Hypothesis

Albumin's role in steroid hormone action and the origins of vertebrates: is albumin an essential protein?

Michael E. Baker*

Department of Medicine, 0623B, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0623, USA

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Abstract Albumin, the major serum protein, binds a wide variety of lipophilic compounds including steroids, other lipophilic hormones and phytochemicals that bind to hormone receptors. Albumin has a low affinity for these lipophilic compounds. However, due to albumin's high concentration in serum, albumin is a major carrier of steroids and lipophilic hormones and regulator of their access to their receptors. Moreover, albumin functions as a sink for phytochemicals, which prevents their binding to hormone receptors and other cellular proteins, protecting animals from disruptive phytochemical-mediated endocrine effects. We propose that these properties of albumin were important in protochordates and vertebrates about 550 to 520 million years ago, just before and during the Cambrian. At that time, animal body sizes and exposure to phytochemicals in food were increasing, and animals in which albumin expression was high had a selective advantage in surviving and reproducing in the presence of toxic phytochemicals. This hypothesis that albumin has essential function(s) in mammalian endocrine physiology can be tested by comparing the effects of phytochemicals in Nagase rats that have 1/1000 the normal albumin concentration or in mice in which the albumin gene is knocked out with those in normal rats and mice.

Key words: Albumin function; Vertebrate evolution

1. Introduction

The concentration of albumin in human serum is about 45 mg/ml (0.67 mM), making albumin, by far, the major protein in serum. Albumin's principal functions are considered to be regulating the osmotic pressure in blood and transporting fatty acids and other lipophilic compounds [1,2]. Whether albumin is essential for survival of humans and other mammals has been questioned because analbuminemic humans and rats appear to function normally [3–5]. Evidently, the concentration of other serum proteins increases to maintain osmotic pressure and act as carriers for fatty acids and other lipophilic molecules. However, analbuminemic humans and rats have 1/1000 the normal albumin levels, so they are not truly without albumin, leaving the question of whether albumin is essential unresolved.

Here, I propose functions for albumin in vertebrate physiology that support an essential role for albumin. Although I focus on the physiological importance of the binding by albumin of steroid hormones and the protective function of albumin in regulating the binding of exogenous lipophilic compounds from plants to hormone receptors, the model is valid for albumin's influence on the binding to receptors of other endogenous lipophilic hormones and exogenous compounds from other sources (e.g. fungi, bacteria, marine environment, synthetic chemicals) and to other targets, such as kinases and dehydrogenases. I propose that these functions of albumin were important early in the evolution of vertebrates, and that albumin has been under evolutionary pressure to have low selectivity or a 'fuzzy recognition' for lipophilic compounds. This contrasts with the high selectivity of hormone receptors for ligands, which usually is a characteristic of an essential protein. Indeed, albumin's low selectivity for lipophilic molecules and albumin's 'commonness' may account for the acceptance of the notion that albumin is not essential. Studies with mice that have the albumin gene knocked out, and thus truly lack albumin, can determine if albumin has an essential role in mammalian physiology.

2. Steroid receptors originated about 600 to 520 million years ago

The adrenal and sex steroids: cortisol, aldosterone, estrogen, testosterone, and progesterone, have a central role in development, reproduction and homeostasis in humans and other vertebrates [6–8]. These steroids act through nuclear receptors, a diverse group of transcription factors that also includes receptors for retinoids, thyroid hormone, prostaglandins and fatty acids, as well as receptors without a known ligand, the orphan receptors [7–11]. The adrenal and sex steroid receptors form a clade in the nuclear receptor family [9–12]. Escriva et al. [11] have presented compelling evidence that nuclear receptors for adrenal and sex steroids, thyroid hormone, retinoids and prostaglandins are a 'recent' innovation that arose in protochordates and the earliest vertebrates; that is prior to or during the Cambrian.

