Determination of Hydrodynamic Properties of Bovine Serum Albumin (BSA)

Rolando A. Curvale, Juan C. Cesco*, Nora B. Debattista and Nora B. Pappano

Instituto de Matemáticas Aplicadas (CONICET-UNSL), and Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, San Luis, Argentina, rcurvale@unsl.edu.ar

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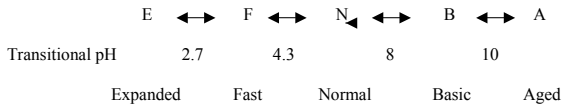
Abstract: This work allows combining theoretical fundamentals along with the development of abilities and skills on applied hydrodynamics. This is a complementary tool used to establish or to confirm the structures and interactions of biomolecules, especially in diluted solutions. In this laboratory, viscosity measurements of bovine serum albumin solutions at different pH values are used to obtain parameters related to form and size of BSA conformers. This practice, which can be performed in three hours, presents the advantage of using low cost reagents and equipment common to many laboratories, which in turn facilitates student participation in their process of learning.

Introduction

Bovine serum albumin (BSA) is the most extensively studied protein due to its important functions, availability, low cost, stability, unusual binding capacity to different ligands, etc [1, 2].

The structure and properties of BSA in solution can be characterized by a versatile conformation that is function of pH, ionic strength, presence of ions, etc. Foster [3] classified BSA conformers as:



Recently, there has been a rise in the use of hydrodynamics as a complementary tool applied to establish or confirm the structure and interactions of biomolecules, especially in diluted solutions. Viscosity measurements can be used to obtain information on form, size and conformation of BSA in solution [4].

Viscosity [η] measurements do not involve technical difficulties and it consists on measuring, with a capillary viscosimeter, the solution flow time (t_s) in relation to the flow time of the used solvent (t_o) [5].

$$\eta_{rel} = \frac{\eta_s}{\eta_o} = \frac{t_s \rho_s}{t_o \rho_o} \tag{1}$$

where η_{rel} is the relative viscosity, ρ is the density, and the sub indexes "s" and "o" correspond to the solution and solvent, respectively.

Intrinsic viscosity [η] can be determined by graphical extrapolation according to different methods. The usual way of doing the concentration dependence is extrapolation of Huggins equation:

$$\frac{\eta_{rel} - 1}{C} = [\eta] + K_H [\eta]^2 C \tag{2}$$

where K_H is the Huggins constant and C is the solute concentration (g/mL) [6].

The Kraemer method uses the following equation:

$$\frac{1}{C} \ln \eta_{rel} = [\eta] - K_K [\eta]^2 C \tag{3}$$

where K_K is the Kraemer constant [7]. The Kraemer extrapolation is usually a complement that reinforces the extrapolated value. However, it frequently occurs that extrapolations do not have a common value at their origin ordinates and is convenient utilize one program of fit [8]. At least four solutions at different concentrations of the biomolecule are required for measurements.

Although there are equations developed to estimate [η] starting from a single concentration of the macromolecule without requiring graphics -known as "single point" equations, these were not satisfactory when applied to the conformational study of the BSA-water system [9].

An interesting method for [η] determination is one that applies the concept of fluidity, φ, (inverse of viscosity). In contrast to flexible polymers, proteins modify their fluidity proportionally to concentration because their behavior is more similar to that of rigid spheres in solution [10]:

$$specific.fluidity = \phi_{sp} = \left(1 - \frac{1}{\eta_{rel}}\right) = [\eta] C \tag{4}$$

The slope of the graph φ_{sp} vs C is [η]. This is the method most applicable to the experimental study of solutes that behave as rigid spheres. Additionally, in protein-water systems, it is less sensible to both, imprecision when determining flow times and the used concentration range [10]. Hence, when Equation 4 is

applied, better results are obtained when determining intrinsic viscosity of BSA-water solutions.

The volume and form of the dissolved macromolecule determine the value of intrinsic viscosity [11]:

$$[\eta] = \nu \overline{V}_{sp} \quad (5)$$

where ν is the "universal function of form" (relation between axes a and b in an ellipsoid of revolution), $\overline{V}_{sp}$ is the volume occupied by the macromolecule including attached and "trapped" water. Equation 5 can be modified in function of measurable parameters:

$$[\eta] = \nu\left(\overline{V} + \delta\overline{V}_0\right) \quad (6)$$

where $\overline{V}$ and $\overline{V}_0$ represent the partial specific volume of the anhydrous macromolecule and water, respectively, and δ is the hydration of the molecule (g water/g dry protein).

Equation 5 can be rewritten in terms of volume as:

$$[\eta] = \frac{\nu N V_m}{M_w} \quad (7)$$

where N is the Avogadro's number, M_W is the molecular weight and V_m is the molecular volume that in turn will present a certain effective radius, namely gyration radius R_G (inferior limit value of the real value):

$$V_m \propto R_G^3 \quad (8)$$

The Mark-Houwink empirical equation relates intrinsic viscosity with molecular weight [12]:

$$[\eta] = K' M_w^{\alpha} \quad (9)$$

where K' is the coefficient in function of solvent polarity and intermolecular forces with the solute, and the α exponent indicates the degree of disentanglement of the macromolecular tangle. The values of viscosity exponent for macromolecules dissolved in a θ solvent is 0.5, while lengthen filaments present values >1. A randomly coiled molecule will present an intermediate value (α ≈ 0.5–0.8).

