

2.4 Synthetic function

2.4.1 Albumin and other carrier proteins

Richard A. Weisiger

Soluble proteins make up 6.4–8.3% of plasma by weight [1], a value comparable to their concentration in cell water [2]. Many plasma proteins are synthesized by the liver. Their concentrations may decrease in the setting of liver disease, malnutrition or protein-losing states such as nephrotic syndrome, severe burns, bowel inflammation, intestinal lymphangiectasia or major bleeding episodes. The most abundant plasma proteins have little or no enzymatic activity. Instead, their function is typically

binding to other molecules, generically referred to as *ligands*. Some plasma binding proteins that have been characterized are listed in Table 1. Most of these proteins bind to only one (or a few related) molecules with high affinity. For example, haemopexin binds haem, retinol-binding protein binds retinol, and transferrin binds iron. In contrast, other binding proteins such as albumin and alpha-fetoprotein are far less selective.

Albumin is the most important serum binding protein in terms of concentration (about 4.2%) [1,3] and number of different ligands bound. The latter include fatty acids [4], unconjugated bilirubin [5], many divalent cations such as copper [6,7], hydrophobic bile acids [6] and a variety of drugs and toxins [6,8–11] (Table 2). Albumin's broad specificity reflects not only

Binding protein	Ligand	References
Afamin	Vitamin E, probably others	[18,65,36]
Albumin	See Table 2	
Alpha-fetoprotein	Long-chain fatty acids, bilirubin, many others	[66]
Avidins	Biotin	[67]
Ceruloplasmin	Copper	[68]
Cholesterol ester transfer protein	Cholesterol	[33]
Corticosteroid-binding globulin	Steroid hormones	[69]
Folate-binding protein	Folic acid	[6]
Haptoglobin	Haemoglobin	[6]
Haemopexin	Haem	[6]
Lipocalins	Retinoids, arachidonic acid, steroids, pheromones	[70]
Lipoproteins	Triglycerides, cholesterol, bile acids, vitamin E	[6]
Phospholipid transfer protein	Phospholipids	[71,72]
Retinol-binding protein	Retinols	[73,74]
Sex hormone-binding globulin	Testosterone, dihydrotestosterone, estradiol	[75]
Thyroxine-binding globulin	Thyroxine	[76]
Transcobalamins	Vitamin B12	[77,78]
Transcortin	Corticoids	[79]
Transferrin	Iron	[6]
Transthyretin	Thyroxine	[73]
Vitamin D-binding protein	Vitamin D	[6]

Table 1 Soluble binding proteins found in plasma.

Table 2 Some molecules bound by plasma albumin.

Amino acids (tryptophan, cysteine)	[17,80]
Bilirubin	[5,6,29]
Cationic metal ions (Ag, Ca, Cd, Co, Cu, Hg, Mg, Mn, Ni, Zn)	[3,6,7,81–83]
Chloride	[6]
Many drugs (e.g. coumadin, digitalis, ibuprofen, diazepam, lidocaine, furosemide, valproic acid, phenytoin)	[6,8]
Medium- and long-chain fatty acids	[6,84]
Certain bile acids (e.g. lithocholate, chenodeoxycholate)	[6,85]
Certain steroid hormones (cortisone, estradiol, progesterone, aldosterone)	[6]
Thyroxine	[6, 86]
Certain toxins (e.g. aflatoxin, digitoxin, 'organic anions')	[9–11]

the presence of several discrete binding sites [6], but also its ability to adapt its three-dimensional conformation in response to binding [12,13], thus creating a better fit between the binding site and the ligand. This ability is termed *conformational adaptability* [14]. Even when no ligands are bound, albumin is constantly sampling from a large library of conformations in a process known as *conformational breathing* [14]. This variability helps to explain why it took so long to crystallize albumin in a form suitable for determining its detailed three-dimensional structure [6,15,16].

Human serum albumin consists of a single polypeptide chain of 585 amino acids with a molecular weight of 66 700 Da [9,17]. It contains six homologous subdomains that individually retain binding capacity when separated by proteolysis [15]. It is part of a multigene family that also includes alpha-fetoprotein, afamin and vitamin D-binding protein [15,18]. Synthesis of albumin is restricted to the liver, and albumin levels typically decline as the severity of liver disease worsens. The serum albumin concentration is an important part of the Child–Pugh score for assessing the severity of liver disease [19], but is not used in the MELD score for determining liver transplantation priority, in part because such critically ill patients often have other conditions (such as gastrointestinal bleeding) that may lower the albumin level [20,21]. Hyperalbuminaemia, when seen, is nearly always due to dehydration rather than increased synthesis.

