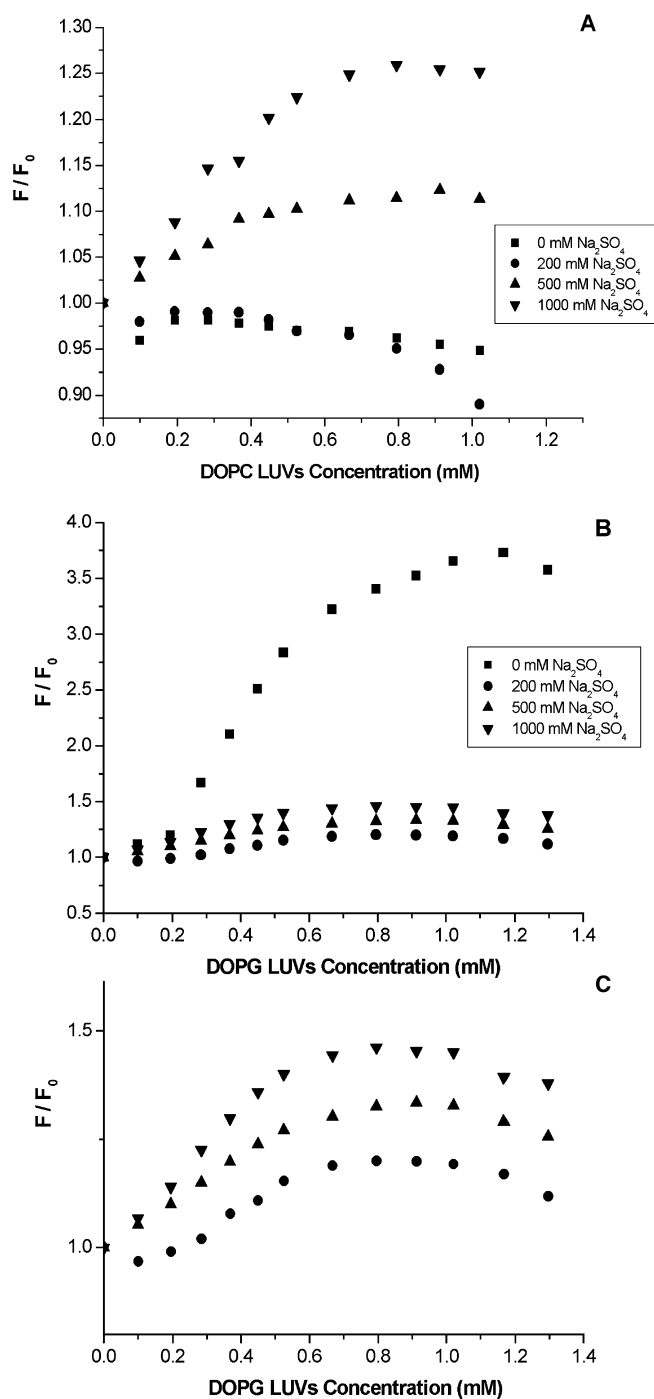


Fig. 3 F/F_0 as a function of a dioleoyl phosphatidylcholine (*DOPC*) and b, c dioleoyl phosphatidylglycerol (*DOPG*) concentration, for different Na_2SO_4 concentrations. In c the ordinate was expanded to clarify the differences for the different sulfate concentrations. Na_2SO_4 (M): 0.2 (*circles*); 0.5 (*up triangles*); 1 (*down triangles*). DOXO concentration 25 μM

phosphate-borate-citrate buffer, pH 7.2. Sodium sulfate was from Merck (Rio de Janeiro, RJ, Brazil). Dioleoyl phosphatidylcholine (*DOPC*) and dioleoyl phosphatidylglycerol (*DOPG*) (Sigma

Chemical Co., St. Louis, MO, USA) large unilamellar vesicles (LUVs) were obtained by evaporating stock chloroform solutions under a stream of nitrogen. The samples were left under vacuum for no less than 2 h, phosphate–borate–citrate buffer was added, and the samples were vortexed for 5 min. LUVs were obtained by extruding the dispersions 30 times through 100-nm-pore polycarbonate filters. The LUVs were kept in ice and used the same day. DOXO binding was assessed by measuring its intrinsic fluorescence [8] with a Hitachi F-4500 spectrofluorimeter. Excitation was at 480 nm and the spectra were scanned from 500 to 750 nm at 22 ± 2 °C.

