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A NEW PROTOCOL FOR PURIFYING HUMAN SERUM ALBUMIN

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ABSTRACT. The possible applications of human serum albumin into the design of blood substitutes with diminished capacity of getting involved in reactions that generate nitrogen reactive species lead us to reevaluate the purification procedures and to propose a modified precipitation procedure that over the economic advantages allows the isolation of other useful fractions.

Keywords: *human serum albumin, hemoglobin, blood substitutes, autooxidation*

INTRODUCTION

Albumin has a large number of biotechnological applications. Among these, we have recently proposedalleviation of prooxidant reactivity in artificial oxygen carriers ("blood substitutes"). Hemoglobin-albumin copolymersindeed displayed a decreased prooxidant activity compared not only to free hemoglobin but also to glutaraldehyde-polymerized hemoglobin [1] Furthermore, tests on animal models and on cell cultures reveal a better performance of hemoglobin-albumin copolymers, compared to simple hemoglobin polymers [1,2].

Many methods of recovering albumin from serum or plasma are described in the literature, one of the most used at large scale being Cohn's cold precipitation with ethanol [3]. Other methods include decreasing the ionic strength of plasma [4] or addition of fatty acids in order to precipitate globulins, addition of zinc ions or trichloroacetic acid to cold ethanol, or ammonium sulfate precipitation [5] combinethe low temperature ethanol precipitation with ammonium sulfate fractionation [6] or with chromatography [7,8,9,10,11], or utilization of polyethylene glycol as precipitation reagent [6]. A protocol is described here for purification of human serum albumin, and proof of concept is given as to its potential use as antioxidant in hemoglobin-based blood substitutes.

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RESULTS AND DISCUSSION

Human serum albumin was prepared using combined fractionation methods with ammonium sulfate and ethanol as presented Figure 1. This method has the advantage of obtaining highly purified albumin with the possibility of recovering other plasma proteins without the risk of irreversible denaturation. Compared to the Cohn cold ethanol precipitation method [3] this method is more cost-effective due to the fact that less ethanol and cooling agent are required for albumin isolation.

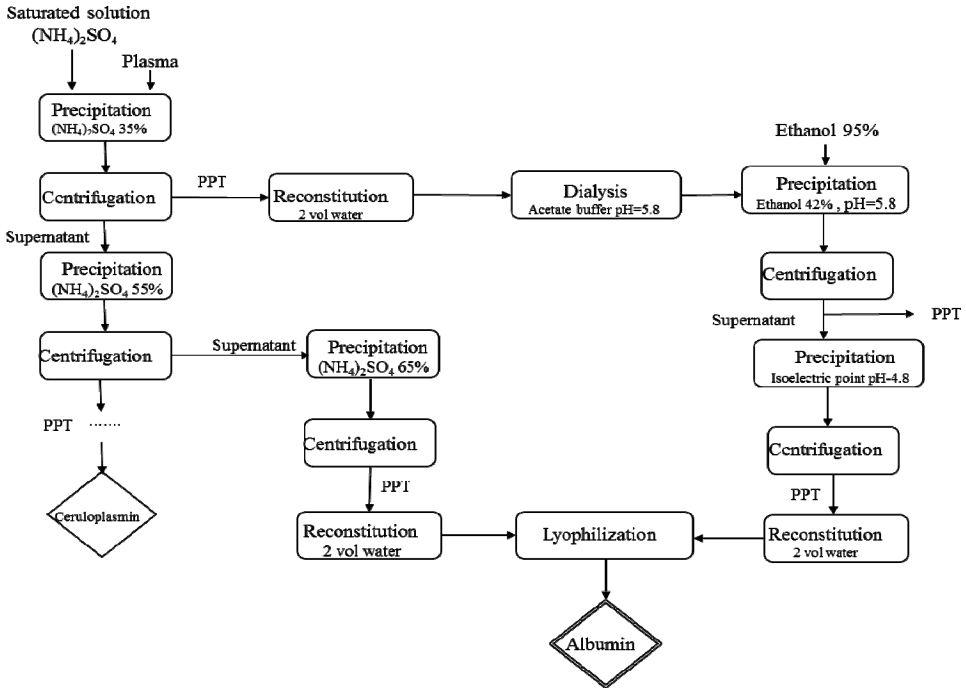

Figure 1. Human serum albumin preparation - operation flow chart.

Another advantage of the proposed method is its potential to be applied at large scale due to the relatively simple operations which do not require any considerable investment in equipments. Purification methods that include steps like electrodencatation [12], gel-filtration, ion exchange and affinity chromatography [7,10,11,13] or heating plasma in the presence of ethanol and stabilizers [14] are rarely applied in industry.