Functions of albumin and other plasma binding proteins

Convection

The primary function of blood is to transport molecules to and from tissues by convection (flow). Without this function, important metabolites such as glucose and oxygen would become depleted, and products of metabolism such as carbon dioxide and lactate would accumulate to toxic levels. The most dramatic example of failure of convective transport is cardiac

arrest, which causes rapid and severe damage to most tissues. However, selective failure of convective transport can occur even with normal cardiac output if binding protein concentrations are low. For example, a very low haemoglobin level selectively blocks convective transport of oxygen, causing ischaemia. Congenital absence of transcobalamin blocks vitamin B12 transport, causing brain damage [22,23]. Congenital retinol-binding deficiency blocks the transport of retinol, causing atrophy of the retina [24]. Congenital haptoglobin deficiency is associated with seizures caused by the accumulation of haemoglobin and its degradation products in the brain [25]. Interestingly, congenital lack of serum albumin causes few manifestations in otherwise healthy persons, probably because other plasma proteins with overlapping binding specificities compensate for its absence [26]. Nevertheless, critically ill patients with hypoalbuminaemia have greatly decreased survival rates even with normal nutrition, and replacing albumin seems to improve their survival [27].

The rate at which a molecule is transported by the bloodstream is proportional to its concentration. For lipophilic and amphipathic molecules, this concentration is very low unless binding proteins are present, reflecting their limited solubility in water and their strong tendency to bind to tissues. Plasma binding proteins may increase the rate of convective transport of their ligands by many orders of magnitude. They are often called *carrier proteins* in recognition of this important role.

It may seem counterintuitive that protein binding can make a ligand more available to tissues. Indeed, bilirubin is cleared by liver much more efficiently in the absence of albumin than in its presence [28], suggesting that the opposite should be true. However, the maximum possible concentration of unbound bilirubin in plasma is so tiny, about 7 nM [29], that normal blood flow cannot deliver nearly enough bilirubin to the liver to keep up with the rate of production, even if the efficiency of removal is 100%. On the other hand, the concentration of albumin-bound bilirubin in plasma is large enough that only a small fraction needs to be removed to keep up with bilirubin production. In summary, protein binding makes ligands more available to sites of metabolism and excretion within the body by increasing their rates of convection from one tissue to another.

By a similar mechanism, binding proteins are also important in hormone signalling. Thyroid and steroid hormones are relatively hydrophobic and tend to bind strongly to tissues [30,31]. In the absence of binding proteins, these hormones bind to the first cells they encounter [30,31], thus preventing rapid signalling to other cells. By competing with cellular binding sites for these hormones, plasma binding proteins for thyroid [32], steroid [6,33] and other lipophilic hormones maintain a uniform circulating pool that bathes all cells with similar hormone concentrations, allowing changes in hormone levels to propagate rapidly and uniformly throughout the body [30]. In general, the activity of a hormone is proportional to the unbound ('free') hormone concentration [34]. Plasma hormone binding proteins buffer this concentration against rapid changes while promoting a uniform distribution throughout the body.

Diffusion

Convection alone cannot deliver dissolved molecules to the cell surface. No matter how vigorously the extracellular fluid is 'stirred' (e.g. by flow or turbulence), a very thin layer of unstirred plasma always remains just above the plasma membrane [35]. The only way for soluble molecules to cross this layer is by diffusion. The thickness of this layer varies among tissues. It is relatively small in the liver, where fenestrations in the endothelial cells allow plasma proteins direct access to the subendothelial (Disse) space [36]. Erythrocytes passing through the liver sinusoids may 'massage' the endothelium, thus both increasing the rate of exchange of plasma with the subendothelial space [36] and reducing the thickness of the unstirred layer. In contrast, cells in less metabolically active tissues may be located one or more cell diameters from the nearest capillary, requiring metabolites to diffuse much greater distances to reach them.

