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Thermodynamic analysis of binding between drugs and glycosaminoglycans by isothermal titration calorimetry and fluorescence spectroscopy

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ABSTRACT

The thermodynamics of the interaction of positively charged drug molecules with negatively charged glycosaminoglycans (GAGs) is investigated by isothermal titration calorimetry (ITC) and fluorescence spectroscopy. The drugs considered are propranolol hydrochloride, tacrine, and aminacrine, and the polymers used as model GAGs are dextran sulfate, chondroitin sulfate, and hyaluronic acid. The ITC results show that the interaction between drugs and GAGs is via direct binding and that GAGs bind to drugs at one set of sites. Large negative values of heat capacity change (ΔC_p) are observed upon binding of GAGs to drugs. Such negative ΔC_p is not expected for purely electrostatic interactions and suggests that hydrophobic and other interactions may be also involved in the binding process. These results are corroborated by fluorescence spectroscopy measurements, which show that specific drug/GAG complex formation is accompanied by a clear enhancement of the fluorescence intensity. The results highlight the importance of the formation of drug/GAG complexes as a primary step for the drug delivery process into cell membranes. It is concluded that the interactions are dependent on the nature of both GAG and drug and this is a fact to be taken into account when new drugs are designed.

1. Introduction

A wide variety of molecules of pharmacological interest have surface-active properties due to their amphiphilic nature. To achieve their target in the intracellular medium, their hydrophilic and hydrophobic moieties firstly interact with the surface of the cellular membrane and its components. This interaction plays a fundamental role for the biological phenomena.

Amphiphilic drugs may adopt different structures which are dependent, for example, on the pH, temperature, ionic strength, and concentration, as well as the molecular shape and the hydrophilic–hydrophobic balance (Schreier et al., 2000). Many surface-active drugs associate and bind to membranes in a detergent-like manner (Schreier et al., 2000). Among others, non-steroidal anti-inflammatory drugs (Rades, 1997), anticancer drugs (King et al., 1989), analgesics (Attwood et al., 1997), anticholinergics (Yokoyama et al., 1994), and

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β-blockers (Ruso et al., 1999) are amphiphilic drugs which contain one or more aromatic nuclei. Propranolol hydrochloride (propranolol), 9-amino-1,2,3,4,-tetrahydroacridine (tacrine), and 9-aminoacridine hydrochloride (aminacrine) belong to this class of drugs. Propranolol is a synthetic non-selective B-adrenergic receptor blocking agent, therapeutically referred to as a membrane stabilizer due to its hydrophobicity (Attwood and Lemmer, 1979) tacrine is an acetylcholinesterase inhibitor used in the treatment of Alzheimer's disease; and aminacrine is a highly fluorescent therapeutic anti-infective dye used clinically as an antiseptic and experimentally, as a mutagen and also as an intracellular pH indicator. These drugs have a very similar chemical structure and their pK_a are 9.2, 9.8, and 9.99 for propranolol, tacrine, and aminacrine, respectively (Drayton, 1990).

Amphiphilic drug molecules have been extensively studied in relation to drug-model cell membrane (lipids/liposomes) or to drug–protein interactions. Glycosaminoglycans (GAGs) are highly negatively charged biomolecules (like sulfated polysaccharides) that are found in the extracellular matrixes of many tissues, and on the surface of cells (Schlessinger et al., 1995). They are involved in diverse biological processes such as neural development, gene delivery, and have also been linked to the pathology of the Alzheimer's disease (Laabs et al., 2005; Capila and Linhardt, 2002; Ruponen et al., 1999; Büow and Hobert, 2004). Recently, we have investigated the interaction of this kind of drugs with phospholipid modified-monolayers at a liquid–liquid interface in the presence of dextran sulfate (DS), which is used as model for GAGs (Santos et al., 2005a,b, 2007a,b). In general, these results have qualitatively shown that the presence of DS affects the drug transfer through the lipid monolayer.