3. Hormones and a closed circulatory system

A key innovation in protochordates and vertebrates was a closed circulatory system in which hormones are synthesized in one organ and transported in the blood to target cells, containing a cognate hormone receptor. Binding of the hormone to its receptor regulates the transcription of genes that evoke a characteristic physiological response. Proteins in the blood are important in transporting hormones to target cells. These carrier proteins include sex hormone binding globulin (SHBG), which binds estradiol and testosterone, corticosteroid binding globulin (CBG), thyroxine binding globulin

*Fax: (1) (619) 534-1424.
E-mail: mbaker@ucsd.edu

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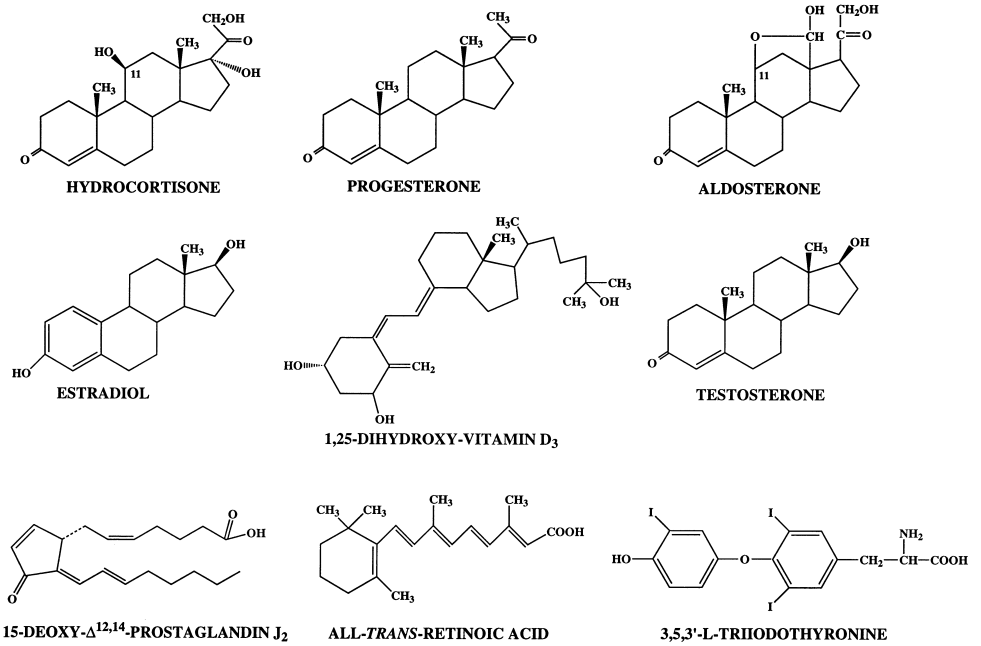

Fig. 1. Compounds that activate nuclear receptors and bind to albumin.

(TBG) and retinol binding protein (RBP). Neither SHBG, CBG, TBG nor RBP is homologous to albumin. However, TBG and CBG are distant homologs: human TBG and CBG are 41% identical.

Our search of GenBank revealed that only the mammalian sequences of SHBG, CBG and TBG have been determined (data not shown). A similar search found RBP sequences in amphibia and trout. However, albumin is found in the lamprey [13], a cyclostome that arose close to the origins of vertebrates. Cyclostomes and mammals last shared a common ancestor about 450 million years ago, suggesting that albumin arose either in a protochordate or early in the origins of vertebrates about 600 to 520 million years ago, prior to or during the Cambrian. Thus, it is likely that an ancestral albumin and RBP were present during the origins of various ligand-activated nuclear receptors in protochordates and vertebrates. At that time, we propose that albumin was the principal carrier for steroids and regulator of access to their receptors as well as a protector of steroid receptors from occupancy by phytochemicals.

4. Albumin can regulate access of steroids and other ligands to nuclear receptors

Albumin binds a wide variety of hydrophobic ligands including steroids, fatty acids, retinoids, thyroid hormone, prostaglandins and antibiotics [1,2] (Fig. 1). The equilibrium dissociation constants (K_ds) for steroids are in the 10^{-6} M to 10^{-4} M range. In contrast, steroids, retinoids and thyroid hormone have K_ds of 10^{-10} M to 10^{-9} M for their nuclear receptors; steroids and thyroxine have K_ds from 10^{-10} to 10^{-8} for SHBG, CBG and TBG. Retinol has a K_d of about 10^{-6} for RBP. However, despite albumin's low affinity for steroids, albumin's high concentration enables it to bind most of the estradiol and a substantial part of testosterone in male and non-pregnant female serum in the presence of SHBG [14,15].