Diffusion is the random motion of dissolved molecules across a concentration gradient. Although the unstirred plasma layer is typically very small, it can greatly limit the rate at which small molecules move into and out of cells [35,37]. According to Fick's law, the steady diffusional flux (J) is proportional to the concentration difference of the diffusing molecule (ΔC) and its diffusion constant (D) (a measure of its rate of random motion) and inversely related to the square of the thickness of the layer (x) (Eq. 1).

$$J = \Delta C D / x^2 \quad (1)$$

Because of their low solubility in water [29,38], many hydrophobic molecules such as fatty acids and bilirubin cannot even approach a sufficient concentration in plasma to drive observed rates of uptake across this layer in the liver and other metabolically active tissues [37]. In much the same way that they increase convection, binding proteins also increase the diffusional flux of their ligands by increasing the amount of dissolved ligand available for diffusion [37]. While it is true that binding to the protein lowers the ligand's diffusion constant by a factor of about 10, this is more than compensated for by the increase in the soluble concentration (often by many orders of magnitude). These higher concentrations allow for larger values of ΔC , resulting in a net increase in the diffusion rate [37].

Interactions with cell membranes

Cellular uptake of protein-bound ligands may occur by several possible mechanisms. Some binding proteins randomly release their ligands in the general vicinity of the target cell membrane, while others deliver them carefully to specific sites on the cell surface or even within the cell. Many cells have receptors for binding proteins on their surface (e.g. transferrin [6]). Binding to these receptors is typically followed by receptor-mediated endocytosis of the entire protein–ligand complex, dissociation of the ligand from the binding protein in the acid lysosomal compartment and return of the binding protein to the plasma

[6]. In other cases, however, the complex may serve a signalling function. Sex steroid-binding protein allows certain sex hormones to activate adenyl cyclase without ever entering the cell by reversibly binding to a specific G protein-linked receptor on the cell surface [39]. Only certain sex hormones are able to activate the receptor when bound to the binding protein [39].

Cellular uptake may also draw on a pool of unbound ligand at the cell surface that is rapidly replenished by dissociation from the binding protein. Dissociation may either be spontaneous or catalysed by an interaction of the binding protein with the cell surface. If sufficiently slow, the rate of dissociation may limit the rate of cellular uptake by allowing the unbound ligand concentration to become depleted at the cell surface. Dissociation-limited uptake has been demonstrated for avidly bound molecules such as bilirubin and fatty acids [37,40]. Indeed, the affinity of albumin for bilirubin and long-chain fatty acids may represent a compromise between the need to limit the toxicity of these molecules (which favours more avid binding) and the need for rapid dissociation of the ligand from albumin prior to uptake (which favours less avid binding). Under physiological conditions, the hepatic uptake rate of fatty acids and bilirubin is influenced by many processes (including the rates of plasma flow into the liver, diffusion across the unstirred plasma layer, transport across the plasma membrane, diffusion through the cell cytoplasm and metabolism or excretion), each of which has a roughly comparable rate [41]. The detailed process by which binding proteins facilitate diffusion of their ligands across extracellular water layers was first defined by Bass and Pond [42] and has since been validated in a variety of physiological settings [43–45].

Limiting toxicity

Many molecules that bind to plasma proteins are potentially toxic. This includes endogenous molecules such as bilirubin and long-chain fatty acids, heavy metal ions such as iron and copper, and exogenous drugs and toxins such as digitalis, aminoglycosides and warfarin. These molecules cause little or no toxicity while bound to plasma proteins, but interfere with critical cell functions when present at sufficient concentrations within tissues. Binding to plasma proteins provides a relatively safe place to store these molecules while they await cellular metabolism or elimination.

For example, children with congenital absence of hepatic bilirubin uridine diphosphate (UDP)-glucuronal transferase may develop brain damage (kernicterus) from the accumulation of high levels of unconjugated bilirubin in neural tissues [46]. Bilirubin inhibits important cellular functions such as the Na^+/K^+ -ATPase and peptide phosphorylation in the brain [47,48]. However, brain damage does not occur until the storage capacity of plasma albumin is exceeded [6,46]. Likewise, long-chain fatty acids can cause damage to cell membranes and inhibit some enzymes when present at high concentrations [49–51]. However, high plasma concentrations are needed to

support maximum cardiac and skeletal muscle output. Binding of more than 99.99% of the plasma fatty acids to albumin greatly reduces this potential toxicity while keeping the fatty acids in a form that is rapidly available for metabolism. Finally, in patients with haemochromatosis, liver and other tissues do not become loaded with iron until after transferrin is saturated [52], allowing the formation of small iron complexes that are rapidly cleared by the liver [53,54]. Without binding proteins such as transferrin, iron ions release toxic free radicals as they repeatedly cycle between the ferrous and ferric states [55].

