European Journal of Pharmaceutical Sciences 46 (2012) 435–445

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Insights into the binding of the drugs diclofenac sodium and cefotaxime sodium to serum albumin: Calorimetry and spectroscopy

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ARTICLE INFO

Article history:
Received 7 February 2012
Received in revised form 15 March 2012
Accepted 20 March 2012
Available online 29 March 2012

Keywords:
Bovine serum albumin
Diclofenac
Cefotaxime
Calorimetry
Binding thermodynamics
Spectroscopy

ABSTRACT

Understanding physical chemistry underlying drug–protein interactions is essential to devise guidelines for the synthesis of target oriented drugs. Binding of a non-steroidal anti-inflammatory drug, diclofenac sodium (DCF) and an antibiotic drug, cefotaxime sodium (CFT) belonging to the family of cephalosporins with bovine serum albumin (BSA) has been examined using a combination of isothermal titration calorimetry (ITC), differential scanning calorimetry (DSC), steady state and time resolved fluorescence and circular dichroism spectroscopies. Binding affinity of both DCF and CFT with BSA is observed to be of the order of 10^4 M^{-1}, with the binding profiles fitting well to the single set of binding site model. The disagreement between calorimetric and van't Hoff enthalpies indicates non-adherence to a two-state binding process which could be attributed to changes in the conformation of the protein upon ligand binding as well as with increase in the temperature. Circular dichroism and the fluorescence results, however, do not show any major conformational changes upon binding of these drugs to BSA, and hence the discrepancy could be due to temperature induced conformational changes in the protein. The results of ionic strength dependence and binding in the presence of anionic, cationic and non-ionic surfactants indicate, involvement of more that a single type of interaction in the binding process. The ITC results for the binding of these drugs to BSA in presence of each other indicate that the binding sites for the two drugs are different, and therefore binding of one is not influenced by the other. The DSC results provide quantitative information on the effect of these drugs on the stability of serum albumin. The combined calorimetric and spectroscopic approach has provided a detailed analysis including thermodynamics of the binding of DCF and CFT with BSA qualitatively and quantitatively.

1. Introduction

It is imperative to understand the molecular basis of drug–protein interactions because it greatly influences the biological activity of a drug. The knowledge of the nature and magnitude of drug–protein binding can help us understand and also modulate the pharmacokinetics and pharmacodynamics of a drug. Once introduced into the system, drugs are bound to various blood constituents like cells, proteins, etc. Within the plasma proteins serum albumin is the most important carrier for a broad spectrum of exogenous and endogenous ligands (Kratochwil et al., 2002). Since the therapeutic effect of a drug is directly related with the free concentration of drugs in the plasma (Briand et al., 1982), it is of central pharmacological interest to study the drug–albumin binding. Detailed knowledge of the drug binding site can provide necessary information about the distribution of the drug in the body, its usage along with the other drugs and other competitive natural catabolites (Brodersen et al., 1988). Serum albumins, being present in very high concentration (~40 mg ml^{-1}) in the blood plasma are the major macromolecules chiefly responsible for maintaining the blood osmotic pressure and the pH (He and Carter, 1992; Peters, 1996). Serum albumin often increases the apparent solubility of hydrophobic drug in plasma and modulates its delivery to cells in vivo and in vitro (He and Carter, 1992). Serum albumin has two major albumin binding sites as described by Sudlow and co-workers (Sudlow et al., 1975, 1976). Site I, also called as the warfarin binding site to which drugs such as warfarin, azapropazone and phenylbutazone bind, is located in subdomain IIA of human serum albumin (Sugio et al., 1999). Site II, which is known as benzodiazepine binding site is located in subdomain IIIA and binds several indole derivatives and benzodiazepines with a high degree of structural specificity (Muller and Wollert, 1979; Sjoholm et al., 1979). Site I has been characterized as a larger binding area consisting of overlapping sites of warfarin and azapropazone (Fehske et al., 1981), and the drugs which usually bind to this site are more bulky heterocyclic molecules. On the other hand, site II is more of a narrow hydrophobic binding pocket and the drugs which bind to it are aromatic carboxylic acids, largely ionized at

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http://dx.doi.org/10.1016/j.ejps.2012.03.007

physiological pH (Chamouard et al., 1985). Consequently, a clear understanding of the interaction of a drug with serum albumin, and characterization of the binding interactions gives us information which is essential for the rational drug-design process.

Diclofenac sodium (DCF) (monosodium 2-[(2,6-dichloroanilino) phenyl acetate]), a non-steroidal anti-inflammatory drug [NSAID] (Fig. 1), is known for its high biological activity and possesses high potential against pain and rheumatic inflammations (Brogden et al., 1980). It is used as an effective antipyretic and analgesic against acute febrile sore throat and also relieves episodic tension-type headache even at low doses (Kenawi, 2006). Its pharmacological effects are attributed to the inhibition of the conversion of arachidonic acid to prostaglandins, which are the mediators of the inflammatory processes (Jobin and Gagnon, 1971). Moser and co-workers (Moser et al., 1990) have suggested that the high activity of DCF is due to its geometry; the 69° twist that exists between the two phenyl rings, which are tilted at ~109°. Binding of DCF with serum albumins has been reported in literature (Rahman et al., 1993; Dutta et al., 2006), the detailed energetics of binding and effect on the conformation of proteins is not available.