Similarly, the high concentration of albumin enables it to regulate binding of steroids to nuclear receptors. For example, serum albumin regulates the access of estradiol and its metabolite, estriol, to the estrogen receptor [16]. This effect of albumin depends on its different affinity for estradiol and estriol [1,2,14,15]. Estriol has 1/3 the affinity of estradiol for the estrogen receptor. However, in the presence of albumin, the affinity of estriol is twice that of estradiol for the estrogen receptor because estriol has a lower affinity than estradiol for albumin. Walent and Gorski [17] and Arnold et al. [18] also found that albumin could reduce the binding of estradiol to the estrogen receptor.

5. Albumin controls the access of phytochemicals to the estrogen receptor

Animals accumulate various lipophilic compounds in their blood when they consume plants. Some of these compounds such as carotenoids and linoleic acid are important nutrients. Other compounds may have toxic effects due to binding to either hormone receptors [19–21] or enzymes [22–26]. A class of phytochemicals that are of special interest due to their endocrine effects are flavonoids, which have some structural similarity to estrogens (Fig. 2). Flavonoids bind to estrogen receptor [19–21] and to aromatases [22–25], which convert testosterone to estradiol. Albumin reduces the binding of flavonoids and other lipophilic compounds to the estrogen receptor [18,27–29]. This property of albumin would be important in regulating the action of a protoestrogen receptor early in the evolution of vertebrates and their protochordate ancestors.

The synthesis of flavonoids and other animal-toxic compounds by plants is an example of coevolutionary interaction between animals and plants [30–32], in which plants synthesize a chemical that is toxic to animals, and then animals evolve a defense, and then a new compound is synthesized in plants that will retard its consumption by animals. Animals defend against these toxic compounds by degrading the toxic phyto-

