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# Binding of iodide to bovine serum albumin and protamine studied with an ion-selective electrode

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Binding of iodide ion to bovine serum albumin and protamine was studied using an iodide ion-selective electrode. Binding was found to be significant with bovine serum albumin but insignificant with protamine. The data for the iodide–bovine serum albumin system were treated according to the Klotz equation and the fit was found to be quite good. Number of iodide ions bound per bovine serum albumin molecule was calculated as one and the equilibrium constant for this binding was determined to be $5{\cdot}3 \times 10^3$. Insignificant binding to protamine and single binding to each bovine serum albumin molecule were explained in terms of the amino acid compositions and secondary structures of these proteins.

## INTRODUCTION

It is of interest to study binding of anions to proteins to obtain information about the resulting structure modification of the proteins. Klotz *et al.* (1946) studied binding of organic anions, methyl orange and azo-sulphathiazole by bovine serum albumin and derived the so-called Klotz Equation. This equation can be given as

$$\frac{1}{r} = \frac{K}{n}\frac{1}{[\mathrm{A}]} + \frac{1}{n} \qquad (1)$$

where $r$ is the ratio of moles of bound anion to the total moles of protein, $n$ is the maximum possible number of bound anions per protein molecule and $K$ is a constant to be determined experimentally. $K$ depends on the nature of the anion as well as on the character of the protein. [A] is the equilibrium concentration of free anion. Scatchard *et al.* (1959) reported binding of small anions by serum albumin. They used chloride, fluoride, thiocyanate and trichloroacetate as small anions, bovine serum albumin and human serum albumin as protein. Their data were treated according to a type of non-linear form of Klotz equation which is also known as the Scatchard equation. Mangoni *et al.* (1969) studied the interaction of fluorine with serum albumin. More recently, Luehrs and Johnson (1986) reported a study on binding of fluoride ion to egg albumin. The latter workers used an ion-selective electrode to measure the amount of fluoride ion bound to protein. A study of interactions of two proteins, namely bovine serum albumin (BSA) and protamine, with water and urea in relation to the denaturation process was reported previously from this laboratory (Kaya, 1987; Ayrancı & Kaya, 1990). Another study on the interactions of five major amino acids present in the structure of BSA and protamine with water and guanidine hydrochloride using viscosity and apparent molar volume measurements was also reported from this laboratory by Belibağlı and Ayrancı (1990). The purpose of the present study is to investigate binding of iodide ion by BSA and protamine using an ion-selective electrode and to examine the validity of the Klotz equation for the binding. The reason for choosing these two proteins is their water solubility and also the possibility of correlation of this work with previous works mentioned above.

## MATERIALS AND METHODS

### Materials

Crystallised BSA (Sigma A-7906) and protamine from salmon (Sigma P-4005) were obtained from the Sigma Chemical Company. They were kept in a desiccator at 0°C when not in use. NaI used as the iodide ion source and $NaNO_3$ used as ionic strength adjuster were reagent grade. Water used in all experiments was doubly distilled.

### Method

Free iodide ion was determined using an Orion Model 701A pH/mV meter equipped with an Orion iodide ion-selective electrode. Details of the procedure for using

ion-selective electrodes as applied to analytical measurements of chloride and nitrate in foods were given by Ayranci and Balci (1993). First, a calibration curve of measured potential in mV as a function of logarithm of concentration was obtained using standard $10^{-4}$, $10^{-5}$ and $10^{-6}$M NaI solutions. Two millilitres of 5M $NaNO_3$ solution was added to all standard solutions as an ionic strength adjuster. An excellent straight line was obtained as a calibration curve with the three standard iodide solutions. For binding studies, 100 ml $2 \times 10^{-4}$M protein solution was supported with 2 ml 5M $NaNO_3$ as ionic strength adjuster. In determining the concentration of protein solution, the average molecular weights of BSA and protamine were taken as 66 000 g $mol^{-1}$ and 5000 g $mol^{-1}$, respectively (Kaya, 1987). Then, increasing amounts of $8 \times 10^{-4}$M NaI solution were added. After each addition, potential was read from the meter when the equilibrium was reached. A constant reading from the meter was taken as an indication of achievement of equilibrium. This reading was converted into iodide ion concentration using the calibration curve.

pH of BSA solutions was found to remain constant at 7·2 after the addition of ionic strength adjuster, whereas that of protamine solutions showed a slight change from 11·2 to 10·9 after the addition of ionic strength adjuster throughout the binding studies. A buffer was not used to prevent introducing more charged species into solutions, since pH remained almost constant during binding studies. pH measurements were made with an Eyela pH controller FC-10.