Cefotaxime sodium (CFT) (Fig. 1), commonly known as claforan, belongs to the family of broad spectrum antibacterial drugs called cephalosporins. It has an active nucleus of β-lactam ring which results in a variety of antibacterial and pharmacologic characteristics and is widely used to treat gonorrhea, meningitis, pneumococcal, staphylococcal and streptococcal infections. Its antibacterial activities result from the inhibition of mucopeptide synthesis in the cell wall of mainly gram negative bacteria. There are previous reports in the literature where this class of antibiotics was found to bind to serum albumin with diverse affinity, however, there are discrepancies in the published results (McNamara et al., 1990; Tawara et al., 1992; Nerli et al., 1997; Briand et al., 1982; Markovich and Avereva, 1985).

Several interactions between antibiotics and NSAIDs have been described in the literature and they suggest that anti-inflammatory drugs could increase antibiotic efficacy by altering their pharmacokinetics (Catella-Lawson et al., 2001; Tsivou et al., 2005; Joly et al., 1998; Marks, 1991; Rocca and Petrucci, 2012). Conversely, some antibiotics are able to alter metabolism and/or kinetics of some NSAIDs (Rocca and Petrucci, 2012). In fact, enhancement of the therapeutic effect of some of the cephalosporins has been reported in the presence of DCF (Joly et al., 1998; Marks, 1991; Rocca and Petrucci, 2012). DCF and CFT are the drugs which could be administered together, because of the wide spectrum of diseases they cover, so it is of interest to study their binding efficacy in presence of each other, to the serum albumin. In the present study, ITC has been used to quantitatively determine the thermodynamic parameters accompanying the binding of DCF and CFT with BSA. The nature of interactions involved in the binding process has been investigated by studying the binding as a function of temperature and in the presence of salts and surfactants. The possibility of overlapping binding sites for DCF and CFT has also been examined by conducting combinatorial experiments. The binding has also been studied using fluorescence spectroscopy and compared with the ITC results. The conformational changes in the protein and its stability have been investigated by CD spectroscopy and differential scanning calorimetry.

2. Experimental methodology

2.1. Materials

Fatty acid free BSA, diclofenac sodium salt, cefotaxime sodium salt, sodium chloride, sodium dodecyl sulfate (SDS), triton X-100 (TX-100) and hexadecyl trimethyl ammonium bromide (HTAB) were purchased from Sigma–Aldrich Chemical Co., USA. A Sartorius BP 211D digital balance of 0.01 mg readability was used for the mass measurements. The water used for preparing the solutions was double-distilled and further deionized using a Cole-Parmer research mixed-bed ion exchange column. All the experiments were performed at pH 7.0 in 10 mM phosphate buffer. The protein stock solutions were prepared by extensive overnight dialysis at 4 °C against the buffer. The reported pH is that of the dialysate measured on a Standard Control Dynamics pH meter at room temperature. The concentration of BSA was determined on a Shimadzu double-beam UV 265 spectrophotometer at 280 nm using the extinction coefficient value corresponding to $A^{1\%}_{1cm} = 6.8$ (Sober and Harte, 1973).

2.2. Isothermal titration calorimetry (ITC)

Experiments on binding interaction of the drugs with BSA were done on a VP-ITC titration microcalorimetry system (MicroCal, Northampton, MA). All solutions were thoroughly degassed before use by stirring under vaccum in a Thermovac unit supplied with the instrument. The sample cell was loaded with 0.045 mM protein solution or buffer. The titrations were carried out using a 250 μL autopipet, filled with the respective drug solution (0.675 mM DCF or 1 mM CFT), keeping the stirring speed fixed at 300 rpm. Each experiment consisted of 10 μL consecutive injections of the drug solution at durations of 20 s each with a 4-min interval. Control experiments were performed at the same concentrations of the

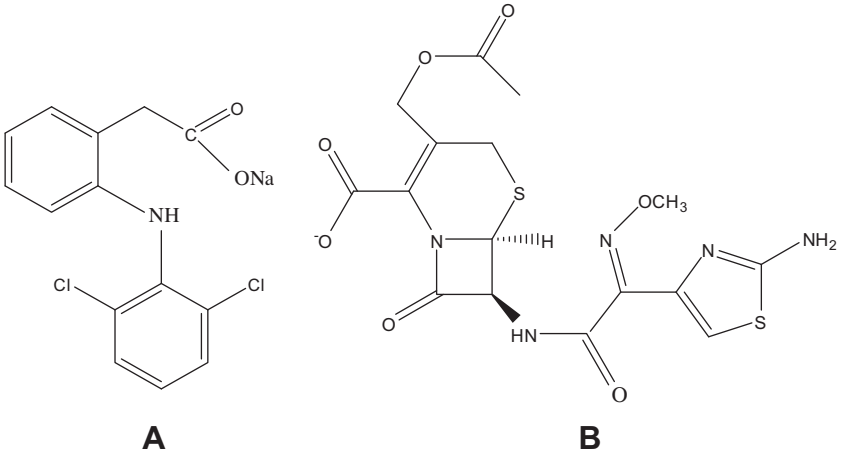

Fig. 1. Structure of diclofenac sodium (A) and cefotaxime sodium (B).