

Review

Serum albumins—Unusual allergens[☆]

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ABSTRACT

Background: Albumins are multifunctional proteins present in the blood serum of animals. They can bind and transport a wide variety of ligands which they accommodate due to their conformational flexibility. Serum albumins are highly conserved both in amino acid sequence and three-dimensional structure. Several mammalian and avian serum albumins (SAs) are also allergens. Sensitization to one of the SAs coupled with the high degree of conservation between SAs may result in cross-reactive antibodies in allergic individuals. Sensitivity to SA generally begins with exposure to an aeroallergen, which can then lead to cross-sensitization to serum albumins present in food.

Scope of review: This review focuses on the allergenicity of SAs presented in a structural context.

Major conclusions: SA allergenicity is unusual taking into account the high sequence identity and similarity between SA from different species and human serum albumin. Cross-reactivity of human antibodies towards different SAs is one of the most important characteristics of these allergens.

General significance: Establishing a relationship between sequence and structure of different SAs and their interactions with antibodies is crucial for understanding the mechanisms of cross-sensitization of atopic individuals. Structural information can also lead to better design and production of recombinant SAs to replace natural proteins in allergy testing and desensitization. Therefore, structural analyses are important for diagnostic and treatment purposes. This article is part of a Special Issue entitled Serum Albumin.

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1. Introduction

Serum albumins (SAs) are multifunctional proteins which are highly conserved in both sequence and structure. SAs are α -helical proteins with several disulfide bridges that stabilize the structure [1]. The molecule, which has three domains, is very flexible and may change its conformation easily in order to bind many diverse ligands [2,3]. SAs are also thermolabile and undergo denaturation with relative ease in milk or beef extracts, leading to the formation of water-insoluble aggregates [4].

SAs are used in many medical laboratory applications; most likely very few, if any, molecular biologists have never worked with bovine

serum albumin (BSA) at least once. SAs are also present in animal products that are a major component of the human diet. While most people tolerate exposure to foreign serum albumins well, a small fraction of people develop allergic reactions to mammalian and/or avian SAs [5]. Serum albumins are also present in animal dander and it is highly likely that contact with this dander is the major source of health problems associated with SAs in humans. Up to approximately 30% of patients allergic to animal dander exhibited IgE reactive toward serum albumins [6]. Moreover, people do not have to be exposed directly to mammalian or avian sources of SA to become sensitized, as some allergens, such as Fel d 1, are transferred on the clothes of animal owners in public places [7,8]. The amount of allergen transferred, in many cases, is sufficient to sensitize people without direct exposure to the allergen source.

This review presents information on serum albumin allergenicity in relation to sequence and structure conservation of these proteins. Although the Allergome database (www.allergome.org) currently identifies 26 different SAs as potential allergens, this review focuses on the 18 SAs (14 mammalian and 4 avian) to which humans are most often exposed (see Supplementary Materials for the list and sequence alignment). Six of the analyzed albumins are registered as allergens by the World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee (Table 1).

Abbreviations: BSA, bovine serum albumin; FSA, feline serum albumin; HSA, human serum albumin; IgG, immunoglobulin G; IgE, immunoglobulin E; MMR, measles–mumps–rubella; MMRV, measles–mumps–rubella–varicella; PSA, porcine serum albumin; SA, serum albumin

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Although SAs are not a major cause of allergic sensitization, they compose a very interesting and unusual group of allergens. Foremost, they have sequences that are similar to the sequence of human serum albumin (HSA) and surprisingly they are able to sensitize atopic individuals. As shown previously, there are relatively few allergens with sequence identities to human homologs greater than 50–60% [9,10]. Moreover, due to the high similarities of their sequences, SAs are often cross-reactive, which can cause additional health risks to allergic individuals.

2. Sequence and structure conservation

Mammalian SAs have high sequence identities (72–82%) and similarities (83–88%) relative to HSA (Table 2 and also Supplemental Fig. 2). Cat and dog SAs are the most similar to HSA, while rodent SAs are the least similar among the mammalian species described in this review. Avian serum albumins display a relatively high similarity to the human protein (61–63%) and moderate sequence identity (46–49%). High sequence similarity between different SAs also results in similar structures (Fig. 1). There are currently four mammalian serum albumins with experimentally-determined structures: BSA, horse SA, HSA and rabbit SA [2,3,11].

High sequence identity increases the likelihood of cross-reactivity between serum albumin allergens due to their structural similarity. Table 2 indicates that sequence identity between all mammalian serum albumins is higher than 70%, the threshold typically required for cross-reactivity [13]. However, the mean 50% sequence identity shared between mammalian and avian SAs is well below the typical limit for cross-reactivity.

3. Bovine SA

BSA is one of the most well known proteins because of its widespread availability, similarity to HSA, use in medical formulations, and incorporation into many medical and biochemical assays. It is present in beef [14] and cow's milk [15]. BSA is used as a component of many vaccines [16,17], such as MMR, MMRV, Varicella, and Zoster, and is an important part of the culture medium used in artificial insemination. It is also studied because of its allergenicity; BSA has been identified as a minor allergen in bovine dander and serum [18].

Cow's milk allergy is one of the most common food allergies, together with allergies to eggs, fish, and peanuts. In most cases childhood allergy to cow's milk does not persist into adulthood; only 10–15% of young children diagnosed with the allergy remain so over five years of age [19,20]. A majority of patients with persistent milk allergy are also allergic to bovine serum albumin [21]; IgE from the affected patients was shown to be reactive toward several mammalian danders and meats. These patients have a greatly increased risk of developing rhinoconjunctivitis or asthma due to animal epithelia.

