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BUFFER CAPACITY OF BOVINE SERUM ALBUMIN (BSA)

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Abstract

In this work, we have studied the acid-base features of bovine serum albumin (BSA). Buffer capacity, as well as dilution, temperature and salt addition effects were determined. We have found that buffer capacity can be co-related with BSA conformational changes.

Keywords: bovine serum albumin, buffer capacity, conformers.

Resumen:

En este trabajo, estudiamos las propiedades ácido-base de la seroalbúmina bovina (BSA), determinamos su capacidad buffer, los efectos de la dilución, la temperatura y el agregado de sales.

Encontramos que la capacidad buffer puede correlacionarse con los cambios conformacionales de la BSA.

Palabras clave: albúmina de suero bovino, capacidad buffer, confórmeros.

Introduction

Since long, many efforts have been made in order to acquire knowledge on proteins. In this sense, bovine serum albumin (BSA) has been frequently the model of choice for physical chemical studies, due that it is an easily available polypeptide [1,2]. BSA is constituted by the twenty essential amino acids within a structure that contains 583 units [2]. The molecular weight for BSA, calculated from different techniques, is ranged from 66411 to 66700 Da and "the best value" in solution is 66500 Da [2,3].

The structure and properties of BSA in solution can be characterized by a versatile conformation that is a function of pH, ionic strength, presence of ions, etc. Foster [4] has classified BSA conformers as:

Conformer:	**E**	**F**	**N**	**B**	**A**
Transition pH:	**2.7**	**4.3**	**8**	**10**	**10**

Ferrer et al. [5] have demonstrated that BSA (conformer N) has a globular, compressed structure, with a triangular heart shape. The N↔F transition is an abrupt expansion occurring at pH 4.3 [6]. E conformer's is found in the 3.4 - 2.75 pH range. At pH 9, albumin changes conformation to B basic form and after 3 or 4 days, another isomerization known as form A occurs.

Amino acids have one (or more) anionic, cationic group and a lateral chain that may be ionizable. Because of this, amino acids can act as buffers in aqueous solution.

In this work, we study BSA's buffer capacity, knowing that amino acid sequence and tridimensional structure define properties in proteins.

IUPAC [7] considers buffer capacity as the capacity of a given solution to resist a change in pH when a strong acid or a strong base is added.

Kolthoff [8] introduced the term "buffer capacity" in the place of "buffer value" which Van Slyke [9] had used to quantitatively express the buffering effects of in-solution electrolytes and defined it as:

$$\beta = \frac{dC_B}{dpH} \qquad \text{ó} \qquad \beta = -\frac{dC_A}{dpH} \quad (1)$$

Where C_B and C_A represent the concentrations (in equivalents) of the added base "B" and acid "A". Buffer capacity is always positive. It is a non-lineal continuous function; it is a derivative [10] (simply, the reciprocal of the titration curve slope). Buffers are studied in the 3 - 11 pH range because the function tends to a relative maximum when it comes out of this range.

Buffer action is a consequence of the equilibriums among the solvent (water), weak acids (HA), weak bases (B), ampholytes ($Z^{\pm}$) and ions into which different species are converted to when in aqueous solution [11]:

$$HA^{z+1} + H_2O \rightleftharpoons A^z + H_3O^+$$
$$B^z + H_2O \rightleftharpoons BH^{z+1} + HO^-$$
$$Z^{\pm} + H_3O^+ \rightleftharpoons Z^+ + H_2O$$
$$Z^{\pm} + OH^- \rightleftharpoons Z^- + H_2O$$

The properties of buffer solutions can be suitably explained in terms of the equilibriums involved. It is an additive property that can be resolved as a series of terms including each active component:

$$\beta = \beta_{OH^-} + \beta_{H^+} + \sum \beta_i \qquad (2)$$

The first two combined terms refer to the buffer capacity of water and the last one, β_i, includes all buffer species present in solution.