Plasma binding proteins also influence drug effectiveness and toxicity. Warfarin causes toxicity by blocking the formation of clotting factors in the liver, an effect that is used therapeutically by the drug coumadin. However, the full effect of a daily dose of coumadin does not become manifest until after the plasma binding sites on albumin have been loaded. Persons with hypoalbuminaemia are more susceptible to warfarin toxicity [56,57]. Hypoalbuminaemia is also a risk factor for aminoglycoside toxicity [58]. In a similar way, plasma binding increases the amount of a dietary toxin that must be ingested to produce toxicity. The important role of plasma binding in modulating tissue concentrations of drugs is underscored by the fact that measurement of unbound drug concentrations is increasingly used to monitor therapy [59]. Monitoring unbound concentrations is particularly important in patients with disturbed plasma binding due to hypoalbuminaemia or the presence of competitive inhibitors of binding such as uraemic toxins or certain drugs [59].

Targeting ligands to specific tissues

Binding proteins may help to direct their ligands to specific tissues that express receptors for the protein. This mechanism makes optimum use of ligands that are in limited supply or may cause toxicity if delivered to the wrong tissues. For example, transcobalamins target vitamin B12 to cells expressing the transcobalamin receptor [60], and transferrin targets iron to cells expressing the transferrin receptor [6]. When expressed, the receptor for sex steroid-binding globulin renders cells sensitive to the hormonal effects of certain steroid hormones [39].

Oncotic functions

Albumin provides approximately 80% of the colloid osmotic pressure in normal blood, reflecting its high concentration and relatively low molecular weight. Significant decreases in serum albumin levels due to liver failure, bleeding, nephrotic syndrome or protein-losing enteropathy are associated with the accumulation of fluid in extracellular compartments (e.g. oedema, ascites) [61]. Cerebral oedema may be life threatening and reflects not only low albumin levels, but also increased permeability of the cerebral vessels [62]. Administration of albumin reduces the reaccumulation of ascites after paracentesis [63] and has been used to treat cerebral oedema as well [64].

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2.4.2 The liver and coagulation

Maria T. DeSancho and Stephen M. Pastores

Introduction

The liver plays a major role in haemostasis, as most of the coagulation factors, anticoagulant proteins and components of the fibrinolytic system are synthesized by hepatic parenchymal cells. Additionally, the reticuloendothelial system of the liver helps to regulate coagulation and fibrinolysis by clearing these coagulation factors from the circulation. Finally, because the liver is a highly vascularized organ with vital venous systems draining through the parenchyma, liver diseases can affect abdominal blood flow and predispose patients to significant bleeding problems.

The aetiology of impaired haemostasis resulting from abnormal liver function is often multifactorial and may include impaired coagulation factor synthesis, synthesis of dysfunctional coagulation factors, increased consumption of coagulation factors, altered clearance of activated coagulation factors and quantitative and qualitative platelet disorders. In this chapter, we will review the normal physiology of haemostasis, describe the role of the liver in the haemostatic system and discuss the coagulation abnormalities that may occur in patients with liver disease and during liver transplantation.

Physiology of haemostasis

Normal haemostatic balance is dependent on a complex interplay between procoagulant, anticoagulant and fibrinolytic proteins. Initiation of coagulation begins when tissue factor (TF) is exposed after an injury to the vessel wall (Fig. 1). TF forms a

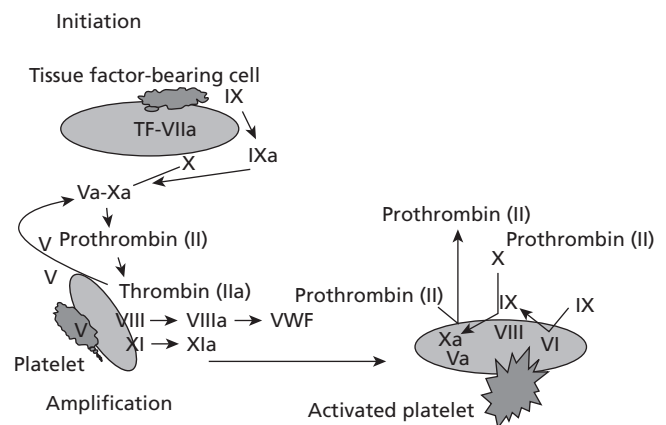


Fig. 1 Tissue factor (TF) is the major initiator of coagulation. Tissue factor-bearing cells include stimulated monocytes, endothelial cells and vascular smooth muscle cells. Exposure of TF to blood is rapidly followed by the formation of a complex between TF and factor VIIa that activates both factor IX and X, leading to generation of thrombin. Thrombin also activates factor V, VIII, XI and platelets.