In this laboratory work, a viscosimetric study of bovine serum albumin conformational changes is performed, obtaining in addition information on the macromolecule's size and form.

Materials and Methods

This laboratory experience requires the following: an Ubbelohde "suspended level" viscosimeter (aproximately120 seconds of water flow time), densimeter, UV-VIS spectrophotometer, thermostatic bath, pH meter, chronometer, thermometer and glass materials.

Fresh BSA concentrated solution (1%) is prepared by dissolving BSA (lyophilized powder) in boiled distilled water. Following, the solution is filtrated and concentration can be verified by spectrophotometry at 278 nm (ε=0.667 $L.g^{-1}$ cm^{-1}).

Each viscosity measurement uses 20 mL of solution. pH values (2.5–4–7.4–8.2–10) are adjusted with micro additions of HCl or HONa 1M.

Results and Discussion

Experiments are performed at the established pH values in order to obtain bovine serum albumin conformers E, F, N, B and A. In each case, measures of both flow times and density of the different BSA percentage concentrations are taken.

- The obtained data allow to calculate relative viscosity, η_{rel} (Equation 1), and from it the specific fluidity. An additional value is predetermined at concentration = 0.
- The slope in the graphical representation of Equation 4 delivers intrinsic viscosity, [η].
- The universal function of form ν is determined from Equation 6 for each conformer, by using previously reported data on partial specific volume of both the anhydrous macromolecule and water, molecule hydration [13], in addition to calculated values of intrinsic viscosity. Considering that all bovine serum albumin conformers correspond to a prolate type ellipsoid of revolution, the a/b value is obtained by graphical interpolation of ν in function of the axial relation of the ellipsoids of revolution given by Van Holde [11].
- The molecular volume V_m and the gyration radius R_G for each pH are assessed from Eqs. 7 and 8, respectively.
- Applying the Mark-Houwink empirical relation (Eq. 9) delivers the α exponent.

The Table 1 presents the values of hydrodynamic properties of different BSA conformers applying viscosity measurements, determined by a group of students.

The secondary structure of an isolated molecule of polyethylene in solution is that of a random tangle, while proteins have a very organized disposition determined -in great extent- by hydrogen bonds, taking the form of helixes or sheets in several molecule segments [15, 16]. At present, it is accepted that the BSA *N* conformer in solution presents the form of an equilateral triangle of 80×80×80 A° and 30 A° of deepness (heart shape) [14].

Analysis of the data presented in the Table 1 allow to infer that:

- The low values of intrinsic viscosity observed in the pH range between 4 and 10 correspond to slightly hydrated compact macromolecules. However, the *E* conformer shows a high [η] value due to the molecule's unfolding (a/b = 8.7), which corroborates that intrinsic viscosity depends on the relation between volume and molecular weight (Eq. 6).
- The molecular volume and gyration radius for all BSA conformations is similar, thus confirming globular structure.
- The α exponent values in the Mark-Houwink Equation indicate that all conformers are randomly coiled.

Additionally, students can determinate the following:

- The approximate value of the isoelectric point of BSA (4.7), by plotting relative viscosity (1%) versus pH.

Table 1. Hydrodynamic properties of different BSA conformers

	CONFORMER				
	E	F	N	B	A
pH	2.7	4.2	7.4	8	10
[η] mL/g	14.66	5.73	4.77	6.52	6.18
ν	12.91	5.05	4.20	5.74	5.44
(a/b)	8.7	4.1	3.3	4.5	4.2
Ellipsoid dimensions Å [11,12]	250 × 21	130 × 40	80×80×80×30	----	----
$V_{m\ Å^3}$	125.397	125.300	125.417	125.436	125.452
$R_{G\ Å}$	50.05	50.04	50.06	50.06	50.06
α	0.68	0.59	0.58	0.60	0.60

Additonal Data: $M_W = 66500\ g/mol;\ \bar{V}_0 = 1/\rho_0 = 1/0.997;\ \bar{V} = 0.734 mL/g; \delta = 0.4;\ K' = 7.93\times10^{-3}\left(25^\circ\ \mathrm{C}\right)$ [13].

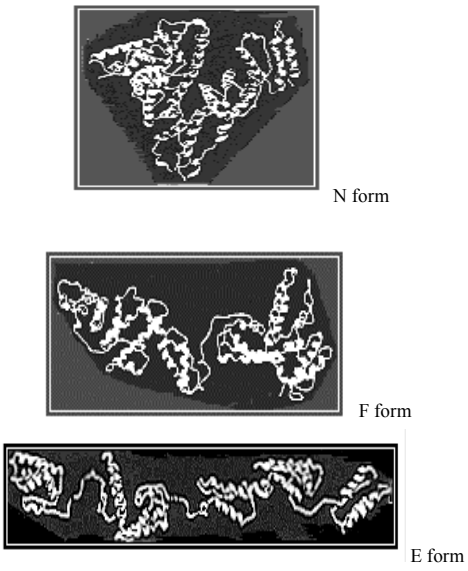


Figure 1. Ribbon diagram of serum albumin in its N form, and in its proposed F and E forms [17].

- The effect of ionic strength on BSA, by using NaCl 0.15M solution.

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