In the case of several in-solution co-existing buffer species, the general expression is [12]:

$$\beta = 2{,}303\left(\frac{K_w}{[H^+]} + [H^+] + \sum \frac{C_{buf} K_a [H^+]}{(K_a + [H^+])^2}\right) \qquad (3)$$

Where K_w represents the dissociation constant of water, K_a are the acid dissociation constants and C_{buf} is the concentration (molality) of each buffer species. In the case of the poly-ampholyte BSA, this calculation is impracticable due to the number of dissociation constants involved in addition to their internal interactions.

Dilution Effects

If a buffer is effective in regulating acidity of a given solution, it should also be insensitive to changes in the total concentration of its components. In order to express dilution effects in a quantitative fashion, it is convenient to define the dilution value ($\Delta pH_{1/2}$) as the pH variation undergone by a solution with C_i initial concentration when diluted with an equal volume of pure water [13].

$$\Delta pH_{1/2} \equiv (pH)_{C_{i/2}} - (pH)_{C_i} \qquad (4)$$

Dilution value is positive when pH rises with dilution and negative when it lowers.

Effects due to salt addition

When a neutral salt is added to a buffer solution, the ionic strength of the solution increases and, consequently, the activity coefficients of ions diminish. It should be also considered that the added salt could interact with the buffer species. There are studies showing that the BSA-binding ability of ions depends on the particular ion, BSA net charge and pH [14,15].

Temperature effects

Although temperature influence on concentration can be despised, activity coefficients and dissociation constants are affected by temperature.

Materials and Methods

BSA (powder, lyophilized, defatted and deionated, purity grade >98%) was provided by Fedesa-UNSL. Titrations were performed on fresh BSA solutions in distilled and degassed water [16]. The BSA concentrations at study were 0.75, 1.5, 3.0, 6, 12 and 15×10^{-4} molal. Titers were obtained using HCl and NaOH approximately 1 molal and additions (50 µL) were done using a Metrohm Heriseau E415 dosifier. Temperature was regulated using a LAUDA thermostatic bath at

25 and 37 °C (± 0.1). pH determinations were performed using a BOECO BT-500 microprocessor, BA 25 Gel electrode, precision 0.02 pH units. After each run, the electrode was washed with 1% pepsin acid solution and recalibrated.

Results

Titration curves for hydrogen ion within proteins can be obtained experimentally with considerable precision [17, 18, 19]. In the case of BSA, it has been shown that these curves do not show hysteresis and are reversible in the 2.5 to 10.5 pH range [2, 20]. The BSA molecule is flexible and can quickly change its shape [21].

Titration's were performed at 25 °C and 37 °C over the six concentrations above mentioned. Some of these results are shown in Figure 1.

