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Calorimetric investigation of diclofenac drug binding to a panel of moderately glycated serum albumins

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ABSTRACT

Glycation alters the drug binding properties of serum proteins and could affect free drug concentrations in diabetic patients with elevated glycation levels. We investigated the effect of bovine serum albumin glycation by eight physiologically relevant glycation reagents (glucose, ribose, carboxymethyllysine, acetoin, methylglyoxal, glyceraldehyde, diacetyl and glycolaldehyde) on diclofenac drug binding. We used this non-steroidal anti-inflammatory drug diclofenac as a paradigm for acidic drugs with high serum binding and because of its potential cardiovascular risks in diabetic patients. Isothermal titration calorimetry showed that glycation reduced the binding affinity K_a of serum albumin and diclofenac 2 to 6-fold by reducing structural rigidity of albumin. Glycation affected the number of drug binding sites in a glycation reagent dependent manner and lead to a 25% decrease for most reagent, expect for ribose, with decreased by 60% and for the CML-modification, increased the number of binding sites by 60%. Using isothermal titration calorimetry and differential scanning calorimetry we derived the complete thermodynamic characterization of diclofenac binding to all glycated BSA samples. Our results suggest that glycation in diabetic patients could significantly alter the pharmacokinetics of the widely used over-the-counter NSDAI drug diclofenac and with possibly negative implications for patients.

Introduction

Serum albumin is the major drug binding protein in plasma and greatly influences the pharmacokinetics of drugs that bind to it. Changes in the drug binding capacity of serum albumin can have profound effects on the free drug concentration and can consequently increase adverse effects or reduce therapeutic efficacy. Elderly diabetic patients could be particularly sensitive to serum glycation effects on drug binding. In the United States more than 25% of the population >65 years of age has diabetes mellitus (Boyd et al., 2005) and a more than 50% of these patients receive treatment for additional chronic morbidities (Kirkman et al., 2012). Polypharmacy in these patients is frequent and could be complicated if drug-binding properties of serum albumin are altered to an extent that becomes pharmacological relevant.

In this study we investigate with spectroscopic and calorimetric methods, how glycation of serum albumin modifies its interactions with diclofenac. We propose a mechanism by which glycation modulates diclofenac drug binding and discuss the possible pharmacological relevance of serum glycation.

Serum albumin readily undergoes chemical modification by glycation and its half-life of approximately three weeks is sufficient to allow significant accumulation of glycation damage. Protein glycation is the non-enzymatic, chemical modification of amino acid side chains by reactive carbonyl compounds. It affects mostly lysine and arginine side chains. Physiological relevant glycation reagents include reducing sugars, such as glucose and ribose, as well as oxo- and hydroxy aldehydes such as glyoxal, methylglyoxal or glycol aldehyde. These compounds are formed as metabolic by-products and are form at elevated rates in tissues under oxidative stress, at sites of inflammation, or under conditions involving renal dysfunction (Thornalley et al., 1999; Ahmed et al., 2003; Krautwald and Munch, 2010; Rabbani et al., 2007). Increased glycation of serum proteins is well documented in diabetic patients (Peacock et al., 2008; Inaba et al., 2007; Meerwaldt et al., 2008; Perkins et al., 2012), but also occurs in patients with cardio-vascular disease (Pu et al., 2007; Zieman and Kass, 2004; Cooper, 2004), arthritis (de Groot et al., 2011; Vytasek et al.,

Abbreviations: Ace, acetoin; BSA, bovine serum albumin; CD, circular dichroism; CML, carboxymethyllysine; DCF, diclofenac; DF-BSA, defatted bovine serum albumin; Dia, diacetyl; DSC, differential scanning calorimetry; GA, glyoxylic acid; Gla, glycolaldehyde; Glc, glucose; Gly, glyceraldehyde; ITC, isothermal titration calorimetry; MG, methylglyoxal; Rib, ribose.

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2010), kidney failure (Mostafa et al., 2007), or liver cirrhosis (Sebekova et al., 2002).

Diabetic patients have notoriously elevated levels of glycated blood proteins. Levels of albumin glycation average ~25% of total serum albumin in diabetic subjects and can exceed glycation levels of 40% or 50% in individual cases (Peacock et al., 2008; Inaba et al., 2007; Pu et al., 2007). Because many elderly diabetic patients receive pharmacotherapy for chronic comorbidities it is important to investigate how glycation alters drug binding properties of serum proteins.

The binding of many drug molecules to whole plasma, isolated plasma proteins and in particular serum albumin has been studied in great detail (Vallianatou et al., 2013; Zhu et al., 2008; Zsila et al., 2011). Biophysical studies investigating drug binding to serum albumin use both the human and the closely related bovine protein as a valid substitute (Sulkowska, 2013; Han et al., 2012). These studies have identified two major drug binding sites (Sudlow I and II) and at least four additional secondary drug binding sites in serum albumin (Ghuman et al., 2005; Ascenzi et al., 2006; Zsila, 2013).

The binding of several drug molecules to glycated serum albumin has been investigated (Oettl and Stauber, 2007; Rondeau et al., 2010; Baraka-Vidot et al., 2012; Joseph et al., 2011, 2010; Trynda-Lemiesz and Wiglusz, 2011), but no consensus mechanism that would allow prediction of changes in binding affinity for particular drugs has emerged yet. Our studies combine structural and thermodynamic experiments to gain a better understanding of the relationship between glycation induced structural changes in albumin and its drug binding properties.