Very rarely, contact with BSA may cause an anaphylactic reaction. It was reported to be responsible for anaphylaxis in an artificial insemination patient [22]. The patient was previously diagnosed with asthma and shown to have subclinical allergy to mammalian

serum and thus cross-reactivity with BSA was suggested to be the origin of the anaphylactic reaction. Another patient with respiratory allergic reactions to feline epithelium had episodes of anaphylaxis after contact with BSA [23]; in this case the patient could tolerate well-cooked beef. More recently, it was reported that inhalation of BSA during work in a laboratory induced asthma in a patient [24].

The observation that thorough cooking of food improves tolerance is quite common in reports describing allergic reactions to SAs. Heat treatment has been shown to modify the allergenicity of beef and BSA and reduce, but not eliminate, their capacity to bind IgE from patients [21,25,26]. However, heat treatment in the presence of reducing agents was able to eliminate IgE binding, suggesting that disulfide bridges were preserving the structure [26,27].

BSA is important in our understanding of SA allergenicity and the cross-reactivity of BSA with SAs from other animals has been studied extensively. The binding of IgG and IgA antibodies was studied by Hilger et al. [28], who found significantly higher anti-BSA titers in individuals suffering insulin-dependent diabetes mellitus. Furthermore, the N-terminal region of BSA degraded first in simulated gastrointestinal fluid. This agreed with the result that the IgG to IgA ratio was altered for this region because the epitope was destroyed before reaching the gut-associated lymphoid tissue. Other studies identified residues 524–598 of BSA as forming the IgE epitope [29], of which residues 524–542 were the most critical for antibody binding. Several previous studies also suggested that this fragment of the BSA sequence was immunogenic [18,30–32]. Fergusson et al. and Wahn et al. also showed that the N-terminal part of the protein is immunogenic, identifying residues 115–184 and 1–306 specifically. In addition, the so-called ABBOS epitope (residues 126–144) was suggested to be responsible for an autoimmune reaction against pancreatic islet cells, which leads to islet cell dysfunction [33]. For a more detailed summary of epitopes relevant to bovine, horse and rabbit albumins see Majorek et al. [2] as well as references therein.

4. Cat and dog SA

Initial reports on the role of cat SA as an allergen were published four decades ago using extracts from cat pelts [34]. Cat and dog SA, which have 81.7% and 79.7% sequence identity (88.4% and 88.0% sequence similarity) to HSA respectively (Table 2), are the two major causes of sensitization of SA allergic individuals. Sensitization to feline and canine epithelia is highly correlated with sensitization to epithelia of other mammals like rabbit, cow, or horse [35]. It was shown that 85% of patients allergic to SAs had IgE reactivity to cat and dog SA [6]. Cross-reactivity between these two SA proteins, which have 87% sequence identity and almost 93% sequence similarity to one another, was demonstrated by Boutin et al. using monoclonal antibodies [36]. In that study, anti-cat SA monoclonal antibody was equally reactive to cat and dog SAs, as was an anti-dog SA monoclonal antibody. Furthermore, the murine monoclonal antibodies used were able to significantly inhibit human IgE binding. It was also shown that three tryptic peptides derived from horse (equine) SA (Equ c 1), composed of residues 21–113, 188–275 and 503–560 respectively, were not only

Table 1
A list of serum albumins that are registered as allergens by the World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee (www.allergen.org).

Albumin source common name	Albumin source Latin name	Allergen name	Route of exposure	Structures in Protein Data Bank
Bovine	<i>Bos domesticus</i>	Bos d 6	Iatrogenic/ingestion/inhalation	3v03, 4f5t
Dog	<i>Canis familiaris</i>	Can f 3	Inhalation	–
Horse	<i>Equus caballus</i>	Equ c 3	Inhalation	3v08, 4f5s, 4f5u
Cat	<i>Felis domesticus</i>	Fel d 2	Inhalation	–
Chicken	<i>Gallus domesticus</i>	Gal d 5	Ingestion/inhalation	–
Guinea pig	<i>Cavia porcellus</i>	Cav p 4	Inhalation	–

Table 2

Sequence identity and similarity between 18 different mammalian and avian serum albumins. Pairwise alignment was calculated using MAFFT [69]. For each alignment, the output was fed into the SIAS server, which calculated sequence identity (blue) and similarity (red) using the default parameters (<http://imed.med.ucm.es/Tools/sias.html>). Higher percentages correspond to darker shading.