In the chosen purification protocol the first fractionation step was precipitation with ammonium sulfate at 35%, at 5^0 Celsius, under stirring for 15 minutes, followed by a 15-minute centrifugation at 9000 rpm. Precipitate

was collected, re-suspended in two volumes of acetate buffer pH=5.8 and 150mM sodium chloride, and dialyzed. The supernatant was further precipitated with ammonium sulfate at 55% and centrifuged at 12000 rpm for 15 minutes; the second precipitate was kept for further purification procedures required for ceruloplasmin isolation while the supernatant was collected, precipitated with ammonium sulfate at 65% and centrifuged at 12000 rpm for 25 minutes; the precipitate was reconstituted with two volumes of ultrapure water and lyophilized.

In order to increase the recovery percentage after dialysis the fraction was precipitated with ethanol at 42% concentration, pH=5.8 at -5^0 Celsius and centrifuged for 30 minutes at 12000 rpm; the supernatant, containing mainly albumin was further precipitated at isoelectric point pH=4.8. After stirring for 1 hour the mixture was allowed to age overnight before centrifugation; the obtained paste was reconstituted with two volumes of ultrapure water and lyophilized.

Figure 2 shows the results obtained by polyacrylamide gel electrophoresis analysis applied to various samples saved during fractionation: the supernatant saved from the precipitation with 55 % concentration ammonium sulfate (1) contains a large amount of different proteins while the precipitate collected after precipitation with 65% ammonium sulfate (2) contains as major fractionHSA which is accompanied by two other proteins with similar molecular weight.The fraction isolated after precipitation with ethanol at pH=5.8(3,4) containsthree other proteins along with the HSA. The fraction obtained by precipitation at the isoelectric point contains as major component the albumin (5,6).If compared with commercially available HSA (AppliChem, purity grade min. 96%) on an SDS-PAGE gel, the fraction obtained with the above procedure shows a reasonable purity (Figure 3).

Figure 4 illustrates a proof-of-principle case where HSA can be useful as antioxidant in a blood substitute preparation. Thus, glutaraldehyde-polymerized hemoglobin[16]was subjected to an autooxidation experiment over the course of eight hours. UV-vis spectra were recorded during the incubationat 37 °C in PBS buffer of poly-hemoglobin, in the absence or presence of HSA and BSA, respectively. These spectra illustrate the degree of autooxidation (transition from the physiologically-useful oxy form to the oxidized met, ferric form) in terms of decreases in absorbance at 540 and 580 nm (characteristic of oxy hemoglobin), alongside increases in absorbance at 630 nm (characteristic of met hemoglobin). The autooxidation degree for each time point was definedas $\frac{A_{630,t} - A_{574,t}}{A_{630,oxi} - A_{574,oxi}}$.

After four hours incubation, this ratio was 0.76 for the polymerized hemoglobin; larger values (hence suggestive of less autooxidation over the course of the experiment) were computed for the samples containing HSA

(0.82) and BSA (0.85). The BSA data are in line with our previous observations on its ability to slow down autooxidation in blood substitutes [1,15];it is, however, the first time that HSA was tested in the same context. It appears that HSA behaves similarly to BSA (as expected), although a slight difference does appear to exist in favor of the BSA.

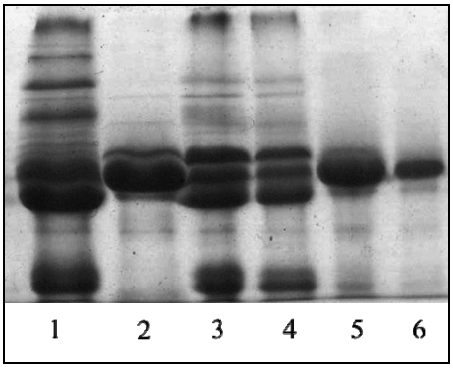

Figure 2. Monitoring the human serum albumin purity by 14% SDS-PAGE.
1 – supernatant from precipitation with 35% ammonium sulfate,
2 – precipitate from precipitation with 65% ammonium sulfate,
3,4 – supernatant from precipitation with ethanol;
5,6 – precipitate from precipitation at isoelectric point.

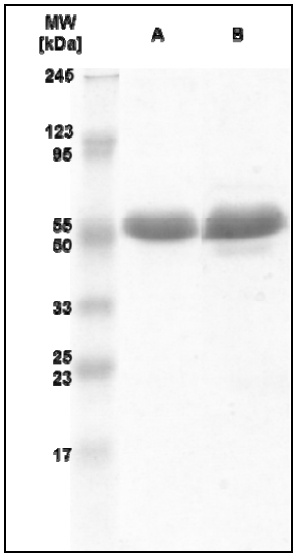

Figure 3. Comparison of commercially available HSA (lane A, 6 ng) with purified human serum albumin (lane B, identical to the fraction in lane 6, Figure 2) on a 14% SDS-PAGE gel.