Results and discussion

Sulfate caused pronounced DOXO self-association, as indicated by a decrease in fluorescence intensity (Fig. 2)

The results are in agreement with reports describing ionic-strength-dependent anthracycline self-association [9]. Extensive DOXO aggregation has been found in the presence of high (of the order of a hundred to a thousand millimolar) sulfate and citrate concentrations. Large supramolecular fibrous bundles were formed at DOXO concentrations 100 times lower than its aqueous solubility [4, 5].

The fluorescence of anthracyclines is enhanced upon binding to lipid bilayers [8]. Making use of this property, we assessed the interaction of DOXO with LUVs by calculating the F to F_0 ratio, where F and F_0 correspond to the fluorescence intensity in the presence and absence of LUVs, respectively (Fig. 3). It should be recalled that F_0 decreased with increasing sulfate concentration (Fig. 2).

In the absence of sulfate, a much greater increase in F/F_0 was observed in the presence of DOPG (Fig. 3b) than in the presence of DOPC (Fig. 3a). This indicated a much more pronounced interaction of the drug with negatively charged than with zwitterionic membranes, in agreement with previous observations [10, 11]. Sulfate affected the binding of DOXO to the bilayers in different ways. As found for the partitioning of the local anesthetic tetracaine into zwitterionic micelles [7], increasing sulfate

concentrations promoted an enhancement of DOXO binding to DOPC (Fig. 3a).

In contrast, the binding to DOPG was reduced upon addition of 200 mM sulfate (Fig. 3b). In this case, a competition occurs between DOXO and sodium ions for binding to the negatively charged phospholipid via electrostatic interactions, promoting a decrease in drug binding. Indeed, this behavior has been found for other anthracyclines [12]. Ionic strength effects should cause a decrease in drug binding with increasing salt concentrations. However, the opposite effect is seen in Fig. 3b and c; in Fig. 3c, the scale was expanded for the data for 200, 500, and 1,000 mM sulfate to show more clearly that DOXO binding to DOPG increases with increasing sulfate concentration. Thus, the ionic strength effect is counteracted by the Hofmeister effect, that promotes enhanced binding.

By exerting its effect on water structure the kosmotropic ion sulfate was expected to decrease DOXO's solubility and to increase its partition into the less polar environment of the bilayers. This hypothesis was confirmed with regard to the drug's solubility. However, the interplay between several phenomena determines the final result with respect to partitioning. Thus, while DOXO's partitioning into zwitterionic DOPC LUVs increased with increasing sulfate concentration, in the case of DOPG the initial increase in ionic strength caused a decrease in DOXO binding. Nevertheless, at higher sulfate concentrations, the Hofmeister effect prevailed, leading to a larger extent of drug binding.

In conclusion, the results show that, by affecting water structure, the kosmotrope sulfate can modulate both drug solubility and partitioning into lipid membranes. These phenomena are of potential use in the design of drug delivery systems.

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References

- Schreier S, Malheiros SV, De Paula, E (2000) *Biochim Biophys Acta* 1508:210
- Capranico G, Butelli E, Zunico F (1995) *Cancer Res* 55:312
- Mankhetkorn S, Dubru F, Hesschenbrouck J, Fiallo M, Garnier-Suillerot A (1996) *Mol Pharmacol* 46:532
- Li X, Hirsh DJ, Cabral-Lilly D, Zirkel A, Gruner SM, Janoff AS, Perkins WR (1998) *Biochim Biophys Acta* 1415:23
- Lasic DD, Ceh B, Stuart MCA, Guo L, Frederik PM, Barenholz Y (1995) *Biochim Biophys Acta* 1239:145
- Collins KD, Washabaugh MW (1985) *Q Rev Biophys* 18:323
- Ferreira GSS, Périgo DM, Politi MJ, Schreier S (1996) *Photochem Photobiol* 63:755
- Gallois L, Fiallo M, Laigle A, Priebe W, Garnier-Suillerot A (1996) *Eur J Biochem* 241:879
- Menozi M, Valentini L, Vannini E, Arcamone F (1984) *J Pharm Sci* 73:766
- Speelmans G, Staffhorst RWH, de Kruijff B (1997) *Biochemistry* 36:8657
- Henry N, Fantine E, Bolard J, Garnier-Suillerot A (1985) *Biochemistry* 24:7085
- Burke, TG, Sartorelli AC, Tritton TR (1988) *Cancer Chemother Pharmacol* 21:274