	Similarity (%)																		
	Human	Bovine	Cat	Dog	Donkey	Horse	Pig	Goat	Sheep	Rabbit	Guinea pig	Hamster	Mouse	Rat	Chicken	Duck	Pigeon	Turkey	
Identity (%)	Human	100.0	85.5	85.5	85.5	85.5	85.5	85.5	85.5	85.5	85.5	85.5	85.5	85.5	61.1	63.3	62.0	62.2	
	Bovine	75.6	100.0	87.7	87.2	83.5	83.4	87.1	96.1	96.4	83.7	80.8	81.3	79.6	80.5	58.8	60.6	59.6	60.0
	Cat	81.7	77.7	100.0	92.8	86.3	85.6	87.2	87.8	87.7	86.6	85.8	86.1	83.6	84.8	59.6	61.5	60.1	60.6
	Dog	79.8	76.0	87.0	100.0	86.0	85.3	86.5	88.4	88.0	86.0	84.4	85.1	82.9	84.8	60.1	62.3	61.8	61.7
	Donkey	76.4	73.8	77.9	76.0	100.0	99.0	84.6	84.4	84.0	83.4	83.4	84.8	81.7	83.6	57.8	60.4	58.9	58.9
	Horse	76.1	73.9	77.6	75.7	98.6	100.0	83.9	84.2	83.9	82.9	82.7	84.2	81.3	83.0	57.8	60.1	58.7	58.6
	Pig	74.9	79.2	78.9	77.7	76.3	75.8	100.0	86.1	86.3	83.9	82.7	82.5	81.0	82.4	58.3	60.3	58.7	59.6
	Goat	74.5	92.1	76.4	77.6	75.1	75.1	77.7	100.0	99.3	84.1	81.7	81.7	80.1	80.8	59.1	61.1	60.4	60.6
	Sheep	74.7	92.1	76.7	77.6	75.5	75.5	77.5	98.5	100.0	83.6	81.5	81.2	80.0	80.7	59.0	61.0	60.3	60.5
	Rabbit	74.2	71.2	75.3	74.3	70.5	70.5	73.1	72.1	72.1	100.0	83.2	85.4	82.2	82.5	60.0	63.0	62.0	62.0
	Guinea pig	72.0	69.7	75.7	73.3	72.1	71.7	72.3	70.5	70.5	71.4	100.0	85.4	83.2	84.4	58.1	60.4	59.6	59.8
	Hamster	76.1	69.9	76.7	74.0	75.3	75.3	72.6	70.0	70.2	73.6	75.7	100.0	89.9	92.0	58.6	61.1	60.4	59.8
	Mouse	72.1	69.5	73.5	71.9	71.4	71.2	70.2	69.2	69.2	71.7	73.5	82.7	100.0	95.2	58.6	61.3	60.1	59.5
	Rat	73.2	70.0	75.2	72.9	72.4	72.4	72.3	69.7	69.5	72.3	73.8	84.2	89.6	100.0	59.0	61.3	60.6	59.8
	Chicken	46.1	43.2	45.1	45.8	42.7	42.7	41.9	43.4	43.4	44.9	43.2	42.4	43.2	43.8	100.0	88.3	85.0	90.7
	Duck	48.6	45.3	47.5	47.9	45.2	45.0	44.1	45.5	45.7	48.4	45.8	45.8	46.7	46.9	84.5	100.0	90.2	91.5
	Pigeon	46.5	43.5	45.7	46.5	44.1	44.1	42.1	44.1	44.3	46.3	44.8	45.3	45.2	46.0	79.6	84.2	100.0	88.1
	Turkey	47.2	44.8	46.0	46.8	43.8	43.6	42.9	45.0	45.1	46.3	44.1	43.8	45.0	45.0	87.2	86.4	81.2	100.0

able to inhibit the binding of IgE and IgG antibodies to horse SA, but also to cat and dog SA, demonstrating that the antibodies were binding to structurally similar epitopes [37].

Recombinant cat and dog SA have been produced in *Escherichia coli* and were found to retain the antigenicity of the natural extracts [38,39]. This is especially important for standardization of the material used for