## RESULTS AND DISCUSSION

The amount of iodide ion bound to BSA was determined by taking the difference between iodide added to protein solution and iodide measured at equilibrium. The ratio of moles of bound iodide to the moles of total protein present provided the value of $r$ in eqn (1). The measured free iodide concentrations correspond to [A] in eqn (1). Figure 1 shows a plot of $1/r$ against $1/[I^-]$ for binding of iodide ion to BSA. Linear regression analysis of data provided a slope of $1{\cdot}8 \times 10^{-4}$, a $y$-intercept of 0·98 and a correlation coefficient of 0·998. The value of $n$ which was defined as the maximum number of bound anion per protein molecule was found to be 1·0 from the intercept. Experimentally observed $K$ constant of eqn (1) was calculated to be $1{\cdot}9 \times 10^{-4}$ from the slope.

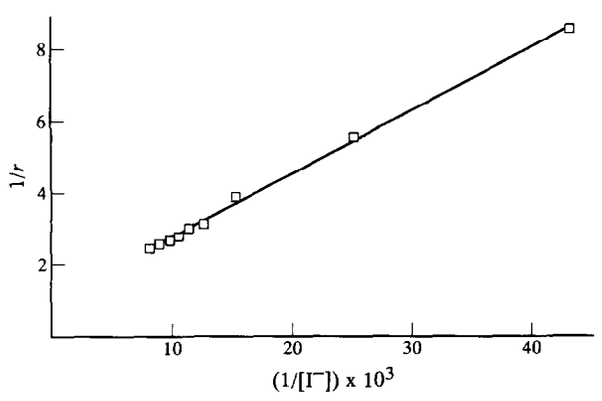


**Fig. 1.** A plot of $1/r$ against $1/[I^-]$ for binding of iodide ion by BSA.

Since the maximum number of iodide ion bound per BSA molecule is just one, binding occurs in one step. The experimentally observed $K$ constant can easily be converted into the equilibrium constant corresponding to the following reaction:

$$BSA + I^- \rightleftharpoons [BSA.I]^-$$

simply by taking the reciprocal of $K$ according to the following equation which was derived by Klotz *et al.* (1946):

$$k_i = \frac{n - (i-1)}{i} \times \frac{1}{K} \qquad (2)$$

where $k_i$ is the equilibrium constant for the $i$th step. So, the single binding process in the present case has an equilibrium constant of $5{\cdot}3 \times 10^3$.

Binding of iodide to BSA is expected to occur at a positively charged centre of the protein molecule. When the amino acid composition of BSA is examined (Kaya, 1987), the presence of about 18 amino acids with percentages ranging from 0·53 to 14·48 can be seen. Therefore, it is very difficult to assign any specific side chain of an amino acid as a binding site. BSA is known to have a considerable amount of helix structure. So, some of the candidate binding sites might be in the interior parts of $\alpha$-helix structure which are not accessible by iodide ion. Therefore, this ion is probably being bound to a positively charged site on the exterior parts of the helix structure. It should be noted that although only a single anion is bound to BSA, it is bound quite strongly as indicated by the large equilibrium constant.

It was found that the difference between the amount of iodide added to protamine solution and the amount of free iodide measured at equilibrium was insignificant. This indicates no binding or a very weak binding of iodide ion to protamine. Previously, it was found by Ayranci and Kaya (1990) that protamine does not contain any helix structure and it is mainly in the random coil form. So, although all the sites in protamine are accessible to iodide ion, the lack of observation of binding indicates that protamine does not contain an easily available positively charged centre. Furthermore, it is well known that iodide ion is the largest halide ion and is assumed to be spherical. The single negative charge is evenly distributed over the whole sphere. So, the binding ability of iodide ion is rather weak and this fact can partly explain the lack of binding to protamine and only a single binding to BSA.

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