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Review

Human serum albumin isoforms: Genetic and molecular aspects and functional consequences☆

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ABSTRACT

Background: At present, 67 different genetic variants of human serum albumin and proalbumin have been molecularly characterized at the protein and/or gene level.
Scope of review: This review summarizes present knowledge about genetic and molecular aspects, functional consequences and potential uses of the variants.
Major conclusions: The frequency of bisalbuminemia in the general population is probably about 1:1000, but it can be much higher in isolated populations. Mutations are often due to hypermutable CpG dinucleotides, and in addition to single-amino acid substitutions, glycosylated variants and C-terminally modified alloalbumins have been found. Some mutants show altered stability in vivo and/or in vitro. High-affinity binding of Ni^{++} and Cu^{++} is blocked, or almost so, by amino acid changes at the N-terminus. In contrast, substitution of Leu90 and Arg242 leads to strong binding of triiodothyronine and L-thyroxine, respectively, resulting in two clinically important syndromes. Variants often have modified plasma half-lives and organ uptakes when studied in mice.
General significance: Because alloalbumins do not seem to be associated with disease, they can be used as markers of migration and provide a model for study of neutral molecular evolution. They can also give valuable molecular information about albumins binding sites, antioxidant and enzymatic properties, as well as stability. Mutants with increased affinity for endogenous or exogenous ligands could be therapeutically relevant as antidotes, both for in vivo and extracorporeal treatment. Variants with modified biodistribution could be used for drug targeting. In most cases, the desired function can be further elaborated by producing site-directed, recombinant mutants. This article is part of a Special Issue entitled Serum Albumin.

1. Introduction

Human serum albumin (HSA) is the most abundant plasma protein and comprises 60–65% of the total plasma protein in humans, and many observations propose the existence of an important link between the concentration of HSA and health [1]. Due to a large number of acidic (98 Glu + Asp) and basic residues (83 Lys + Arg), the protein is highly soluble in aqueous media. Thus, its concentration in plasma is ca. 0.6 mM (4% w/w), but solutions of 20% (w/w) can be made for clinical use.

HSA is produced in the hepatocytes without prosthetic groups and covalently bound lipid or carbohydrate and is released continuously at a rate of 14 g/day. Adults have 120 g of albumin in the circulation, but an even larger pool of HSA is found in the extravascular spaces although at a lower concentration. Because of its high intravascular concentration, one of the principal functions of this protein is to support the oncotic pressure, which aids in keeping the blood within the circulation. Due to its many titratable amino acid residues, HSA has a significant buffering capacity. The uneven content of acidic and basic residues results in a net charge of −15 at physiological pH, a fact that renders HSA important for the Donnan effect in the capillaries.

Another main function of HSA is to bind and transport numerous endogenous and exogenous compounds, particularly less soluble, hydrophobic ones. In this way a circulating depot of the ligand is formed, and in many cases is the in vivo half-life of the ligand prolonged. In addition, the protein binding can increase the solubility of the ligand and decrease its potential toxicity. Among the exogenous compounds is a wide range of drugs, and binding to HSA affects their absorption, distribution, metabolism and elimination [1–5].

The protein is also an important circulating antioxidant, and this effect can be performed in different ways [6]. HSA can bind the compound and in this way protect it from oxidizing agents. Alternatively, the protein can interact with oxidizing agents and thereby scavenge

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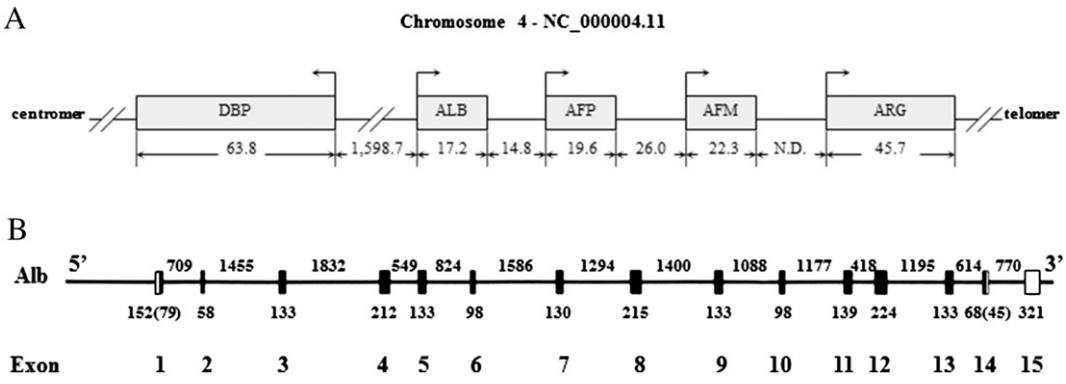

