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Plasma Vitamin D-binding Protein (Gc-globulin): Multiple Tasks

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The transporter of vitamin D and its metabolites in blood has received increasing attention in recent years, and is recognized to be a member of a gene family that includes albumin and α-fetoprotein. Identical to the group specific component (Gc-globulin) of serum, the protein is a single-chain polypeptide constitutively synthesized in liver that circulates in amounts in far excess of normal vitamin D metabolite concentrations in blood. It plays the major role in the egress of endogenously synthesized vitamin D_3 from skin and appears to restrain D-sterols from too rapid/excessive cell entry. Along with plasma gelsolin, it comprises the plasma actin-scavenger system that facilitates removal of actin, liberated from lysed cells, by depolymerization and prevention of polymerization. Recently, the protein has been shown to behave as a co-chemotaxin specific for the complement peptide C5a, and its sialic acid-free form has been reported to play a role in macrophage activation. The latter functions strongly implicate its participation in inflammation responses. A unifying hypothesis might also suggest the protein to provide focal D-sterol delivery to cells that are important to the resolution of tissue injuries.

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BACKGROUND

First recognized as a group-specific globulin (Gc-globulin) [1] this plasma α-globulin was later identified to be the major transporter of vitamin D-sterols in blood (DBP) [2, 3]. The polymorphism of Gc-globulin has interested population geneticists and forensic pathologists, since three common phenotypes are recognized (in order of increasing electrophoretic mobility): Gc-2, Gc-1 slow and Gc-1 fast. Selective functions for these phenotypes are not clearly known, but a role for Gc-2 in macrophage activation has been reported [4, 5].

The protein is synthesized in hepatic parenchymal cells in a constitutive manner, and plasma levels are stable in adult life (5 μM), except during high-estrogen states when a 50% increase is seen. With very severe hepatic disease, low plasma levels are observed [6, 7]. No relationship between vitamin D-25-hydroxylation in liver and DBP plasma concentrations is recognized, but DBP concentrations can influence "free" concentrations of plasma vitamin D metabolites, presumably by altering their availability for metabolic clearance [3].

Structurally similar to albumin and α-fetoprotein, these proteins are considered to have arisen as a result of the divergent triplication of a single common ancestral gene [6]. The genes for these proteins are linked on the long arm of human chromosome 4 and share a series of conserved cysteine bonds that form a stable configuration within each of three internally homologous domains. Recent studies indicate that the sterol and actin-binding domains are located at the amino and carboxyl ends of the molecule, respectively [8]. In extensive, worldwide screening of human populations, a Gc^0 homozygote has never been identified, strongly suggesting that Gc/DBP deficiency results from a lethal mutation [3, 9].

THE TASKS CONSIDERED

Vitamin D-sterol transport

DBP exhibits a mole per mole, high (5×10^{-8} M) affinity toward 25-hydroxyvitamin D [25-OHD] > 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D] > vitamin D [D]. Endogenous production of D from cutaneous 7-dehydrocholesterol UV-radiation leads to egress of D from skin on DBP [10]. Following its oral absorption, D is carried on lacteal chylomicrons. This route permits hepatic entry of D on chylomicron remnants, but the strongly conservative body retention of D is well-served by its high-affinity carrier, DBP. DBP is

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Table 1. Features of plasma DBP

Size	58 kDa
Concentration	5–6 μM
D-sterol ligand/carrier	mol/mol
Ligand preference	25-OHD > 1,25-$(OH)_2$D > = D
D-sterol occupancy	<5%
Free sterol concentrations:	25-OHD = 0.04% of total
	1,25-(OH)D = 0.4% of total
Actin influence on sterol binding	Not recognized

Table 2. Non-sterol binding features of DBP

Actin-binding	mol/mol (G-actin)
Affinity for G-actin	2×10^{-9} M
Plasma actin clearance	Indistinguishable from that of DBP
Competitive binder	Profilin (10^{-6} M)
Profilactin complex	Dissociated by DBP
C5a binding	Apparently mol/mol
Affinity for C5a	Not known
Cell surface binding	Non-covalent
Cell types	Monocytes, lymphocytes, macrophages, trophoblasts, neutrophils
Source of cell-bound DBP	Plasma
Macrophage activation	Gc-2 or sialidase-treated Gc-1

not currently known to facilitate cellular ingress of D-sterols, but rather provides a physicochemical equilibrium with its sterols that permits their gradual availability to cells. For example, the tightly-bound 25-OHD has a plasma $t_{1/2}$ of 10–14 days in man. In distinction to the normal ligand to binding protein ratios of hormones such as steroid, thyroid, retinol, less than 5% of DBP is occupied by D-sterols [3]. This remarkable excess of binding protein to D-sterols appears to provide a circulating reservoir of these sterols, but some have considered that it may indicate other functions by the protein (see Table 1).

Plasma clearance of DBP appears not to be altered by 25-OHD occupancy, nor sialidase pre-treatment [11]. The labeled protein is catabolized thoroughly to small molecular weight peptides that are found in many tissues. No intermediate-sized fragments are found in plasma.

The relative binding affinity by DBP for vitamin D metabolites provides for a differential availability of "free" sterols [12, 13]. The hormonal metabolite, 1,25-$(OH)_2$D has 0.4% of its total concentration as the "free" sterol, whereas only 0.04% of 25-OHD is readily available to the tissues. Other tasks by DBP (*vide infra*) have not been demonstrated to alter its capacity nor its affinity for D-sterols.