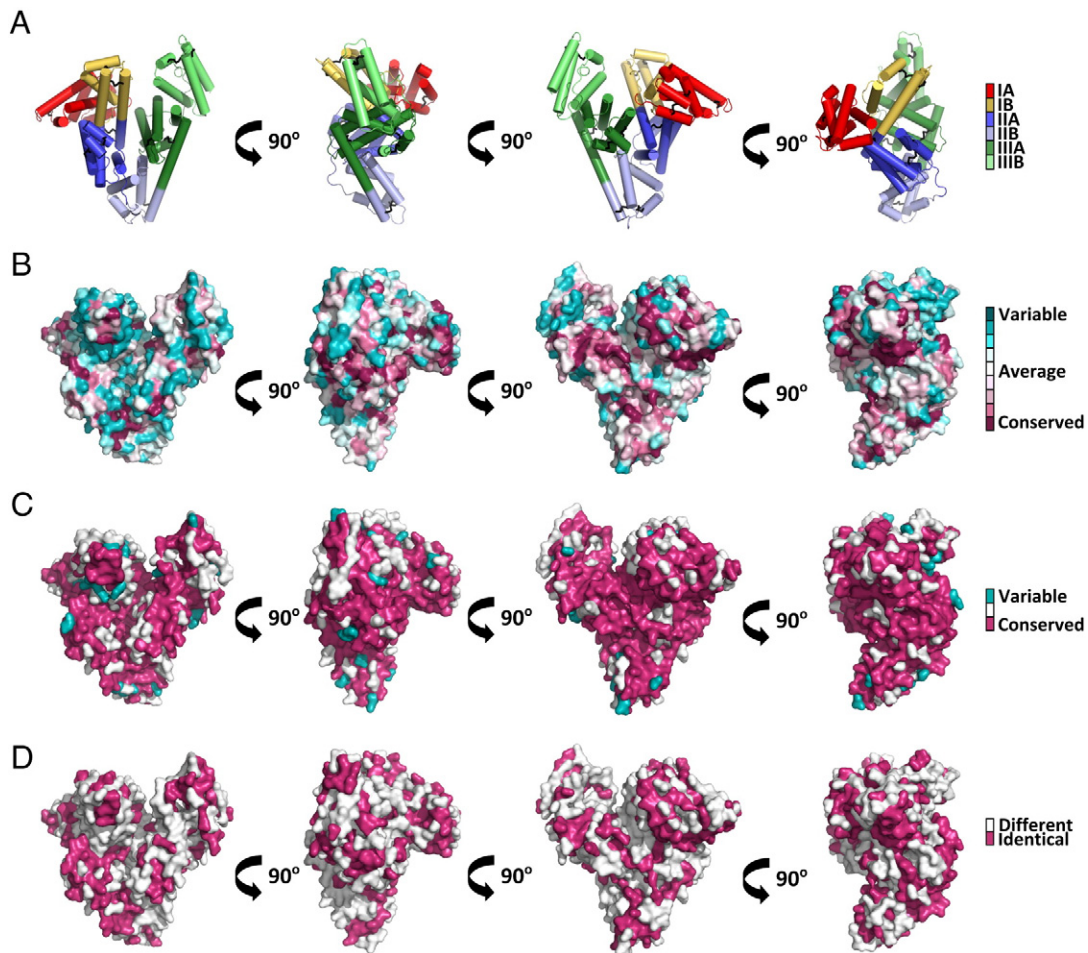


Fig. 1. Model of human serum albumin in cartoon and surface representations. (A) Secondary structure and domain assignment as described by Sugio et al. [11]. Disulfide bonds are shown as sticks. (B) HSA in surface representation colored by sequence conservation between all 18 albumins analyzed in Table 2. Sequence conservation was determined by ConSurf [12] and residues were colored according to the scale on the right. (C) HSA colored by sequence conservation as compared to cat and pig SAs. The colors indicate the number of conserved residues between the 3 aligned SAs according to the scale on the right. (D) HSA colored by sequence conservation with the 4 avian SAs according to the scale on the right. The orientations of the corresponding HSA models are the same on all parts of the figure.

diagnostics. For example, a significant variation in allergen content was found in dog extracts [40].

No serum albumin is considered to be a major allergen (among patients allergic to a given source, a purified allergen from that source to which >50% of patients react is major). However both cat SA (Fel d 2) and dog SA (Can f 3) are considered to be intermediate allergens, with up to 23% and 35% of patients sensitized respectively [41,42]. Interestingly, it has also been observed that dogs can become sensitized to human serum albumin. Some dogs were found to have adverse reactions to a solution of HSA [43] and 7% of healthy dogs had anti-HSA antibodies, despite not being treated with HSA solutions [44].

This shows that sensitization to SAs may be quite complex. For example, Liccardi et al. [45] reported a case in which a patient that did not own any mammalian animals was sensitized to several different SAs. An occupational allergy may also be related to the cross-reactivity between different serum albumins. It was reported that a canine allergic cook showed cutaneous and respiratory symptoms when exposed to raw beef [46]. An immunoblot of the patient's serum showed reactivity toward BSA that was inhibited when incubated with canine dander extract—the blot identified several proteins of between 19 and 25 kDa that were also detected in canine dander extract as well as one of 67 kDa, which was likely dog SA.

5. Other mammalian albumins

Caretakers of research animals are often affected by animal allergens. Between 11 and 44% of such workers may have allergic symptoms related to their occupation [47]. The most relevant allergens originate from rodents; specifically mice, rats, guinea pigs, and rabbits. Both mouse and rat SA were identified as important allergens, with approximately 30% of mouse allergic patients sensitized to SA. Other mammalian serum albumins also affect human health; horse SA was one of the

first albumins reported to be an allergen [48]. It was also the second SA for which the crystal structure was determined [2,3,49]. Another report found that uncooked pork caused oral allergy syndrome [50]. (In this case it is possible the patient was primarily sensitized to horse SA, as the patient's serum reacted strongly to horse dander, but did not react to samples from cat.)

While cow's milk allergy is the most common human allergy to milk, there are cases where people are selectively sensitized to milk from other species. It was reported that a patient was selectively allergic to ovine (sheep) milk with a slight allergy to caprine (goat) milk. It was later found that the patient's IgE antibodies recognized ovine SA [51].

An interesting summary on the cross-reactivity of mammalian albumins was presented by Spitzauer et al. [6]. The authors examined the sera of 60 patients allergic to SAs. The majority of examined patients (85%) had IgE antibodies against cat SA, dog SA, or both. The next most prevalent reactive SA was from guinea pig (75% of the patients), followed by horse SA (70%), and rabbit and hamster SAs (60%). The same studies showed that porcine SA, ovine SA, and rodent SAs reacted with IgE antibodies from the sera of 50% of patients, while only 20% of examined individuals had IgE against BSA and avian SAs. This suggests that neither BSA nor avian SAs was the primary sensitizing SA.