We have previously investigated the structural consequences of serum albumin glycation and found that glycation does weaken the structural integrity of the protein, but does not lead to structural collapse or complete unfolding of the protein (Indurthi et al., 2012; Vetter and Indurthi, 2011). We also showed that the chemical nature of the glycation reagent influences the extent to which the structural properties of serum albumin are altered. Building upon these studies, we hypothesized that glycation of serum albumin affects drug binding in a manner that is specific to individual glycation reagents.

We chose diclofenac as a representative example of a small acid drug molecule with high plasma protein binding. Diclofenac (Fig. 1) binds to two sites on serum albumin with $K_a = 10^5$ to $10^4\ M^{-1}$ affinities, depending on the methodology used to measure binding affinities and the composition of the buffer system used in the experiments (Chamouard et al., 1985; Wang and Wang, 2008; Dutta et al., 2006; Sharma et al., 2012). Diclofenac can interact via ionic and hydrophobic interactions with drug binding sites on proteins (Ji et al., 2002; Russeva and Mihailova, 1996). Diclofenac was also selected because of its wide use as an over-the-counter pain and anti-inflammatory medication. Diclofenac is frequently used to relieve arthritis pains, which is not uncommon in diabetic patients. Non-steroidal anti-inflammatory drugs have been associated with increased cardiovascular risk and recent studies suggest a particularly high overall risk for diclofenac (McGettigan and Henry, 2006, 2011; Moore et al., 2014). Diabetic patients might be at an increased risk of adverse effects of diclofenac due to altered drug binding properties of glycated of serum albumin.

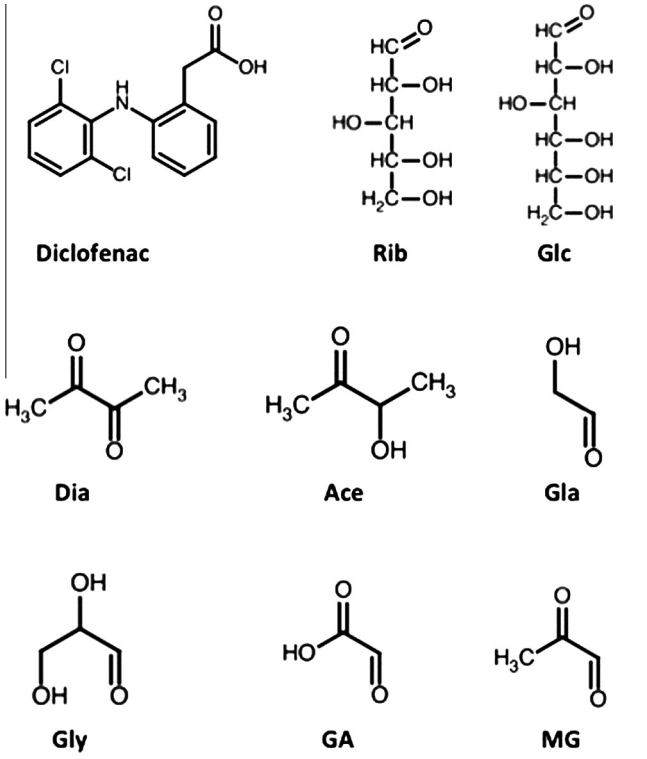

Fig. 1. Chemical structures of the non-steroidal anti-inflammatory drug diclofenac and the glycation reagents used to modify bovine serum albumin for this study. Rib: ribose; Glc glucose; Dia diacetyl (2,3-butanedione); Ace acetoin (3-hydroxybutanone); Gla glycolaldehyde (2-hydroxyacetaldehyde); Gly glyceraldehyde (2,3-dihydroxypropanal); GA glyoxylic acid (oxoethanoic acid); MG methylglyoxal (2-oxopropanal).

2. Materials and methods

2.1. Materials

All chemicals used for glycation of BSA were of purchased from VWR or Sigma Aldrich and of the highest purity available. The reagents were used without further purification. The individual glycation reagents were from the following manufacturers: acetoin (>98%, TCI America), diacetyl (>98%, TCI America), methyl glyoxal (35% aqueous solution, Alfa Aesar), glyoxalic acid (>97% Alfa Aesar), glycolaldehyde (crystalline dimer, Sigma Aldrich), glyceraldehyde (>90%, Sigma Aldrich), glucose (EMD Millipore), ribose (high purity grade, Amresco). Diclofenac was purchased from MP-Biomedicals via VWR.

All reagents used for buffer preparation were of biochemical or molecular biology grade and purchased from VWR or Fisher Scientific. A single batch of non-defatted bovine serum albumin (Amresco, biotechnology grade, crystalline, purity >99% purity, cold ethanol precipitation) was used for all glycation experiments. Differential scanning calorimetry experiments showed the expected thermal unfolding profile for non-defatted BSA. A second non-fatty acid containing BSA preparation (DF-BSA; Amresco, biotechnology grade, heat shock isolated, purity >98%) showed the expected single T_m in thermal unfolding studies and was used as control.

2.2. Preparation of glycated serum albumin

We used eight glycation agents (Fig. 1) to glycate BSA following the procedure previously described by us and by others (32, 33, 43–45]. Briefly, 20 mg/ml of BSA was dissolved in a buffer containing 500 mM sodium phosphate, 1 mM EDTA and 1 mM sodium azide at pH 8.0. The glycation agents were added to the final concentrations indicated in Table 1 and sterile filtered into sterile glass vials. CML modified BSA was prepared using sodium cyanoborohydride to reduce the Schiff base initially formed between the amino groups of lysine side chains and glyoxalic acid. The solutions were incubated at 37 °C for 21 days in sealed sterile glass vials. After incubation samples were dialyzed twice against 200 volumes of