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Biliverdin is the endogenous ligand of human serum α_1-acid glycoprotein

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ABSTRACT

α_1-Acid glycoprotein (AAG), an acute phase component of the human serum, is a prominent member of the lipocalin family of proteins showing inflammatory/immunomodulatory activities and promiscuous drug binding properties. Both three-dimensional structure of AAG and its precise biological function are still unknown and only a few endogenous AAG ligands have been described to date. CD spectroscopic studies performed with commercial AAG and the separated genetic variants revealed high-affinity binding of biliverdin (BV) and biliverdin dimethyl ester to the 'F1/S' fraction of the protein. The preferential accommodation of the right-handed, *P*-helicity conformers of the pigments by the protein matrix resulted in strong induced CD activity, which was utilized for estimation of the binding parameters and to locate the binding site. It was concluded that both pigments are bound in the central β-barrel cavity of AAG, held principally by hydrophobic interactions. Possible biological implications of the BV binding ability of AAG with special emphasis on the heme oxygenase-1 pathway are discussed.

Human α_1-acid glycoprotein (AAG), also known as orosomucoid, is the most important component of the positive acute phase proteins of the plasma [1,2]. The normal concentration of AAG (0.5–1.4 mg/mL; 12–30 μM), which accounts for about 1% to 3% of total protein can rise to three or fourfolds within a day following the unset of an acute phase response [3]. The single polypeptide chain of AAG (183 residues, M_w = 36,000–44,000) is heavily glycosylated, which is responsible for protein microheterogeneity [2]. In addition, the polypeptide chain of AAG exists in two main polymorphic forms called 'F1/S' (ORM1) and 'A' (ORM2) genetic variants encoded by two different genes having 22/183 codon substitutions [4]. The 'F1/S' isoform predominates in a mean molar ratio of 3:1 over 'A' in normal human plasma [5] but variations of this ratio have been reported under pathological conditions [6]. Due to the carbohydrate-based microheterogeneity, the structure of AAG proved to be refractory for X-ray crystallographic determination. Based on genetic analysis, AAG was classified as the member of the lipocalin protein family [7]. Lipocalins, distributed in animals, plants, and even bacteria, are small, single domain, secreted proteins that bind and transport generally hydrophobic, biologically active molecules (e.g. retinoids, steroids) [8]. Ten lipocalins were found in the human body to date [7] among which X-ray/NMR structures of several proteins have been solved [9]. Despite the less than 20% overall sequence similarity among lipocalins, they share a conserved structural motif called the lipocalin fold, which is common to this group of proteins. It is a β-barrel formed by eight antiparallel β-strands winding around a central axis. At the open end of the resulting cup shaped pocket these strands are connected in a pair-wise fashion by four loops, which form the entrance to the central cavity (calyx), the principal binding site of lipophilic ligands [9].

Among the large number of therapeutic agents reported to bind with AAG basic drugs predominate [10]. High-affinity binding of drugs to AAG modulates their pharmacokinetic properties which may have clinical relevances [11–13].

The primary physiological functions of AAG is as yet undetermined, but the protein has been shown to be involved in several inflammatory and immunological processes [1–3]. AAG suppresses the expression of TNF-α, inhibits the activation/migration of neutrophil leukocytes [14,15], the activation of the complement system and platelet aggregation [16], and inhibits of apoptosis and subsequent inflammation [17]. However, the underlying molecular mechanisms of these diverse effects are largely unknown. Taking into account the promiscuous drug binding ability of AAG [10] it can be hypothesized that endogenous substance-AAG binding interactions might be important in biological functions of this protein. In marked contrast with the more than 300 pharmaceutical agents showed to interact with AAG [10], only a few endogenous AAG ligands have been reported to date. Steroid hormones (progesterone, $K_a = 6.2 \times 10^4\ M^{-1}$ [18]; testosterone) and catecholamines (adrenalin, $K_a = 7.4 \times 10^3\ M^{-1}$; noradrenalin, $K_a = 8.3 \times 10^3\ M^{-1}$

Abbreviations: AAG, α_1-acid glycoprotein; AO, acridine orange; BP, bile pigment; BV, biliverdin; $BV(COOMe)_2$, biliverdin dimethyl ester; CD, circular dichroism; CE, Cotton effect; CHLP, chlorpromazine; DMSO, dimethyl sulfoxide; HO, heme oxygenase; ICD, induced circular dichroism; UV/vis, ultraviolet–visible.

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[19]) are long-known but low-affinity ligands of AAG. The platelet activating factor binds strongly to AAG with a K_a value of $4.3 \times 10^6\ M^{-1}$ [20], which is responsible for the AAG mediated inhibition of platelet aggregation. The accommodation of hemin in the hydrophobic pocket of AAG ($K_a = 4.4 \times 10^5\ M^{-1}$) has been deduced from fluorescence results [21]. Recently, Ojala et al. [20] demonstrated the high-affinity AAG binding of some novel endogenous lipids, the inflammatory mediator lysophosphatidylcholine ($K_a = 5.5–6.7 \times 10^6\ M^{-1}$) and lysophosphatidylserine ($K_a = 5.3 \times 10^6\ M^{-1}$).

By using circular dichroism (CD) spectroscopy method, this work demonstrates that the anti-inflammatory and cytoprotective bile pigment biliverdin (BV) and is the endogenous ligand of AAG.

Materials and methods

Materials. BV·HCl and $BV(COOMe)_2$ purchased from Frontier Scientific were used as supplied. CHLP·HCl was obtained from EGIS Pharmaceuticals Ltd. (Budapest, Hungary). AAG and AO were purchased from Sigma (St. Louis, MO, USA) and used without further purification.