6. Avian SAs

As mentioned previously, avian serum albumins display relatively high sequence similarity and moderate identity to HSA (Table 2; Figs. 1D and 2). Chicken serum albumin (α -livetini), which may cause both respiratory and food-allergy symptoms, is the most common allergen among avian SAs. However, the prevalence of IgE sensitization to this allergen is very low [5]. Only 0.14% of allergic individuals are sensitized to this SA, while the same study determined that BSA and dog SA

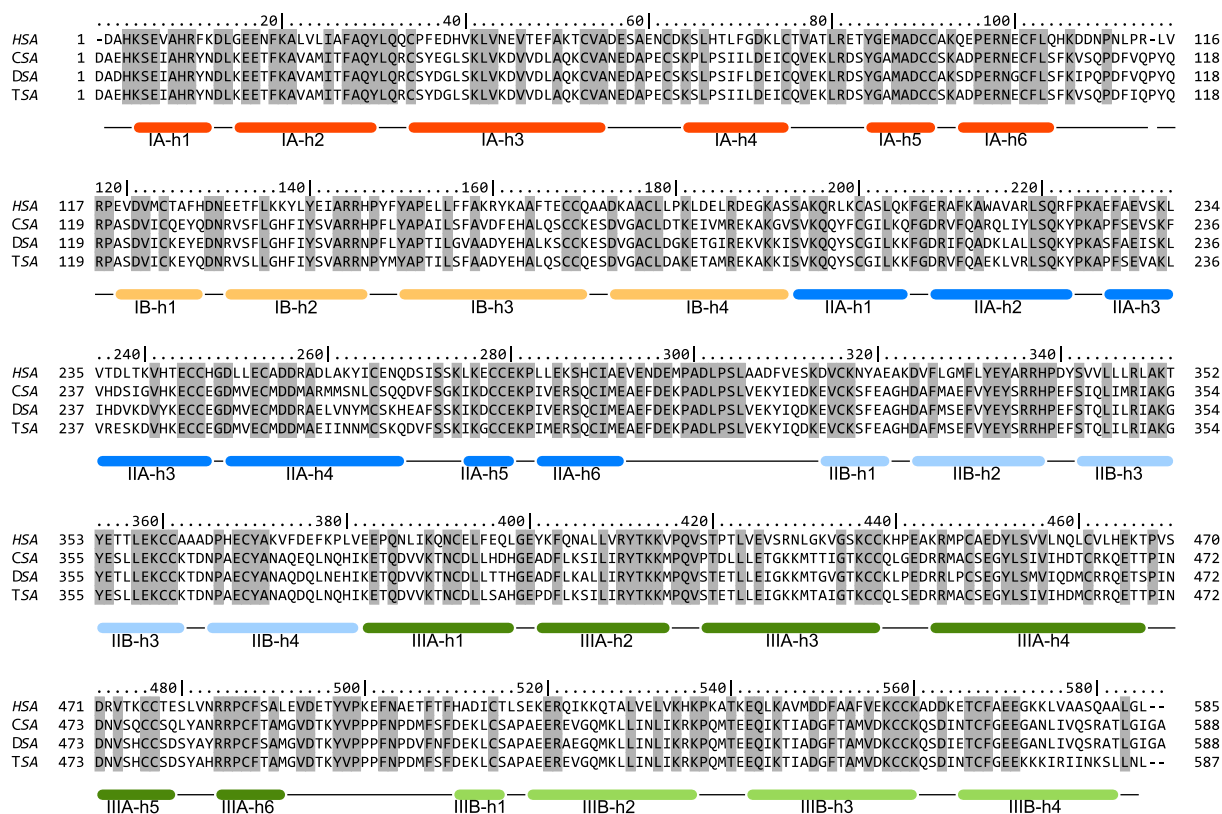


Fig. 2. Sequence alignment of human (HSA), chicken (CSA), duck (DSA) and turkey (TSA) serum albumins. Identical residues are highlighted in gray. Secondary structure elements in HSA are marked. Each color represents a different domain, which are colored with the same scheme used in Fig. 1A. The multiple sequence alignment was calculated with MAFFT [69] using default parameters.