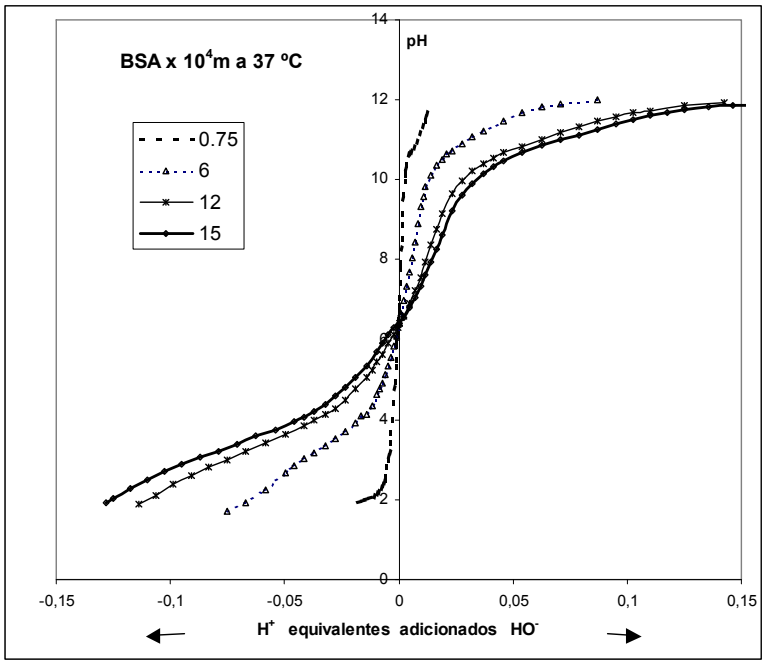

Figure 1. Potentiometric titration of BSA. Concentrations from 0.75 to 15 x 10^{-4} molal at 37 °C. Acid (or base) equivalents added in function of pH.

A similar graphic (with little difference) is obtained when working at 25 °C due that the temperature coefficient that we determined was -0.00033 units of pH per °C.

Using the Microcal Origin 6.0 program, titration curve differentials were obtained and buffer capacity was plotted considering that $\beta = 1/\text{slope}$ [22].

In order to simplify, Fig. 2 shows the study on four concentrations. The curves show a noticeable change when concentration is higher than 3 x10^{-4} molal. It is probable that in the case of dilute BSA solutions (0.75 x 10^{-4} m) the solvent's participation would be considerable. The curves show a broad buffer region with two maximums between pH 2.5 and pH 5, and that corresponds to BSA conformer F; conformer N presents low buffer capacity from pH 5 to pH 8.5; and the B conformer shows an increasing buffer capacity at increasing pH values. A similar graphic is obtained at 25 °C.

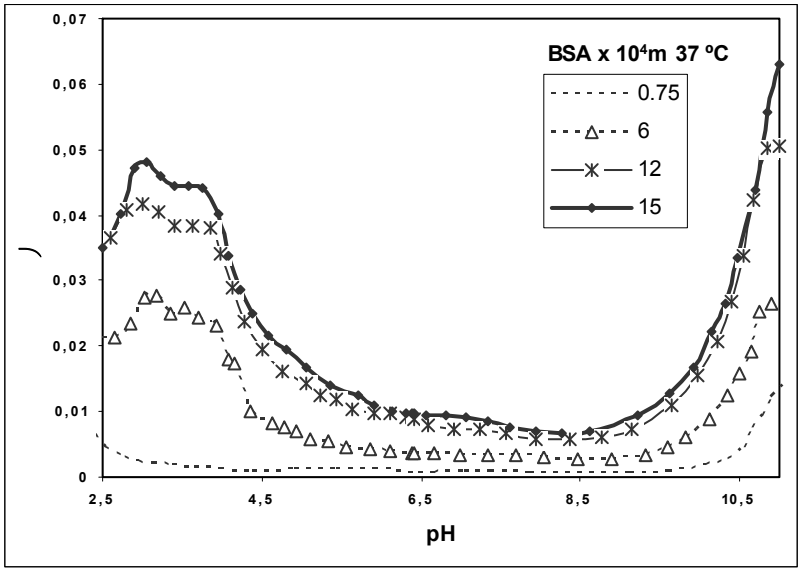

Figure 2. Buffer capacity at different pH values. BSA solutions 0.75 and 15 x 10^{-4} molal at 37 °C.

Van Slike [9] infers the maximum buffer value for monovalent buffers as:

$$\beta_{max} = 0.575C \qquad (5)$$

However, this value can be excessive when the buffer is polyvalent and for example, the maximum value for citric acid is 0.84C.

Fig. 3 shows that the buffer region of the F conformer of BSA shifts to more basic pH values along with increasing addition of KCl, Also, relative maximums change and addition of KCl 1 x 10^{-3} M presents an intermediate behavior.

