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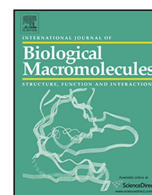

# Comparative studies on the heterogeneity of plasma-derived and recombinant human albumins in laboratory use

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ABSTRACT

We investigated the thiol-redox state, and the relationship between structural characteristics, such as thermal stability, and functional properties, such as cell growth activity, of commercial plasma-derived (pHSA) and recombinant human serum albumin (rHSA) products. In this study, 5 pHSA products (A1653, A9511, A1887, A8763, and A3782) and 2 rHSA products (A9731 and A9986) were obtained from Sigma-Aldrich. Among them, three kinds of HSA products [A1653 (an initial fractionation product), A3782 (a final purified product), and A9731 (recombinant albumin expressed in rice)] were selected for experiments on the thermal stabilities, analyzed by thermal denaturation curves, and cell growth activities of U937 and THP-1 cell lines using the WST-1 reagent. The secondary and tertiary structures of HSA products were similar, whereas a marked difference was observed in their thermal stabilities. The degree of thermal stability of the three representative products was in the order of A9731 (rHSA) > A1653 (pHSA) > A3782 (pHSA), as was the degree of cell growth activity of these products. One possible explanation for the present results is that albumin-bound fatty acids may have influenced the thermal stabilities and cell growth activities of U937 and THP-1 cells.

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## 1. Introduction

Albumin has been extensively studied because of its availability, *i.e.*, its abundance in extracellular fluids (ECF) such as the blood and interstitial fluids, and has often been used as a model protein. Physicochemical and biotechnological studies that have been conducted in the past 2 decades have established the relationship between the structural characteristics and functional properties of serum albumin: the albumin molecule consists of a non-glycosylated single chain of 585 amino acids with 17 disulfide bridges and only one free cysteine at position 34 (Cys-34) [1]. This cysteine represents the largest fraction (>80%) of free thiols in ECFs. The crystal structure of human serum albumin (HSA) is a heart-shaped with three homologous domains connected by a random coil, named domains I, II, and III, and each contains two subdomains, A and B (secondary structure content; 67% α-helix, and almost no β sheets) [2]. Albumin exhibits a wide variety of physiological and pharmacological functions, including osmotic pressure regulation, binding and transport capacities of endogenous and exogenous compounds, and antioxidant properties of human plasma [3]. Regarding the pathophysiological aspects of HSA, the thiol-redox state of HSA is used as a valuable biomarker of oxidative stress [4,5] in many diseases, such as renal disease [6–9], liver disease [10,11], diabetes mellitus [12], senile cataract [13,14], and joint osteoarthritis [15]. Moreover, clinically measured HSA glycation levels may represent a potential clinical utility for diabetes mellitus as well as that of HbA1c, because it reflects glycemia over two to three weeks [16].

Commercially available albumin products separated from mammalian sera have been widely used in both laboratory and clinical fields. Serum albumin, especially bovine serum albumin (BSA), is commonly used in the laboratory field as a standard protein for analytical methods, such as protein concentration analysis and enzyme-linked immunosorbent assay (ELISA). Albumin is often used as a culture medium supplement in cell culture experiments, such as for the generation and maturation of monocyte-derived dendritic cells [17]. HSA is widely used in the clinical field to treat

*Abbreviations:* CD, circular dichroism; DSC, differential scanning calorimetry; DTNB, 5,5′-dithiobis 2-nitrobenzoic acid; ECF, extracellular fluid; ES, embryonic stem; FBS, fetal bovine serum; HMA, human mercaptalbumin; HNA, human nonmercaptalbumin; HPLC, high-performance liquid chromatography; HSA, human serum albumin; iPS, induced pluripotent stem; pHSA, plasma-derived human serum albumin; rHSA, recombinant human serum albumin.

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**Table 1**
Preparation, purification, and special characteristics of each HSA product.

| | Product No. | Preparation/purification | Special characteristics |
|---|---|---|---|
| pHSA (5 products) | A1653 | Cohn fraction V from human serum | 96–99% by agarose gel, remainder mostly globulin |
| | A9511 | 1× crystallized and lyophilized prepared from A1653 using method IV of Cohn et al. [19] | 97–99% by agarose gel |
| | A1887 | Cohn fraction V, heat step, prepared from A1653 | >96% by agarose gel, essentially fatty acid-free (∼0.005%) |
| | A8763 | Prepared from A1653 | Essentially globulin-free |
| | A3782 | Prepared from A8763 | Essentially fatty acid-free (∼0.005%), essentially globulin-free |
| rHSA (2 products) | A9731 (Cellastim$^{TM}$) | Recombinant, expressed in rice | >96% by SDS-PAGE, low endotoxin, cell culture tested |
| | A9986 | Recombinant, expressed in rice | >96% by SDS-PAGE immunoglobulin-free, cell culture not tested |

several diseases, such as burns, hypovolemia, and surgical blood loss [18].

Serum albumin has often been regarded as a single homogenous entity under the above conditions, at least by its molecular weight. The most frequently used HSA product has been "Cohn fraction V" [19] for the initial fractionation of HSA in industrial large-scale preparations. However, commercial serum albumin products, both bovine and human, are known to be heterogeneous, with differences being observed in thiol contents, dimers, and higher oligomers, based on the combination of chromatography and electrophoresis [20]. The micro-heterogeneity of BSA has also been investigated in pH-dependent conformational transitions including N-F and N-B transitions and *N-A* isomerization [21]. This heterogeneity or micro-heterogeneity of the albumin molecule may increase during purification processes *in vitro*, including isolation, concentration, and storage. However, various types of post-translational modifications may cause the heterogeneity of several proteins *in vivo* including serum albumin.