Isolation of genetic variants of AAG. Commercial 'native' AAG samples were subjected for separation of the two main genetic variants. The 'F1/S' and 'A' forms were separated as described previously [22].

Preparation of AAG sample solutions. Protein samples were dissolved in physiological Ringer buffer solution (pH 7.4, 137 mM NaCl, 2.7 mM KCl, 0.8 mM $CaCl_2$, 1.1 mM $MgCl_2$, 1.5 mM KH_2PO_4 and 8.1 mM $Na_2HPO_4{\cdot}12H_2O$). Molar concentration of AAG was determined spectrophotometrically: $\varepsilon_{278nm} = 33{,}500\ M^{-1}\ cm^{-1}$ [23].

CD and UV/vis absorption spectroscopy measurements. CD and UV/vis spectra were recorded on a Jasco J-715 spectropolarimeter at 25 ± 0.2 °C under a constant nitrogen flow. Temperature control was provided by a Peltier thermostat equipped with magnetic stirring. For recording CD spectra, rectangular quartz cells of 1 cm optical pathlength (Hellma, USA) were used. Each spectrum represents the average of three scans (scan speed was 100 nm/min). UV/vis absorption spectra were obtained by conversion of the high voltage (HT) values of the photomultiplier tube of the CD equipment into absorbance units. CD and absorption curves of ligand–protein mixtures were corrected by subtracting the spectra of ligand-free protein solutions.

Calculation of the AAG binding parameters of bile pigments from ICD data. Details of the estimation of the association constants (K_a) and the number of binding sites (n) from ICD data have been described previously [24]. Non-linear regression analysis of the ICD data obtained at different [BP]/[AAG] molar ratios was performed by the NLREG® software (statistical analysis program, version 6.3 created by Philip H. Sherrod).

Results

CD and UV/vis spectroscopic studies of AAG binding of BV and its dimethyl ester

Neither organic or aqueous solution of BV and $BV(COOMe)_2$ display CD activity (Fig. 1). Upon addition of these pigments into sample solutions of AAG, however, strong induced CD (ICD) bands were recorded. Both BV and $BV(COOMe)_2$ displayed a short-wavelength negative (Fig. 1), and a long-wavelength positive (not shown) induced Cotton effects (CE) similar in shape to the UV and vis absorption bands. $\Delta\varepsilon_{max}$ values of $BV(COOMe)_2$ calculated from the total ligand concentration of the sample solution were twofold larger than that of BV. Shape and position of the induced CEs remained the same during the titration of the protein solution with increasing amounts of the pigments. The series of ICD values measured in the negative maximum of the UV CE at increasing [BP]/[AAG] molar ratios were subjected to non-liner regression analysis to estimate the binding parameters. Values of the K_a and the number of binding sites per AAG molecule (n) are shown in Table 1. According to the binding data, AAG association of $BV(COOMe)_2$ is one-hun-

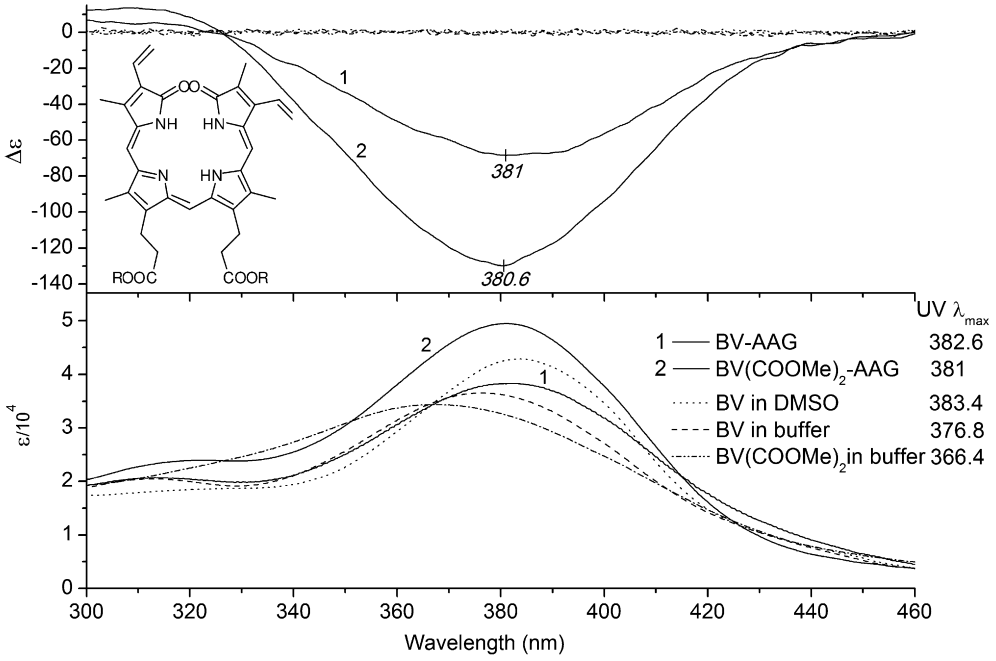

Fig. 1. Upper panel: ICD spectra of 4.4 μM BV·HCl (1) and 5.7 μM $BV(COOMe)_2$ (2) in the presence of 25 and 29 μM native (commercial) AAG. Lower panel: UV/vis absorption spectra of 4.4 μM BV·HCl (1) and 5.7 μM $BV(COOMe)_2$ (2) in the presence of 25 and 29 μM native (commercial) AAG. CD and UV/vis absorption curves of 10 μM bile pigments measured in DMSO (BV, dotted line) and in protein-free Ringer buffer solution (BV, dashed line; $BV(COOMe)_2$, dash-dotted line) are also shown. Inset: the chemical structure of BV (R = H) and $BV(COOMe)_2$ (R = Me).