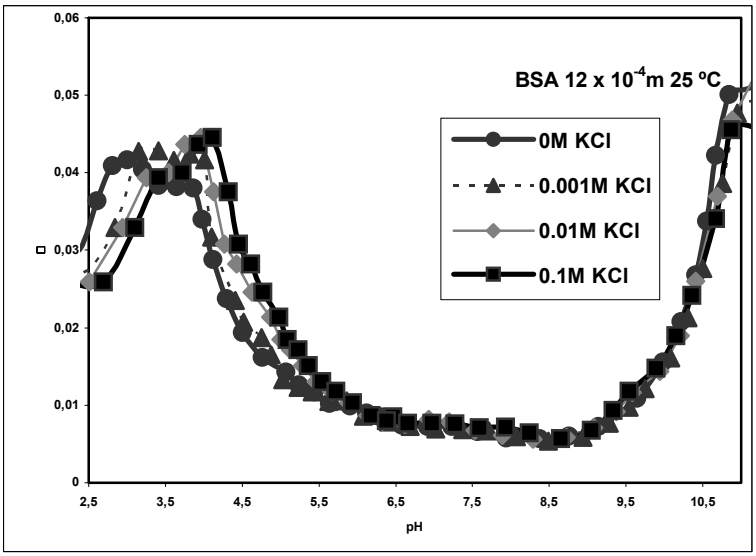

Figure 3. Buffer capacity in function of pH. Effect of the addition of KCl 0.1, 0.01 and 0.001M at 25 ºC.

Summary of buffer features for BSA

***Practical pH range*: 2.5 - 4.5**

***Maximum value at pH* = 3.5 ± 0.5 units: β = 0.046, for BSA 15 x 10^{-4} molal at 37 ºC.**

***Dilution value:* -0.014.**

***Temperature Coefficient:* - 0.00033 units of pH per ºC.**

Conclusions

The experimental study performed in this work was done by acid-base titration of BSA. This molecule contains several different dissociable groups that interact with each other and, thus, charge continuously varies with pH. Hence, we can only measure -after reaching equilibrium- the resulting hydrogen ion concentration.

BSA presents buffer property that, due to electrostatic effects, depends on the "effective" dissociation constants [20].

Fig. 2 shows that the buffer capacity of BSA is low and almost constant at low concentrations (lower than 3 x 10^{-4} molal). Water protolysis probably has influence in this behavior. However, in the case of BSA 6 x 10^{-4} molal (or greater) there are significative buffer capable zones.

The representation of BSA's buffer capacity (Fig. 2) shows the conformational changes adopted by the molecule. In the 4.5 - 8 pH range, (conformer N), presents low buffer capacity [2]. When media acidity rises, the molecule opens separating I+IIA domains from IIB+III domains [1] and thus resulting in conformation F. This transition occurs in two steps: N↔F1↔F [23, 24]. Khan et al. [25] point out that the F1 conformation occurs in the 4.3 - 3.8 pH range. At the first step, some of the carboxyl groups stay protonated due to salt bridge binding that prevent their dissociation. In the second step, they all are protonated. Fig. 2 shows two maximums that occur coincidently in the pH ranges indicated by Khan for F1 and F conformers, and that the latter presents higher buffer capacity.

The N↔B transition is less dramatic and through discrete stages [26]. In fig. 2, a gradual increase in buffer capacity is observed starting from pH 8.5.

The buffer capacity of BSA 15 x 10^{-4} molal is comparable to a classical 0.1 molal buffer.

The presence of salt (Fig. 3) generates a shift in N↔F transition that is 0.5 pH units higher because of the affinity in the union of BSA with Cl^- [27]. Scatchard [28] indicates that this capacity double folds in the 5.2 - 4.2 pH range. Acid expansion is favored by a rise in ionic strength and indicates predominant saline forces. In fig. 5, the shift in transition N↔F1↔F due to addition of KCl can be observed. In addition, F1 and F conformers invert the magnitude of their buffer capacity: conformation F1 is highest.

BSA has a natural tendency for aggregation under stress conditions [29] (pH extremes, concentration, ageing, higher ionic strength, fatty acid impurity, etc.) our working conditions do not allow us to make observations in this regard.