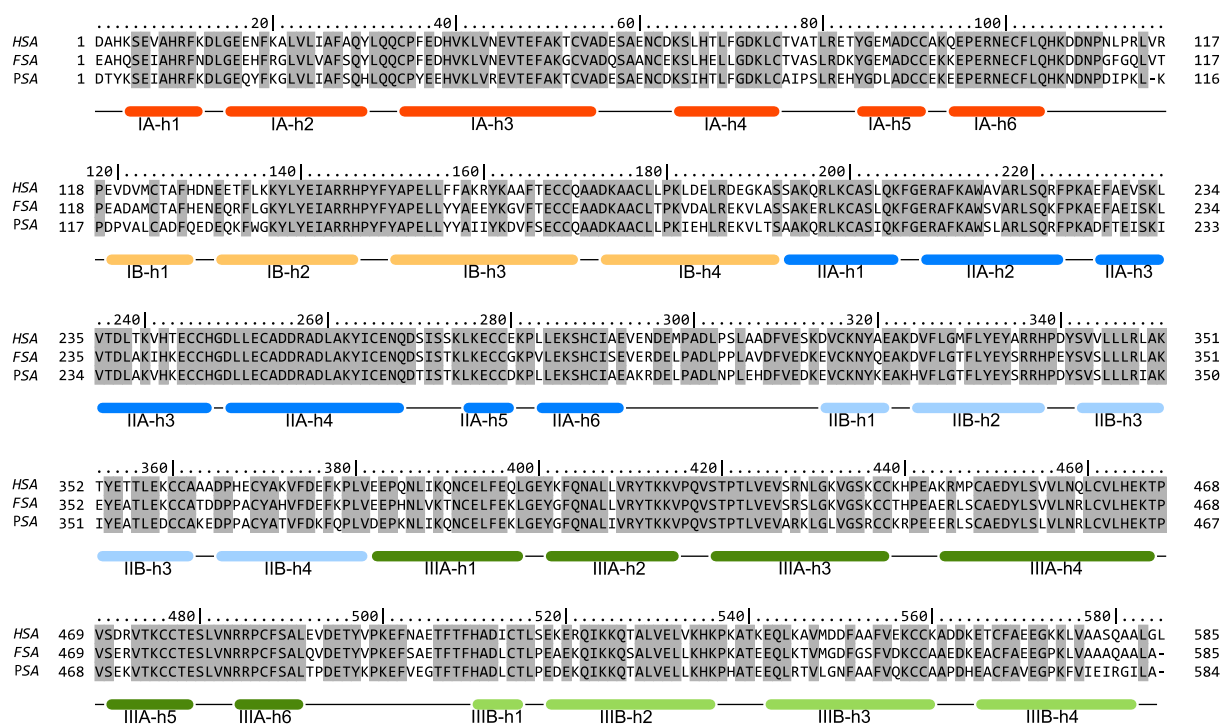


Fig. 3. Sequence alignment of human (HSA), cat (FSA) and porcine serum albumins (PSA). Identical residues are highlighted in gray. Secondary structure elements in HSA are marked. Each color represents a different domain, which are colored with the same scheme used in Fig. 1A. The multiple sequence alignment was calculated with MAFFT [69] using default parameters.

affect 3.6% and 1.9% of tested subjects, respectively. Conversely, other studies [6] have shown that BSA and chicken SA were recognized by IgE of a comparable fraction of serum albumin allergic patients. This discrepancy may be due to experimental methods or may be related to the primary sensitizing SA.

Chicken SA was identified as an inhalant and a food allergen in so-called bird-egg syndrome [52–56]. The studies showed that chicken SA (Gal d 5) was a relevant allergen for patients with both food allergies to egg yolk and respiratory syndromes. Heating chicken SA to a temperature of 90 °C for 30 min reduced IgE reactivity by almost 90%. Tests performed in the homes of allergic individuals confirmed the presence of airborne chicken SA. Bird-egg syndrome may also be triggered by contact with birds kept as pets [57].

7. Cross-reactive disorders

Approximately 20 years ago, it was noted that there is an association between sensitivity to pork meat and feline epithelia [58,59]. This condition is known as cat–pork syndrome, and was mainly reported in Europe. However, it has drawn greater attention in recent years as it is considered an under-recognized allergy in the US [60]. A study by Hilger et al. [41] showed that sensitization to feline SA was in the range of 14–23% in patients with sensitivities to both cat dander and pork, while it was 3–10% for porcine SA. It was estimated that approximately one third of these individuals were likely to be affected by cat–pork syndrome (the two SAs are 78.9% identical and 87.2% similar by sequence; see Figs. 1B and 3).

In the case of cat–pork syndrome, the most likely primary sensitization is to cat SA working as an aeroallergen, followed by cross-sensitization to the food allergen porcine SA [41,60]. The mechanism of sensitization is similar to the one described for pollen–fruit [61,62] or feather–egg syndromes [54,55]. Such a mechanism of sensitization is additionally supported by the observation that IgE reactivity to cat SA was present in all patients who were also reactive to porcine SA

[41]. There are several aspects of the cat–pork syndrome, summarized by Posthumus et al. [60], that make this condition quite distinct. First of all, sensitization to pork has not been reported in children younger than eight years of age. Moreover, allergic reactions to pork are not reported at every encounter of the food and that well-cooked pork is more often tolerated. The most important feature of this syndrome is related to the fact that the reaction to pork begins very soon after eating the meat. This fact allows for discrimination of cat–pork syndrome from the reactions caused by interaction of IgE with galactose- α -1,3-galactose [63–65].

A rare example of cross-reactivity between cat, pork, and chicken serum albumins was recently reported [66]. It was concluded that primary sensitization was most likely to cat SA and the sensitizations to porcine SA and chicken SA were through cross-reactivity of the IgE antibodies. This shows that cross-reactivity between proteins having less than 50% of sequence identity is possible, although rare.

8. Conclusions

Although serum albumins are not considered to be major allergens, they are unusual ones. The fact that some SAs are allergens at all is somewhat surprising given their high sequence identity to human serum albumin. Spitzauer [67] suggested that an allergy to mammalian proteins may indicate the borderline between discrimination of foreign and human proteins, and SAs appear to be a prime example. Serum albumins are mainly respiratory allergens; however, sensitization to SAs may be quite more complex and involve multiple routes of exposure. Cross-reactivity is one of the most important features of these allergens and in many cases serum albumin allergic individuals are sensitized to several albumins originating from different animals. It has been suggested [14] that BSA should be considered an important pan-allergen in allergies to meat and mammalian epithelium. However, patients with allergies to SA can be sensitized by cat SA, dog SA or BSA, although these may not have been the allergens that led to sensitization.

Many studies have shown that heat treatment, especially in combination with a reducing agent, significantly reduces IgE reactivity to SAs. This observation is consistent with clinical reports describing allergic reactions to SAs and the fact that thorough cooking of food improves tolerance in allergic individuals. Therefore, it seems that the three-dimensional structures of albumins are important for antibody binding and allergenicity. It also demonstrates how food processing may have a significant impact on the allergenicity of SAs.