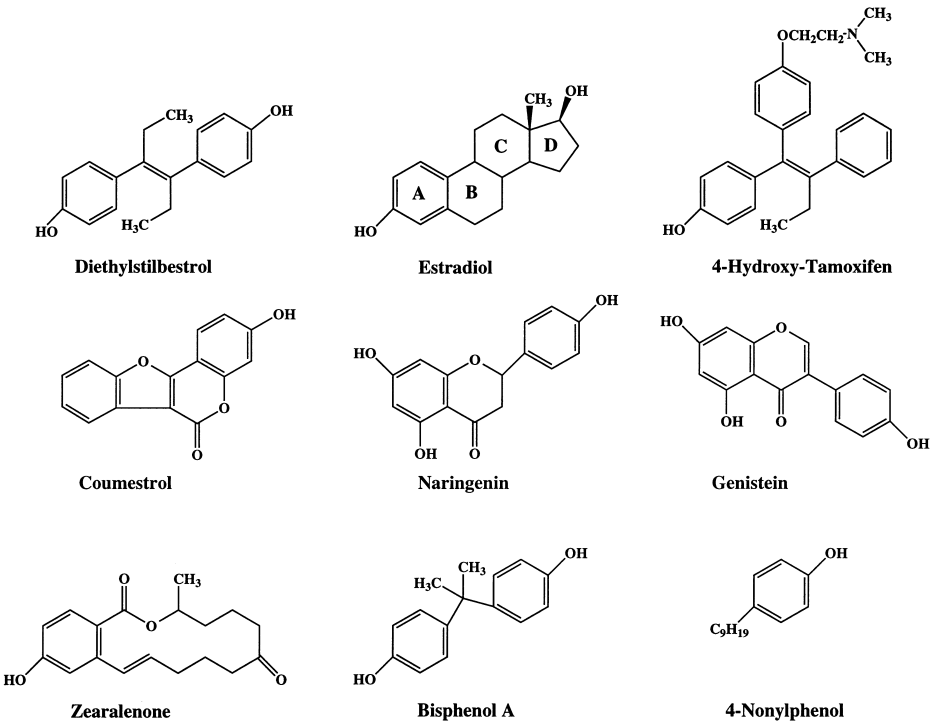

Fig. 2. Comparison of the structure of flavonoids and natural and synthetic estrogens. The phenolic group on the A ring of estradiol is important for binding to the estrogen receptor. The synthetic estrogen, diethylstilbestrol, which lacks a B ring, has a higher affinity than estradiol for the endoplasmic reticulum (ER) [21]. Evidently, the two phenolic groups can form tight bonds with the ER. Various plant and synthetic compounds also contain a phenolic group and cyclic structures or aliphatic groups that enable them bind to the ER. Among the phytochemicals, it is the isoflavonoids, which are found in soy, that have been best studied [19–21]. However, other flavonoids also bind to the estrogen receptor [19,20,27,29,30,32].

chemical, to render it inactive, or modifying it, for example by conjugation with glucuronic acid, which renders it soluble and suitable for excretion.

We propose that albumin has a role in the detoxification process by retarding the binding of phytochemicals and other xenobiotics [28,29,33] to hormone receptors, hormone carrier proteins and enzymes, which allows the inactivation and excretion of phytochemicals to occur without disruptive endocrine effects.

Thus, albumin has important physiological functions as a carrier of steroids and other lipophilic compounds to their nuclear receptors and a regulator of their access to their receptors. Also, albumin protects animals from the disruptive effects due to binding of phytochemicals and xenobiotics to hormone receptors and enzymes. This allows enzymes to metabolize these chemicals for inactivation and excretion. All of these activities of albumin would be important in the survival and expansion of vertebrates during and after the Cambrian.

The Cambrian period from 540 to 520 million years ago is characterized by the 'explosive' appearance of a wide variety of animal body plans that are substantially larger than animals in the preceding Vendian [34–36]. The causes of the Cambrian explosion are not fully understood. Larger animal body sizes are thought to depend on increased atmospheric oxygen, which supported the metabolism necessary for growth of larger animals [37,38]. This larger size requires an increased consumption of plants and small animals and the need to control the toxic effects of poisons in these foods. Even now, we ingest more toxic chemicals from plants than synthetic chemicals [31]. Under this selective pressure, humans and the intestinal bacteria have evolved enzymes that can metabolize these phytochemicals. For example, genistein and daidzein, two isoflavonoids that have estrogenic effects, have a half-life of about 7 h in humans [39].

6. Fuzzy recognition of chemicals by albumin: a selective advantage in the Cambrian

Animals experiencing increased exposure to phytochemicals in the Cambrian required one or more proteins to sequester these chemicals and prevent deleterious physiological effects. Even a protein with low affinity for lipophilic molecules can control their free concentration if the protein concentration is in excess to the lipophilic molecule's concentration [40]. Such a protein needs high aqueous solubility, stability, which can be achieved with disulfide bonds, and not be 'expensive' to synthesize; that is, not require amino acids such as tryptophan, which are not common in food. Albumin has these properties. Moreover, albumin recognizes a wide variety of small molecules with structures consisting of rings or aliphatic chains with different degrees of desaturation [1,2]. A consequence of recognizing many molecules is a low specificity or fuzzy recognition of these molecules. However, fuzzy recognition coupled with albumin's greater than 500 μM concentration in serum means that albumin will exceed the concentration of phytochemicals in serum and prevent unwanted binding of phytochemicals to receptors and enzymes.

It is likely that several proteins had the above attributes of albumin prior to and during the Cambrian. Indeed, insects have proteins in their hemolymph that bind fatty acids, ster-

ols, and other hydrophobic ligands [41]. However, none of the insect protein sequences in the database has sequence similarity to albumin.

The 'choice' of albumin may have been a chance mutation that increased its expression and conferred a selective advantage to an animal that consumed a variety of plants. It would also confer on albumin an osmotic function, which is important in animal physiology. The initial chance choice of albumin for either or both functions in a protochordate set the course for the future function of albumin vertebrate descendants, in which albumin is a high capacity, low affinity binder of lipophilic compounds.

Nagase rats, which have about 1/1000 the normal albumin concentration [5], can be used to investigate an essential function of albumin in the response to phytochemicals, steroids and other lipophilic hormones and xenobiotics. Transgenic mice in which the albumin gene is knocked out would be even better.

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