Actin binding

During the search for the 1,25-$(OH)_2$D intracellular receptor, post-microsomal cytosolic preparations contained a protein–protein complex that exhibited a binding preference of 25-OHD > 1,25-$(OH)_2$D [3]. Later work revealed the complex to be an equimolar binding of DBP with monomeric or globular actin (G-actin) [14, 15]. Long-considered an artefact created by plasma contamination of intra-cellular materials, we now recognize that this complex can be found in plasma of man or animals undergoing tissue injuries and cell lysis [16]. There is a high-affinity between these proteins (nanomolar) and evidence that DBP is the major sequestrant of actin monomers that enter the circulation [17] (see Table 2).

Although multiple intracellular proteins bind actin and regulate microfilaments' disposition [18], only two plasma proteins bind actin avidly. The plasma protein gelsolin can sever actin filaments (F-actin) and nucleate actin. The behavior of DBP toward actin, however, resembles that of the intracellular actin-monomer sequestrants, such as profilin and DNAse-I [19, 20]. DBP appears to share its actin-binding site with profilin, but at a 1000-fold greater affinity [19]. Intracellular injections of DBP can cause reversible dissolution of a cell's microfilaments. In contrast, the actin-binding site for DNAse-I is distinct from that for DBP, and these proteins can form an equimolar, triprotein complex [15].

Current evidence supports the concept of a circulating actin-scavenger system in plasma [16]. The system involves filament-severing (gelsolin) and G-actin sequestration (DBP) activities. *In vitro* studies have demonstrated the complementarity of these proteins' actions in facilitating the depolymerization and inhibiting the polymerization of actin moieties [20, 21]. This system is saturable, since increasing amounts of G-actin, when injected intravenously to rats, will lead to intravascular filament formation, thrombi and microangiopathy [22]. Actin–DBP complexes have been found in plasma from man and animals sustaining injuries/inflammations of tissues (e.g. trophoblastic emboli, severe active hepatitis, acute lung injuries). Some controversy exists about whether actin occupancy of DBP leads to its altered disposition. Plasma survivorship studies have indicated no change or enhanced plasma DBP clearance [17, 23].

Cell associations

Several laboratories have detected DBP on the surfaces of lymphocytes, neutrophils and monocytes from blood. Immunofluorescent, electron-microscopic and surface labeling techniques indicate that bonafide DBP is intimately associated with the plasma membrane (Table 2). A search for membrane receptors for DBP has not been fruitful. Cell-surface DBP resists non-ionic but not ionic detergent extractions. When injected intracellularly, fluorescent-DBP appears to localize to lysosomes during the loss of its striking, depolymerizing effect on stress fibers [24]. A 12–14 kDa DBP-fragment has been identified in neutrophil lysates [25].

Co-chemotaxin

Serum enhancement of the activity of the complement factor, C5a, has been traced to DBP binding of C5a. DBP may protect C5a from proteolysis, thereby extending the biological life of this potent chemotaxin. The binding of C5a by DBP appears to be of low-affinity [26].

Possibly, the association between C5a and DBP may serve in the disposition of DBP-bound actin/sterols. Receptors for C5a on monocyte/macrophages might also attract DBP and its ligands, thereby facilitating their engulfment by these cells. The presence of the 25-OHD-1α-hydroxylase enzyme in activated macrophages also suggests that a DBP-mediated entry of actin (disposal) and 25-OHD (substrate) could both be served, but this remains a speculative idea at present.

Macrophage activator

Some evidence suggests a role for DBP in the activation of macrophages. Lymphocyte membrane-associated glycosidases are thought to remove sialic acid from *N*-acetylgalactosamine, thereby creating this macrophage activator [5]. Since Gc-2 does not contain sialic acid, it may constitute a natural activator. Some rodent osteopetrotic models have been reported to have deficient β-galactosidase activity on their lymphocytes. More information is required to determine the role of DBP in such activities, as well as its impact on macrophage function and osteoclastogenesis.

Other functions

DBP has been reported to bind unsaturated fatty acids, endotoxin liposaccharides and a synthetic mitogen for murine β-lymphocytes [6, 16]. Fatty acid-binding certainly could relate to this well-recognized property of albumin. Additional work is needed to clarify whether DBP is actively involved in such functions.

PERSPECTIVES

In the turtle, Licht and coworkers have discovered a structural homology between the high-affinity thyroxine protein (TBP) and the DBP of mammals [27]. It appears that TBP is the primary binding protein for 25-OHD in turtle (*Trachemys scripta*) plasma. The binding of thyroxine and 25-OHD is distinct and non-competitive. The homologous structure between TBP and DBP is in the amino-terminus, the domain now recognized to harbor the D-sterol binding site [8]. There is no current evidence that turtle TBP possesses actin-binding or co-chemotaxin activity. Full analysis of TBP structure will permit studies on the evolution of these proteins.

The data on hand collectively suggest that DBP can carry out multiple functions. The challenges ahead seem to involve a clarification of how DBP affects the disposition of its multiple, binding partners and vice versa. Such studies should lead to new insights concerning D-sterol transfer as well as mechanisms in repair of injury and inflammation.

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