Calorimetric techniques are very powerful for the study and understanding of biological processes at molecular level. Isothermal titration calorimetry (ITC) can detect the small changes of heat during reaction. It allows for the determination of the thermodynamic parameters such as enthalpy (ΔH), entropy (ΔS), Gibbs free energy (ΔG), heat capacity (ΔC_p), binding constant (K_b), and effective number of binding sites (N) in biological reactions (Bäuerle and Seelig, 1991; Milhaud et al., 1996; Rowe et al., 1998; Ladbury, 2001).

The interactions between drug molecules and GAGs are of great importance for optimizing drug formulations. In this context, and continuing our previous work, ITC is used to determine the thermodynamic parameters of the binding of amphiphilic drugs (propranolol, tacrine, and aminacrine) with model, surface-membrane GAGs such as DS, chondroitin 6-sulfate (CS), and hyaluronic acid (HA) at pH 5, 7.4, and 9, and at two temperatures, 298 and 313 K.

Fluorescence spectroscopy is also used to study of interactions between GAGs and drugs. This complementary technique is based on the fact that the fluorescence of a number of molecules (fluorescent probes) is very sensitive to the characteristics of their immediate surroundings.

Finally, the paper highlights the fact that drug/GAG interactions can be interpreted in a similar way as protein–protein or carbohydrate–protein interactions. To the best of our knowledge, this is the first time that drug/GAG interactions have been explored in this way.

2. Experimental

2.1. Chemicals

The drugs used were aminacrine (Sigma, minimum 98%), tacrine (Sigma, reagent grade), and propranolol (Sigma, reagent grade). The model GAGs used were DS sodium salt from Leuconostoc ssp (DS 500, Fluka, 500 kDa), CS sodium salt from shark cartilage (Sigma, 50–60 kDa), and HA sodium salt from human umbilical cord (Fluka, 3000–5800 kDa) (see Fig. 1). DS contains ~17% sulfur, which is about 2.3 sulfate groups per glucosyl residue, and CS ~7%. The aqueous solutions were prepared in 15–150 mM NaCl (Merck, p.a.) and buffered to the desired pH using 2–20 mM HEPES (Sigma, minimum 99.5% titration) or 2–60 mM phosphate buffer (J.T. Baker B.V., "Baker Analysed"®), NaOH (Merck, p.a.), and HCl (Merck, p.a.). All the chemicals were used as received without further purification. Millipore water (resistivity > 18 Mω cm) was used to prepare all the aqueous solutions and for rinsing.

2.2. Calorimetry

The heat flow resulting from the binding between GAGs and drugs was measured using a high-sensitivity MicroCal isothermal titration calorimeter (VP-ITC, Northampton, MA) in a reaction cell (volume 1.4413 mL) at a stirring speed of 300–450 rpm. Prior to the measurements all the aqueous solutions were degassed under vacuum about 10–15 min for elimination of any air bubbles. In each titration the reaction cell was loaded with GAG solution and sequences of 29–59 successive 10–15 μL aliquot injections were performed using a 250 μL auto-syringe filled with either drug solutions, at 4–6 min intervals between each injection. To correct for the heat effects of dilution and mixing, control experiments were performed at the same concentration of drugs and GAGs. The calorimetric data were analyzed and converted into enthalpy change using MicroCal Origin 5.0 software provided with the instrument. The enthalpy change for each injection was calculated by integrating the area under the peaks of the recorded time and then corrected with the control titrations. The experimental data were fitted to a binding model using a non-linear least squares method with N, ΔH, and K_b as adjustable parameters. The experiments were performed at 298 and 313 K, and at pH 5, 7.4, and 9.

The titrations were carried out in HEPES and phosphate buffers described as follows: GAG solution in the syringe (typically 8.80–2.24 mM), drug (referred as ligand) solution in the reaction cell (typically 2.60–0.33 mM). At the studied pHs and temperatures, the data fitted well to a one set of sites for GAG-into-drug (further fitting details can be found in the supporting information). The molar concentrations in the case of GAGs refer to the concentrations of the monomer (in the case of DS) or dimer units (in the cases of CS and HA) shown in Fig. 1.

2.3. Fluorescence spectroscopy

The intrinsic fluorescence of the interactions between drugs and GAGs were studied using a Perkin-Elmer LB-50 spectrofluorimeter with a 3 mL quartz cell of 1 cm path length. Drug