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Review article

Human plasma lipocalins and serum albumin: Plasma alternative carriers?

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ABSTRACT

Lipocalins are an evolutionarily conserved family of proteins that bind and transport a variety of exogenous and endogenous ligands. Lipocalins share a conserved eight anti-parallel β-sheet structure. Among the different lipocalins identified in humans, α-1-acid glycoprotein (AGP), apolipoprotein D (apoD), apolipoprotein M (apoM), α1-microglobulin (α1-m) and retinol-binding protein (RBP) are plasma proteins. In particular, AGP is the most important transporter for basic and neutral drugs, apoD, apoM, and RBP mainly bind endogenous molecules such as progesterone, pregnenolone, bilirubin, sphingosine-1-phosphate, and retinol, while α1-m binds the heme. Human serum albumin (HSA) is a monomeric all-α protein that binds endogenous and exogenous molecules like fatty acids, heme, and acidic drugs. Changes in the plasmatic levels of lipocalins and HSA are responsible for the onset of pathological conditions associated with an altered drug transport and delivery. This, however, does not necessary result in potential adverse effects in patients because many drugs can bind both HSA and lipocalins, and therefore mutual compensatory binding mechanisms can be hypothesized. Here, molecular and clinical aspects of ligand transport by plasma lipocalins and HSA are reviewed, with special attention to their role as alterative carriers in health and disease.

Contents

Abbreviations: AGP, α-1-acid glycoprotein; apoD, apolipoprotein D; CF, cystic fibrosis; CMPF, 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid; DIS, di-iodosalicylic acid; FA, fatty acid; FABP, fatty acid-binding protein; Gd, glycodelin; HDL, high density lipoproteins; HSA, human serum albumin; LCAT, lecithin-cholesterol acyl transferase; LDL, low density lipoprotein; NGAL, neutrophil gelatinase-associated lipocalin; NPC, Niemann–Pick type C disease; RA, retinoic acid; RBP, retinol-binding protein; SCR, structurally conserved regions; S1P, sphingosine-1-phosphate; TIB, tri-iodobenzoic acid; TTR, transthyretin; VLDL, very low density lipoprotein.

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1. Introduction

In humans, endogenous and exogenous compounds are usually transported by plasma proteins like lipocalins and human serum albumin (HSA) [86,221].

Lipocalins are member of the calycin protein superfamily [96,97,100,104]. To date, the main lipocalins recognized in humans are: α-1-acid glycoprotein (AGP or AAG) [102], α1-microglobulin (α1-m or protein HC) [4], lipocalin type prostaglandin D synthase (PGDS) [251], apolipoprotein D (apoD) [207], apolipoprotein M (apoM) [209], complement component 8γ (C8G) [183], glycodelin (Gd) [68,143], neutrophil gelatinase-associated lipocalin (NGAL, also known as lipocalin 2) [111], odorant-binding protein [43], lipocalin 12 [79], lipocalin 15 [79], retinol-binding protein (RBP) [272], and tear lipocalin (also known as lipocalin 1) [42,208]. Among these, AGP, apoD, apoM, RBP and α1-m are plasma lipocalins. In particular, AGP, apoD, apoM, and RBP act as carriers of endogenous and exogenous ligands [102,207,209,272], while α1-m binds the heme [151,163].

HSA, the most abundant protein in plasma, regulates plasma oncotic pressure, acts as an anti-oxidant, transports many endogenous compounds like fatty acids (FAs) and heme, as well as several acidic drugs thus affecting their pharmacokinetics [86,191].

In plasma, the AGP, apoD, apoM, RBP, and α1-m concentrations are ~2.0×10^{-6} M, ~6.5×10^{-6} M, ~1.0×10^{-6} M, ~1.3×10^{-6} M, and ~2.0×10^{-6} M, respectively [37,45,62,69,147,209], whereas the HSA concentration is ~7.5×10^{-4} M [86] (Table 1). Besides the different plasma concentration, the ligand binding properties of lipocalins appear to be different from those of HSA [131]. In fact, apoD, apoM, RBP and α1-m bind only one ligand molecule, AGP often simultaneously accommodates two ligand molecules, and HSA binds up to 9 equivalents of FAs [86,223] (Table 1). As a result, lipocalins undergo saturation by ligands while HSA is generally not saturated *in vivo* [131,184]. Of note, ligand binding to some plasma lipocalins (*e.g.*, AGP and RBP4) and HSA is regulated both competitively and allosterically [17,61,86,92,94].