Fig. 1. Structure and genomic organization of the albumin family of genes. (A) Organization of the human albumin locus. The relative locations of the genes of the albumin family are shown. The arrows represent the start of transcription. The numbers indicate the size of the genes and intergenic regions (in kilobase pairs). The figure is constructed on the basis of information found in [13,14] and in GenBank (http://www.ncbi.nlm.nih.gov/pubmed). DBP: vitamin D-binding protein (GenBank Gene ID: 2638); ALB: albumin (GenBank Gene ID: 213); AFP: α-fetoprotein (GenBank Gene ID: 174); AFM: afamin (GenBank Gene ID: 1731); ARG: α-fetoprotein related gene, which is inactive in humans. Whether the reverted orientation of the DBP gene has a regulatory or other function has not yet been clarified. N.D.: not detected. (B) Genomic structure of the human albumin gene. The linear map of the gene indicating the location of exons (boxes) and introns (lines) is constructed on the basis of information found in GenBank (http://www.ncbi.nlm.nih.gov/pubmed). The partially filled boxes indicate the partially untranslated regions of exons 1 and 14, and the unfilled box the completely untranslated exon 15. The numbers below the exons indicate the exon size (in bp); numbers in parentheses for exons 1 and 14 represent the number of coding nucleotides. The number between exons represents intron length (in bp).

them. Finally, HSA can bind copper ions with a high affinity and thereby hinder them in initiating an oxidation cascade.

The many functions of HSA also include enzymatic properties [4]. The properties are mainly an esterase-like function and other types of hydrolytic activities. Among the substrates are both endogenous and exogenous compounds such as drugs.

Structurally, HSA is a single polypeptide chain of 585 amino acids and has a molecular mass of 66.5 kDa. The three-dimensional structure of the protein, without or with bound ligands, has been determined crystallographically in several laboratories [7–9], and now the structure is known at a resolution of 2.3 Å [10]. HSA has ca. 67% α-helix but no β-sheet, and it consists of three homologous domains (I–III) that assemble to form a heart-shaped molecule. Each domain is composed of two subdomains (A and B) with distinct helical folding patterns connected by flexible loops. Small-angle X-ray scattering studies of HSA in solution show general agreement with the crystal structure [11]. Also, a combined phosphorescence depolarization-hydrodynamic modeling study has suggested that the overall conformation of HSA in neutral solution is very similar to that observed in crystal structures [12].

2. The albumin gene and superfamily

HSA is a member of the albumin superfamily, which also includes α-fetoprotein, vitamin D-binding protein (Gc-globulin) and afamin (α-albumin). All four proteins are transport proteins with HSA as the quantitatively most important one. The plasma concentration of vitamin D-binding protein and afamin is only about 5 μM and 0.8 μM, respectively. α-Fetoprotein is an important plasma protein in the fetal state, but it is practically speaking absent in healthy, adult persons. Vitamin D-binding protein is the most polymorphic of the four proteins with three common alleles and more than 120 rare variants [13].

Recently, Naidu et al. [14] found a fifth member of this gene family, which they named the α-fetoprotein related gene, because it shows greatest similarity to this family member. However, the gene in human and other primates contains multiple mutations which turn the gene into an inactive pseudogene.

All the genes are single-copy genes, and the four active ones in the human are expressed in a codominant manner, i.e., both alleles are translated. The genes lie on chromosome 4, near the centromere for the long arm, at position 4q11-13 [14,15] (Fig. 1A). The genes for albumin, α-fetoprotein and afamin are tandemly arranged in the same transcriptional orientation; the inactive α-fetoprotein related gene is also oriented in the same way. In human, the distances between the genes for albumin-α-fetoprotein and for α-fetoprotein-afamin are 14.8 and 26.0 kilobase pairs, respectively. The gene for vitamin D-binding protein is less tightly linked, located 1.6 megabase pairs upstream of the 5′ end of the albumin gene and is in the opposite transcriptional orientation [14] (Fig. 1A). The five genes have arisen from a common ancestor through a series of duplication events and are tightly linked in all species where this has been investigated. The HSA gene (NCBI Reference Sequence: NG_009291.1) has 16,961 nucleotides from the putative "Cap" site to the first poly(A) addition site. It is split into 15 exons by 14 intervening sequences, which are symmetrically placed with the three domains of the albumin molecule and are thought to have arisen by triplication of a single primordial domain [16] (Fig. 1B).

The mRNA for HSA (NCBI Reference Sequence: NM_000477.5) encodes a precursor protein (preproalbumin) of 609 amino acid residues. Cleavage of the signal peptide of 18 residues and the propeptide of six residues yields the mature protein of 585 residues.

In this review, we have tabulated the 67 currently known mutations of the HSA gene which result in a circulating variant of proalbumin or albumin (alloalbumins). The protein changes of the mutants will be described according to the HGVS rules, i.e., with a p. followed by the mutation and its position in the sequence of the albumin precursor molecule (preproalbumin) of 609 amino acids (NCBI Reference Sequence: NP_000468.1), which represents the reference sequence. For homology, the numbering of all the amino acid residues discussed in the text will also refer to their position in this reference sequence, not in the mature albumin molecule.

Although alloalbumins do not seem to be associated with disease, they can be used as markers of migration and provide a model for study of neutral molecular evolution. They can also give valuable molecular information about albumins binding sites, antioxidant and enzymatic properties, as well as stability. Mutants with increased affinity for endogenous or exogenous ligands could be therapeutically relevant as antidotes, both for in vivo and extracorporeal treatments. Variants with modified biodistribution could be used for drug targeting. Therefore, understanding the effects of mutations is important, and in the following sections these potential effects will be discussed.

Mutations can also compromise the protein synthesis to such an extent that HSA is completely absent or strongly decreased in