Production of recombinant SAs is important for diagnosis of allergy. The major advantages of recombinant SAs are that they can be produced at high purity and can be more easily standardized in comparison to natural allergens [68]. For example, it has been shown that recombinant cat and dog SAs can be used as a replacement for natural allergen in allergy testing. In addition to diagnostic applications, recombinant SAs may find application in allergy treatment. The use of recombinant allergens is especially desirable due to issues related with quality control of natural allergens and the possibility of engineering non-immunogenic proteins for allergen immunotherapy. Taking this into account, it seems that we cannot avoid the production of recombinant SAs. This is especially true for avian SAs, as there are no reports of a recombinant version of them being produced. In addition to the use of SAs in diagnostic and treatment of human conditions, these proteins may be useful in veterinary applications.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.bbagen.2013.06.016>.

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Serum albumins - unusual allergens

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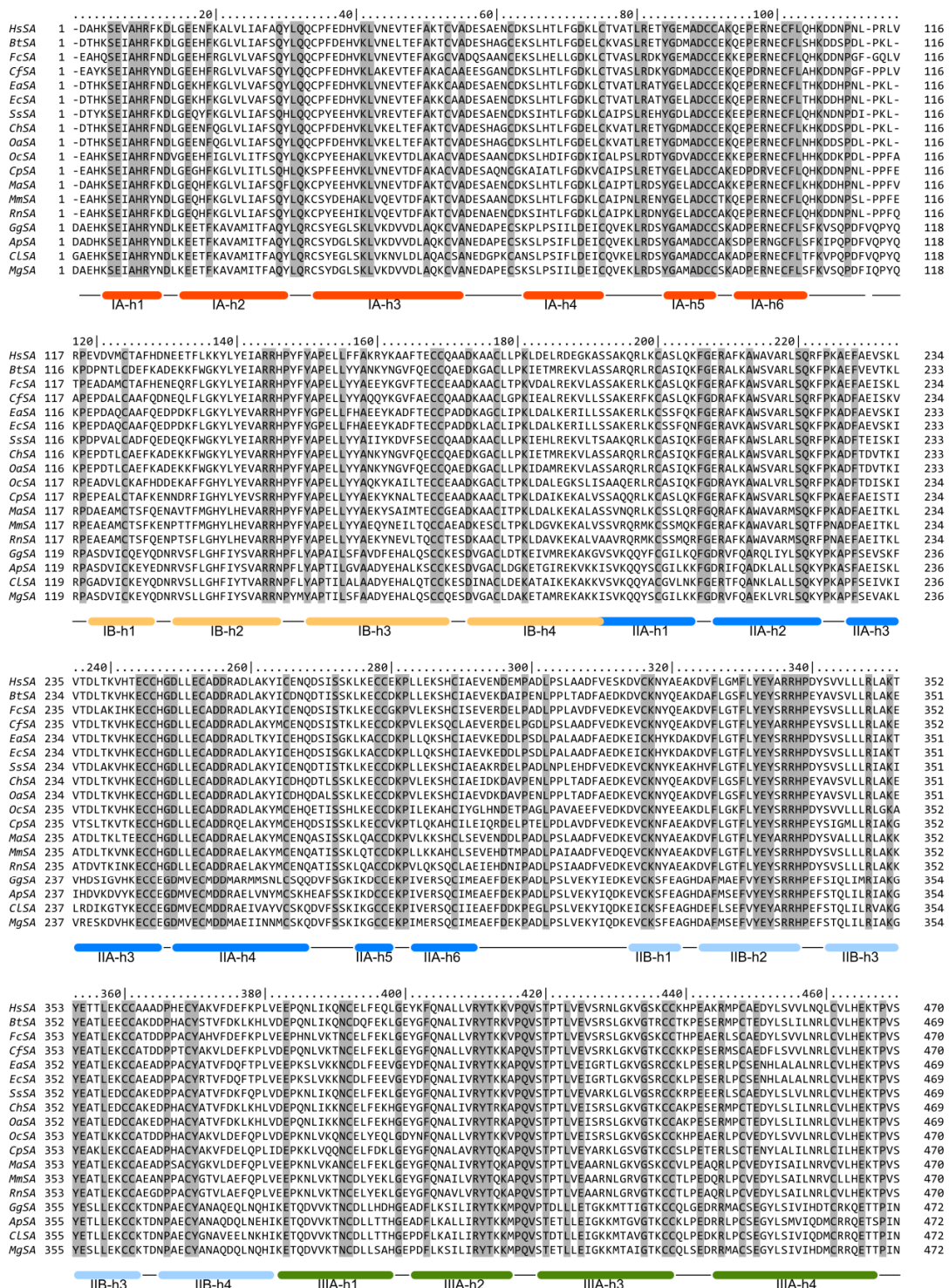
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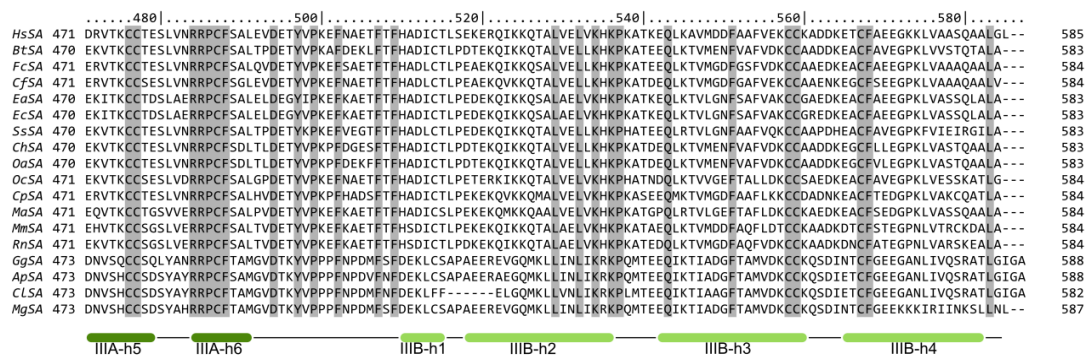
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List of serum albumins used for analysis:

HsSA - Homo Sapiens
BtSA - Bos taurus
FcSA - Felis cattus
CfSA - Canis familiaris
EaSA - Equus asinus
EcSA - Equus caballus
SsSA - Sus scrofa
ChSA - Capra hircus
OaSA - Ovis aries
OcSA - Oryctolagus cuniculus
CpSA - Cavia porcellus
MaSA - Mesocricetus auratus
MmSA - Mus musculus
RnSA - Rattus norvegicus
GgSA - Gallus gallus
ApSA - Anas platyrhynchos
ClSA - Columba livia
MgSA - Meleagris gallopavo

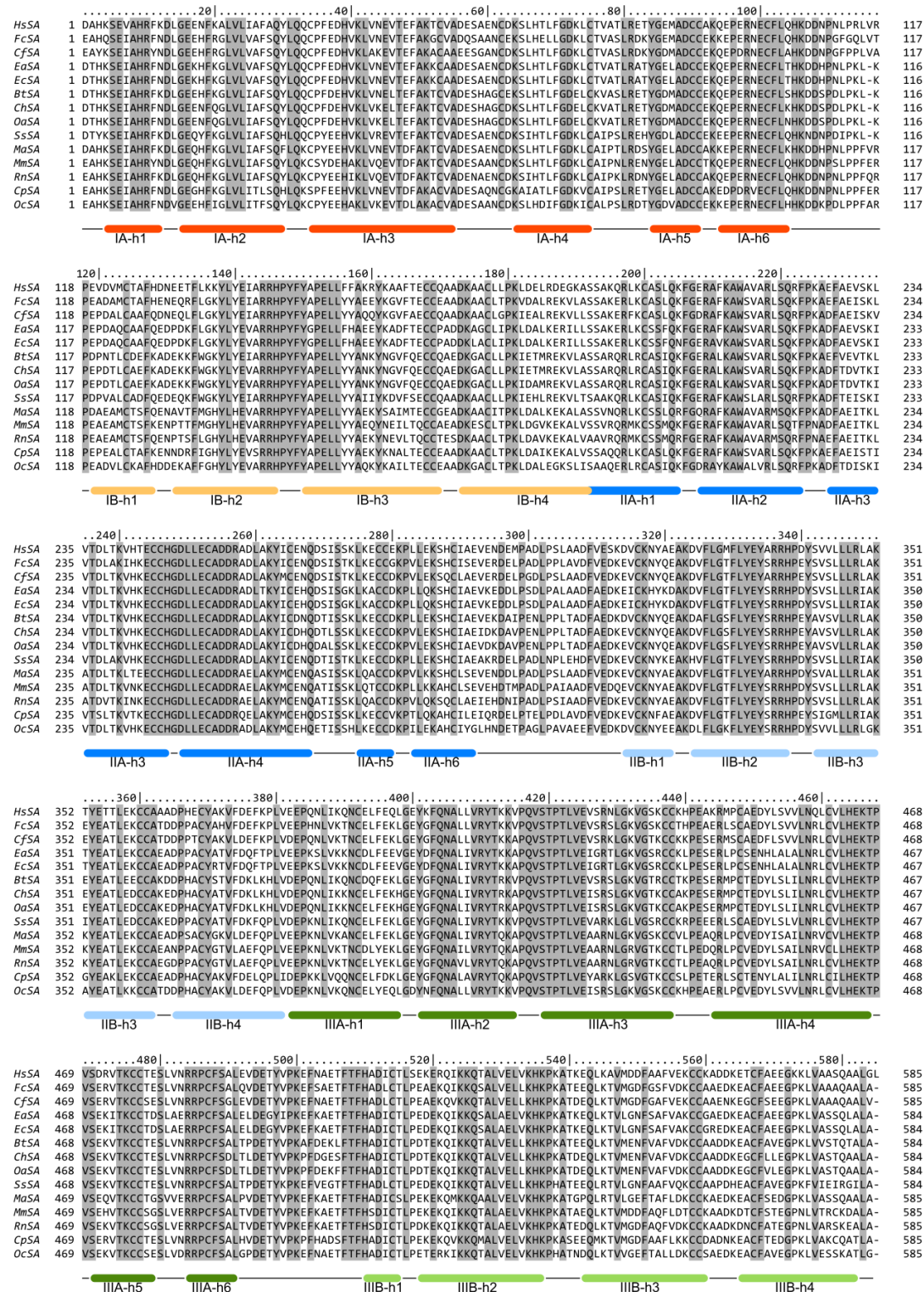
Supplemental Figure 1.





Supplemental Figure 1. Sequence alignment of 18 SAs which were selected as the most relevant for human health. Multiple sequence alignment was calculated with MAFFT (Katoh San-Juan *et al.*, 2013) using default parameters.

Supplemental Figure 2.



Supplemental Figure 2. Sequence alignment of mammalian serum albumins.