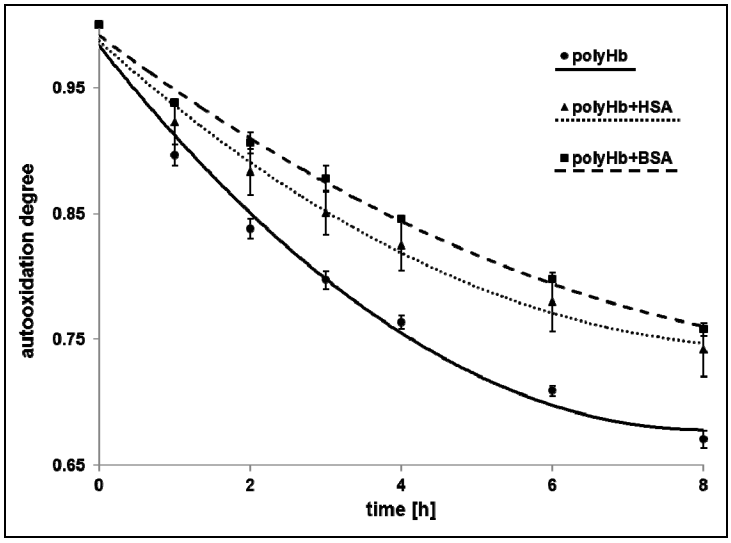

Figure 4. Second order decay of the physiologically-useful oxy form of glutaraldehyde-polymerized hemoglobin upon incubation at 37 °C in PBS buffer, in the presence or absence of HSA and BSA, respectively.

CONCLUSIONS

To conclude, we have reported here a new protocol for purifying human serum albumin from plasma, for use in biotechnological applications such as those involving antioxidant protection in artificial oxygen carriers ("blood substitutes") or chemotherapy.

EXPERIMENTAL SECTION

Proteins were and manipulated in phosphate buffer saline (PBS) unless otherwise mentioned. Human serum albumin (HSA) was purified from plasma following a protocol that combines two fractionation methods namely precipitation with ammonium sulfate and cold ethanol precipitation [3,5]. Human serum albumin was isolated from the fractions remained from ceruloplasmin precipitation with ammonium sulfate. Frozen plasma was obtained from four healthy donors that gave their consent to the sample collection.

The purification of the HSA was monitored by electrophoresis using 14% SDS-PAGE or native PAGE gels, controlled by an electrophoresis power supply Consort EV233. The denaturing SDS gels were stained with Bradford acidic solution for total protein staining. UV-vis spectra were recorded on Agilent 8453 (Agilent, Inc.) instrument.

Polymerized hemoglobin was prepared as previously described [1,13], bovine serum albumin was a commercial preparation identical to that used in our previous experiments on hemoglobin [1].

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REFERENCES

[1] Iacob, B.; Deac, F.; Cioloboc, D.; Damian, G.; Silaghi-Dumitrescu, R. Artif *Cells Blood Substit Immobil Biotechnol* **2011**, *39*, 293-7.

[2] Fedorov, A.N.; Iarochkin, V.S.; Koziner, V.B.; Hachaturian, A.A.; Rozenberg, G.I., *Dokl Akad Nauk SSSR* **1978**, *243*, 1324-6.

[3] Cohn, E.J.; Strong, L.E.; Hughes, J.W.L.; Mulford, D.J.; Ashworth, J.N.*J. Am. Chem. Soc.* **1946**, *68*, 459-475.

[4] Nitschmann, H.; Kistler, P.; Renefer, H.R.; Hassig, A.; Joss, A. *Vox. Sang* **1956**, *1,* 183-200.

[5] Mahn, A; Ismail, M; *J Cromatogr.B,* **2012**, *879*, 3645-8.

[6] Amagasaki, Y.M; Hirakata, E.T., *US Patent 4197238*, **1980**.

[7] Tanaka, K.; Shigueoka, E.M.; Sawatani, E.; Dias, G.A.; Arashiro, F.; Campos, T.C.X.B.; Nakao Braz, H.C. *J Med Biol Res* **1998**, 3111.

[8] Curling, J.M.; Berglof, J.; Lindquist, L.O.; Erkisson, S. *Vox Sang*. **1977**, *33*, 97-107.

[9] Jiang, L.; Hea, L., Fountoulakis, M.; *J. Cromatogr. A,* **2004**, *1023*, 317.

[10] Burnouf, T.; *Bioseparation*, **1991,** *1*, 383.

[11] Burnouf, T.; Radosevich M.; *J Biochem Boiphys Methods*, **2001**, *49*, 575.

[12] Deac, F.; Todea, A.; Silaghi-Dumitrescu, R. In *Metal Elements in Environment, Medicine and Biology Tome IX*; Silaghi-Dumitrescu, R., Garban, G., Eds.; Cluj University Press: Cluj-Napoca, Romania, **2009**.

[13] Hao, Y. *Vox. Sang*.**1979**, *365*, 313-20.

[14] Ouchterlony, O. *Progr. Allergy* **1958**, *5*, 1-78.

[15] Fischer-Fodor, E.; Mot, A.; Deac, F.; Arkosi, M.; Silaghi-Dumitrescu, R. *J Biosci* **2011**, *36*, 215-21.