Design of a robust undergraduate biochemistry laboratory course based on a modified and expanded bovine serum albumin purification scheme

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Synopsis

The ASBMB curriculum for an undergraduate degree recommends a set of skills that can be acquired only through laboratory courses and research experience. Based on a previously reported purification scheme for BSA (Odunuga and Shazhko, 2013), we designed a robust, reproducible, cost-effective, safe and enquiry-based undergraduate biochemistry laboratory course that encompasses many of the skill-sets recommended in the ASBMB curriculum. Our work not only modified certain steps in the scheme, it also included additional steps to enhance student learning and skill acquisition. Salt precipitation, ion exchange and size exclusion chromatography were employed by students to purify BSA from cow plasma. Presence of major contaminants of BSA purification, IgGs and nucleases, were tested in the purified sample by western blotting and DNase I assay respectively. The DNase assay step provides an opportunity for students to learn basic molecular biology techniques such as plasmid isolation and restriction-enzyme digestion. One major addition to the purification process is the bromocresol green-BSA complex assay to precisely quantitate BSA at each step and generate a purification table. Comparison of sequences and other parameters of albumin proteins from common animals provides a bioinformatics twist to student experience. Abundance of albumin from the plasmas of common animals, cow, pig and chicken, allows for variation in the design of the course, and students can work in groups or individually. The course could be designed as a half -semester or full-semester biochemistry laboratory module.

Primary Reference

Odunuga, OO and Shazhko, A (2013), Ammonium sulfate precipitation combined with liquid chromatography is sufficient for purification of bovine serum albumin that is suitable for most routine laboratory applications, Biochemical Compounds, 1:3

Bioinformatics

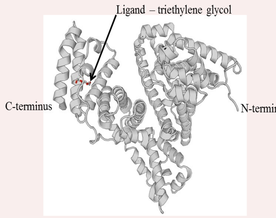


Fig. 1: Structure of mature BSA. Coordinates of BSA crystal structure were downloaded from Protein Data Base and visualized using PV.

Table 1: Comparison of sequences and protein parameters of serum albumin orthologs from common animals. Sequences obtained from Pubmed were analyzed using ProtParam.

Protein Origin	Cow	Pig	Chicken
Length of mature protein	584 amino acids	585 amino acids	590 amino acids
Molecular weight (dalton or g/mol)	66704.2	67016.9	66944.6
Theoretical pI	5.71	5.80	5.35
Total # of (Asp + Glu)	99	98	91
Total # of (Arg + Lys)	84	84	73
Average extinction coefficient ($M^{-1}cm^{-1}$)	41862.5	44842.5	30862.5
Striking observation	-	-	No tryptophan
Phylogenetic tree data	0.09723	0.10996	0.45927
% Identity matrix	100.00	79.28	44.35
	79.28	100.00	43.08
	44.35	43.08	100.00

Salt precipitation

Table 2: Scheme for ammonium sulfate precipitation of albumin from cow plasma.

% $(NH_4)_2SO_4$ saturation	Start volume (mL)	Grams of $(NH_4)_2SO_4$ added*	End volume (mL)
10	5.0	0.2710	4.9
20	4.8	0.2695	4.5
30	4.4	0.2498	4.5
40	4.4	0.2598	4.4
50	4.3	0.2608	4.1
60	4.0	0.2515	4.0
70	3.9	0.2506	3.6
80	3.5	0.2300	3.1
90	3.0	0.2100	3.3

*Determined using ammonium sulfate calculator, at 4 °C (http://www.encorbio.com/protocols/AM-SO4.htm)

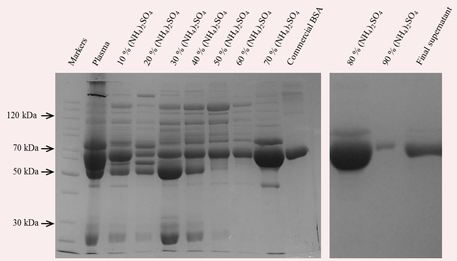


Fig. 2: SDS-PAGE analysis of partial purification of bovine serum albumin by stepwise precipitation with ammonium sulfate. Pellets containing $(NH_4)_2SO_4$-precipitated proteins were resuspended in 20 mM Tris, pH 9 buffer and then resolved on a 10 % SDS-PAGE gel. Majority of the contaminating proteins were removed at 50 % salt saturation.

Anion exchange chromatography

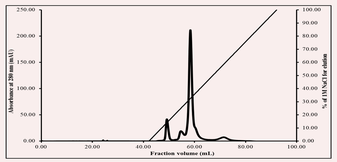


Figure 3A: Linear NaCl gradient elution

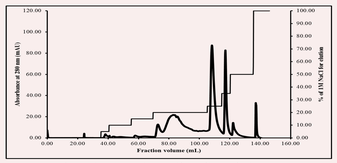


Figure 3B: Stepwise NaCl gradient elution

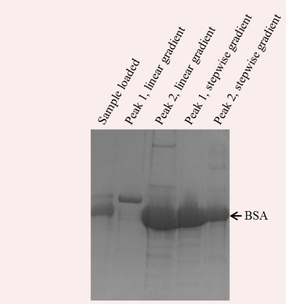
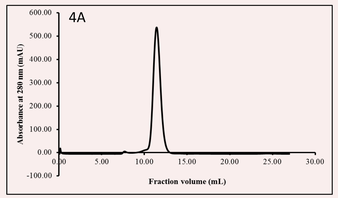
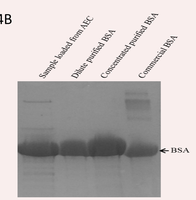