Recombinant proteins have been expressed and purified by various kinds of hosts due to recent advances in biotechnology, and these have been used instead of wild type proteins. Recombinant human serum albumin (rHSA) has also been produced and used in various fields as a substitute for plasma-derived HSA (pHSA) in order to avoid the potential risk of disease infection due to viral or prion contamination [22]. Commercially available rHSA has been shown to be as efficient as pHSA for embryo development [23] and fertilization [24]. However, we recently reported that differences in the ability of rHSA for fertilization in a mouse culture were attributed to the heterogeneity of the product sources including their lot numbers [25].

The aim of the present study was to investigate the heterogeneity of commercially available HSA products, *i.e.*, both pHSA and rHSA, by analyzing the thiol-redox state, and the relationship between the structural characteristics, such as thermal stability, and functional properties, such as cell growth ability, of an *in vitro* cell culture system.

## 2. Materials and methods

### 2.1. Materials

Several kinds of albumin products can currently be purchased because several companies supply them all over the world. However, for the sake of simplicity, all commercial pHSA and rHSA products used in this study were purchased from Sigma-Aldrich Chem. Co. (St. Louis, MO, USA). The product numbers [lot numbers] of pHSA used were as follows; A1653 [5 lots; 076K7555, 128K7535, 039K7555, 049K7535, 030M7034V], A9511 [3 lots; 077K7585, 107K7560, 089K7540V], A1887 [3 lots; 124K7635, 018K7540, 068K7538], A8763 [3 lots; 080K7607, 086K7535, 025K7555], and A3782 [5 lots; 085K7541, 107K7565, 109K7550, 090M7001V, 050M7008V]. These were prepared from normal human serum by a multi-step process. Those of rHSA were as follows; A9731 [2 lots; 061M1563, 091M1137V], and A9986 [031M1353V]. Detailed information of the preparation, purification, and special characteristics of the source materials of these products is summarized in Table 1 (Data listed are taken from the manufacturer's product information sheets). Briefly, for pHSA, A1653 is an initial fractionation product obtained from large-scale pooled human sera by the Cohn's cold ethanol precipitation method V, and the product is known as "Cohn fraction V" [19]. A9511, A1887, and A8763 were purified from A1653 by different purification methods. A3782 is a final purification product prepared originally from A1653 *via* A8763. As a result, A3782 is the most purified HSA product, *i.e.*, essentially fatty acid-free and globulin-free. Therefore, it is the most expensive product in the commercial market (the more a product is purified, the more expensive it is). Both A9731 and A9986 for rHSA are recombinant proteins expressed in rice. A9731 is a very low endotoxin content product that is sold as Cellastim$^{TM}$, which is suitable for cell culture experiments (Table 1). HSA was dissolved in phosphate buffer with saline (pH 7.4), and concentrations were determined with a Hitachi 320S spectrometer (Hitachi Co., Tokyo, Japan), assuming $E$(1%, 1 cm) at 280 nm to be 5.30 [26].

Myoglobin from equine skeletal muscle (M0630—041M7004V), lysozyme from chicken egg white (L6876—061M1328V), ribonuclease A from a bovine pancreas (R6513—070M7007V), papain from papaya latex (P4762—081M7016V), cytochrome *c* from an equine heart (C7752—091M7000V), and α-chymotrypsin from a bovine pancreas (C4129—060M7007V), all used as reference proteins of CD-resolved protein secondary structure, were purchased from Sigma-Aldrich Chem. Co. (St. Louis, MO, USA). All proteins were used without further purification, and were dissolved in phosphate buffer with saline (pH 7.4). Protein concentrations were determined using a Hitachi 320S spectrophotometer (Hitachi Co., Tokyo, Japan). Each absorbance was described elsewhere [27].

For the cell culture experiment, the RPMI1640 medium, L-glutamine, and HEPES were all obtained from Sigma-Aldrich Chem. Co. (St. Louis, MO, USA). Streptomycin sulfate and penicillin G potassium were obtained from Meiji Seika Pharmaceutical Co., Ltd (Tokyo, Japan). Fetal bovine serum (FBS) was purchased from Biowest (Nuaillé, France). The cell proliferation reagent WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1, 3-benzenedisulfonate) was obtained from Roche Diagnostics GmbH (Mannheim, Germany). All other chemicals including 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) were of reagent grade.

### 2.2. Methods

#### 2.2.1. SH titration by DTNB

The SH contents of albumin products were determined by the method of Ellman [28] at pH 8.02 (0.10 M Tris–HCl buffer) and employed DTNB spectrophotometrically at 412 nm ($\varepsilon = 1.36 \times 10^4$ M$^{-1}$ cm$^{-1}$) with a Hitachi 320S spectrophotometer (Hitachi Co., Tokyo, Japan).

#### 2.2.2. High-performance liquid chromatography (HPLC) analysis

The albumin thiol-redox state was measured using a previously reported HPLC method [8]. Briefly, the HPLC system consisted of

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