Considering the common transport function and the different ligand binding properties of plasma lipocalins and HSA, here we review the biochemical properties of these plasmatic proteins focusing on the alterative role of lipocalins and HSA as plasma drug carriers in health and disease.

2. Plasma lipocalins

Lipocalins are a family of extracellular proteins described in bacteria, plants, vertebrate and invertebrate organisms [96,101]. They are composed of ~170 amino acids with a similar tertiary structure and play a role in the storage and transport of compounds like vitamins, steroids, and metabolic products [99,227] (Table 1). Lipocalins recognize cell surface receptors and are involved in the immune response and in the maintenance of cell homoeostasis [98,99,101,227].

2.1. Lipocalin structure

Lipocalins share several common molecular recognition properties being characterized by a large cup-shaped cavity formed by a β-barrel as the central folding motif. This β-barrel contains eight anti-parallel β-strands (labelled A-H) with a three-dimensional structure suitable for ligand binding. While a peptide segment closes the *N*-terminus of the β-barrel, the *C*-terminus is open to the solvent, thus providing ligand access to the hydrophobic cavity. Although lipocalins often bind ligands in a one-to-one molar ratio [98,99,101,227] (Fig. 1), the simultaneous binding of two ligands to AGP has been reported [91,94,278].

Six of the seven loops connecting the strands, labelled L2-L7, are short β-hairpins. The seventh loop, named L1, is large and partially closes the ligand-binding site. Remarkably, a considerable variation in the amino acid composition, as well as in the conformation and length of the polypeptide segments, has been described among members of the lipocalin superfamily; these differences represent the molecular determinants of ligand binding specificity. Overall, the lipocalin fold is characterized by three structurally conserved regions (SCRs): *i*, the region comprising strand A and the 3_{10}-like helix (*i.e.*, SCR1); *ii*, the region encompassing strands F and G, and the connecting loop L6 (*i.e.*, SCR2); and *iii*, the region encompassing the strand H and its connecting residues (*i.e.*, SCR3) [227] (Fig. 1).

Lipocalins have a variable number of disulfide bonds. Among them, the one that links the *C*-terminal region to the β-barrel is conserved. However, some human lipocalins show an unpaired Cys residue that forms a covalent bond with another protein [47,48].

2.2. α-1-Acid glycoprotein

AGP is a 42 kDa glycoprotein characterized by ~45% carbohydrates. Although AGP plasma concentration (~2.0×10^{-6} M) is lower than that of HSA, it is relevant in the transport of neutral and basic drugs [131,147] (Table 1).

AGP is composed of 183 amino acids; two variants, *i.e.* F1/S and A, have been identified. These variants are encoded by the *ORM1* and *ORM2* genes, respectively, and differ in 22 amino acid residues. More than 100 forms of AGP occur in human serum depending on both amino acid substitutions at positions 32 and 47 and carbohydrate composition. Diverse physiological and pathological states, like pregnancy, acute inflammatory conditions, severe rheumatoid arthritis, and hepatitis cause modifications in the AGP glycosylation status, thus affecting its biological properties [102,133] (Table 1).

The F1/S genetic variant represents the 70% of the AGP protein in plasma, while the A variant represents only the 30% [91]. Remarkably, each variant binds selectively a specific drug. Indeed, binedaline, dipyridamole, and warfarin are recognized by the F1/S variant, while disopyramide, imipramine, and methadone bind only to the A variant. However, some chemicals like chlorpromazine, flunitrazepam, progesterone, and propranolol can bind both AGP variants [56,125]. These variants display the typical lipocalin fold, where loops 1, 2, 3, and 4 (connecting β-strands A/B, C/D, E/F, and G/H, respectively) delimitate the access to the ligand binding site [161,176,223]. The ligand binding cavity of the F1/S variant of AGP is composed by three lobes, among which lobe I is large, non-polar, and can lodge hydrophobic ligands, whereas lobes II and III are small and negatively charged. The binding pocket of the A variant is composed only by lobes I and II [176]. The geometry of the AGP ligand binding cavity is compatible with the structure of basic and neutral