Figure 3C: SDS-PAGE analysis of further purification of bovine serum albumin by anion exchange chromatography

Resuspended BSA from ammonium sulfate purification was precipitated with cold acetone to remove fats. The protein precipitate was resuspended in 20 mM Tris, pH 9 to a concentration of 0.1 mg/mL. The diluted protein was loaded onto a Q column at flow rate of 0.5 mL/min, up to half the binding capacity of the column. Elution was carried out using either a linear or stepwise NaCl gradient buffered in 20 mM Tris, pH 9. The anion exchange step removed transferrin whose size is close to that of BSA.

Size exclusion chromatography





BSA samples eluted from anion exchange column were pooled, concentrated and fractionated on a Superose 12 10/300 GL column in 20 mM Tris, pH 9, 100 mM NaCl buffer. Chromatogram, figure 4A, showed a single prominent peak that corresponded to the size of BSA. Analysis on SDS-PAGE , figure 4B, revealed purified BSA essentially devoid of contaminants.

Purification table

Table 3: Albumin bromocresol assay at various steps of purification. of BSA

Purification Technique	Sample Vol (mL)	mg Protein per mL of sample	Total Protein (mg)	mg of Albumin per mL of sample	Total Albumin content (mg)	% Albumin in sample	% Yield	Purification fold
Crude Extract - cow plasma	3.00	75.0	225	47.0	141	62.7	100.	1.00
Ammonium sulfate precipitation	5.00	13.5	67.5	9.60	48.0	71.1	34.0	1.13
Anion exchange chromatography	1.00	15.4	15.4	12.3	12.3	79.9	8.72	1.27
Size exclusion chromatography	0.547	15.0	8.21	12.7	7.90	96.2	5.60	1.53

Albumin was assayed in protein solutions by measuring the absorbance of the complex it forms with bromocresol green at 630 nm. A standard graph was generated using a range of dilutions of commercially purified albumin. Note: All numbers were recorded or calculated to 3 significant figures, total protein (mg) = sample vol (mL) * mg/mL protein in sample, total albumin content = sample vol (mL) * mg/mL albumin in sample, % albumin = total albumin content (mg)/total protein*100, % Yield = total albumin content of current step/total albumin content of crude extract*100, Purification fold = % yield of current step/% yield of crude extract

Assay for contaminants

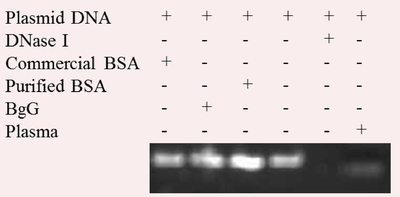


Figure 5A: Analysis of purified bovine serum albumin for nuclease contamination using DNase I assay. Protein samples were analyzed in separate assay mixtures for the presence of contaminating nuclease activity. Ten times the amount of BSA added to routine restriction digestions were used to ensure that any trace amount of DNase would be detected. After 10 min of incubation at 37 °C, the reaction mixtures were resolved on a 1% agarose gel.

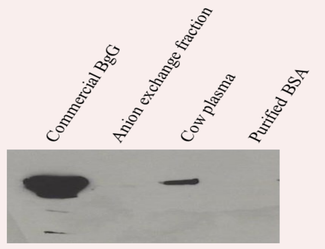
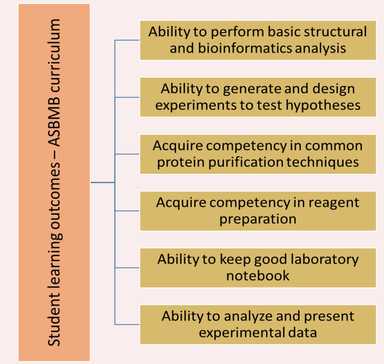


Figure 5B: Aliquots of cow plasma, purified BSA (100 µg each), commercially purified BSA (50 µg) and bovine gamma globulin (BgG, 10 µg) were resolved on 10% SDS -PAGE. The proteins were transferred onto nitrocellulose membrane by western blotting. Presence of IgG was detected by incubating the membrane with HRP-conjugated goat anti bovine IgG diluted at a ratio of 1:5000 in blocking reagent, followed by chemiluminescence.

List of Equipment

1. Analytical Balance
2. Centrifuge (refrigerated or non-refrigerated)
3. Refrigerator or Cold Room
4. PAGE apparatus
5. Western Blotting apparatus
6. UV-Visible Spectrophotometer
7. FPLC or pre-packed column with syringe or peristaltic pump
8. Computers
9. General laboratory equipment and glassware



Acknowledgements

The Robert A. Welch Foundation Grant (AN-0008)
SFA's Office of Research and Sponsored Programs
SFA Beef and Swine Centers
Eastex Veterinary Clinic—Nacogdoches, Texas
All participating students