The main function of BSA is stabilizing the physical environment of the blood; is a rather weak buffer in physiological pH range, but, in extra vascular fluids assume importance [30]. PH extremes (<3, >10) of course, so the molecule no longer considered to be “native”.

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References

[1] D.C. Carter, J.X. Ho, *Advances in Protein Chemistry* **1994**, *45*, 153.

[2] T. Peters, All about Albumin. Biochemistry, Genetics and Medical Applications Elsevier Inc. 1995.

[3] F.L. Gonzalez Flecha, V. Levi, *Biochem. Molec. Biol. Educ*. **2003**, *31*, 319.

[4] J.F. Foster, Albumin, Structure, Function and Uses. Academic Press. 1997.

[5] M.L. Ferrer, R. Duchowicz, B. Carrasco, J. Garcia de la Torre, U. Acuña, *Biophysical Journal* **2001**, *80*, 2422.

[6] N.J. Leonard, J.F. Foster, *J. Biol. Chem*. **1961**, *236*, 2662.

[7] IUPAC. The Compendium of Analytical Nomenclature. Pergamon. Elmsford New York. 1977.

[8] I.M. Kolthoff, C. Rossemblum, Acid-Base Indicators. Macmillan. New York. 1937.

[9] D.D. Van Slike, *J. Biol. Chem*. **1922**, *52*, 525.

[10] E.T. Urbansky, M.R. Schock, *J. Chem. Educ*. **2000**, *77*, 1640.

[11] R.G. Bates, Determinación de pH. Teoría y Práctica. John Wiley. 1973.

[12] B.P.P.M. Borkowki, Chemical calculator. 2005. www.chembuddy.com/ .

[13] R.G. Bates, *Anal. Chem*. **1954,** *26*, 871.

[14] D.J. Winzor, *Analytical Biochemistry*, **2004**, *325*, 1.

[15] G. Scatchard, J.S. Coleman, A.L. Shan, (1957) *J. Am. Chem. Soc*. **1957**, *79*, 12.

[16] R.C. Playle, *Sci. Total Environ*. **1998**, *219*, 147.

[17] C. Tanford, J.C. Kirkwood, *J. Am. Chem. Soc*. **1957**, *79*, 5333.

[18] J.F. Foster, Albumin: Structure, function and uses. Putman Ed. Academic Press. New York. 1960.

[19] J. Steinhardt, J. Krijn, J.G. Leidly, *Biochemistry* **1971**, *10*, 4005.

[20] C. Tanford, S.A. Swanson, W.S. Shore, *J. Am. Chem. Soc*. **1955**, *77*, 6414.

[21] C. Tandford, *J. Am. Chem. Soc*. **1950**, *72*, 441.

[22] G.W. Tindall, *LCGC North America* **2002**, *20*, 114.

[23] M. Sogami, S. Era, S. Nagaoka, H. Inouye, *Int. J. Pept. Protein Res.* **1982**, *19*, 263.

[24] S. Era, H. Ashida, S. Nagaoka, H. Inouye, M. Sogami, *Int. J. Pept. Protein Res*. **1983**, *22*, 333.

[25] M.Y. Khan, A. Salahuddin, *Eur. J. Biochem*. **1984**, *141*, 473.

[26] B.J. ´t Hart, J. Witting, J.J. de Gier, *Biochem. Pharm*. **1986**, *35*, 1005.

[27] M. Dockal, D.C. Carter, F. Ruker, *J. Biol. Chem.* **2000**, *275*, 3042.

[28] G. Scatchard, W.T. Yap, *J. Am. Chem. Soc*. **1964**, *86*, 3434.

[29] A. Brahma, C. Mandal, D. Bhattaharyya, *Biochim. Et Biophys Acta* **2005**, *1751*, 159.

[30] J. Figge, T.H. Rossing, V. Fenci, *Lab. Clin. Med.* 1991, *117*, 453.