Asian Journal of Chemistry Vol. 13, No. 1 (2001), 61–65

Study of the Conformation Changes of Blood Serum

R.P. VIDYARTHI,* AMRENDRA KUMAR, JAGESHWAR CHAURASIA and ARUNCHAL CHAURASIA
Department of Chemistry, L.S. College, Muzaffarpur-842 001, India

pH and conductometric titration of blood serum (Goat's) in 5% aqueous medium gave two inflexions and the corresponding two breaks with N/20 HCl and only one inflexion/break with N/20 NaOH solution. In 0.25 M KCl there was only one inflexion in both acid and alkali. In 8 M urea solution the number of inflexion/breaks rose to three. The coulombic interactions between oppositely charged sities in the protein chain seems to have screened, at least in part by a neutral salt like KCl present in large concentration as also shown by the fact that the fall of pH by the addition of (5 mL) of acid is more in aqueous medium than in KCl solution.

Urea being a powerful disruptor of hydrogen bond the protein chain would, in all probability, exist as a random coil conformation and the study of pH titration curves revealed the acidic and basic groups having three step reactions in it. The nature of the titration curve is also altered in urea solution. The initial pH of blood serum rose from 7.2 to 7.9 showing there by the protons of the NH_3 cations in the zwitter ions be immobilised by hydrogen bond formation with urea as suggested by Donovan, Laskowski and Scherage. Expansion of the protein random coil in the denatured form also seems to occur in the pH titration.

INTRODUCTION

Proteins are linear polyampholytes with the principal chain in the molecule made up of peptide linkages. Depending largely on the pH and the ionic strength of the medium, many acidic and basic groups dissociate simultaneously from the polyampholyte molecules and the macroscropic state of equilibrium which is observed, is the result of a large number of inter-related hydrogen ion equilibria in which many protogenic group participate. In the case of bovine serum albumin (BSA), Tanford[1] found a more or less continuous change in the curvature of a Linderstrom Lang[2] plot of

$$(pH - \log \frac{r_i}{n_i - r_i}) \text{ versus Z drawn from the equation,}$$

$$pH = pK^i_{int} + \log \frac{r_i}{n_i - r_i} - 0.868\ WZ$$

over the entire acid range. Where pK^i_{int} is the intrinsic dissociation constant of its group. Dissociation of only COOH group would occur in this Rands and

Tanford[1] observed that, here, the change in curvature was due to a variation of W with pH. This was also supported by the measurements of the hydrodynamic radius of the BSA molecule by light scattering[3], viscometry[4] and sedimentation[5] method.

In the case of plasma albumin, Foster and Aoki[6] found a transformation of the protein from the native to an isomeric form (N-F transformation) in the region of carboxyl titration. The carboxyls in the F form had different dissociation characteristics from those in the N form. The number of these different types of carboxyls seemed to correspond to the number of NH_2 groups in the protein. Perlmann[7] working with pepsinogen, found a shift in the pH at the maximum buffer capacity when the temperature was varied suggesting the conformational changes arising from (+ –) interactions between histidinium and carboxyl groups which were considered. The importance of the influence of coulombic interactions between oppositely charged sites in determining the potentiometric behaviour of polyampholytes have been discussed by Mazur, Silberberg and Katchalsky[8]. A netural salt, like KCl present in large concentration would be expected to screen, atleast in part, electrostatic interaction between charged sites. Studies of H^+ ion equilibria in blood serum (Goat's) have been rather meagre. Most of the work which has been concerned only with the studies of their conformation. The present investigation was undertaken mainly with a view of fill in this gaps.

pH and conductometric titrations were performed in aqueous, in 8 M urea solution and again in 0.25 M KCl solution pH titration was also done.

The idea behind the contemplated titration in 8 M urea is that, urea being a powerful disrupt or of hydrogen bonds the protein chain would, in all probability exist as a random coil in 8 M urea and it would be interest to see what modification, if any, of the titration behaviour of the acidic and basic groups was caused by this kind of a change in the conformation of the molecule.

EXPERIMENTAL

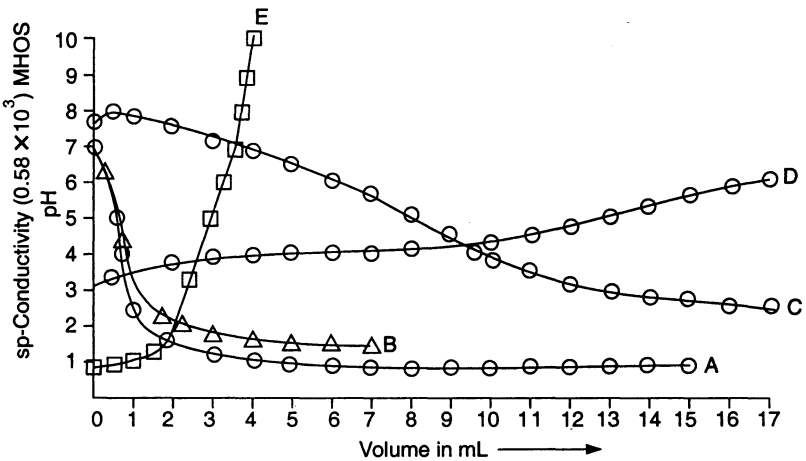
pH and conductometric titrations were done at room temperature; N/20 HCl solution and N/20 NaOH solutions were used for titration. HCl solution was prepared from a constant boiling 6 N HCl by weight. KCl was E. Merck sample and urea was B.D.H., A.R. 20 mL. of 50% blood serum (Goat's) obtained by decantation of fresh goat's blood and decolourisation with active charcoal was taken in the titration vessel as solution A. In the solution B, there is 20 mL of 0.25 M KCl solution and solution A. Solution C is solution A and 20 mL of 8 M urea solution.

pH titration was performed with a systronic pH meter type 322. This has accuracy of 0.05 pH. After each addition of drops of acid or alkali, it was stirred and sufficient time was given so as to get reading at equilibrium value. Systronic conductivity meter type 302 having a direct readings dial and a magic eye blue free null indicator was used.

RESULTS AND DISCUSSION

pH and conductometric titration curves are shown in Fig. 1 and Fig. 2. Fig 1

contains the acid titration and Fig. 2 the alkali titration. In the acid titration of Fig. 1 there are curves for pH titration of blood serum denoted as solution A. Solution A and 8 M urea and solution A with 0.25 M KCl. The corresponding conductometric titration curves are also there. No conductometric titration in the medium of 0.25 M KCl was performed because KCl solution being highly conductive the small addition of acid or alkali did not change the conductivity. There was number of inflexions in the pH titration curve; these inflexions are supported by corresponding breaks in the conductometric titrations. So these two titrations are mutually supporting and supplementing each other.



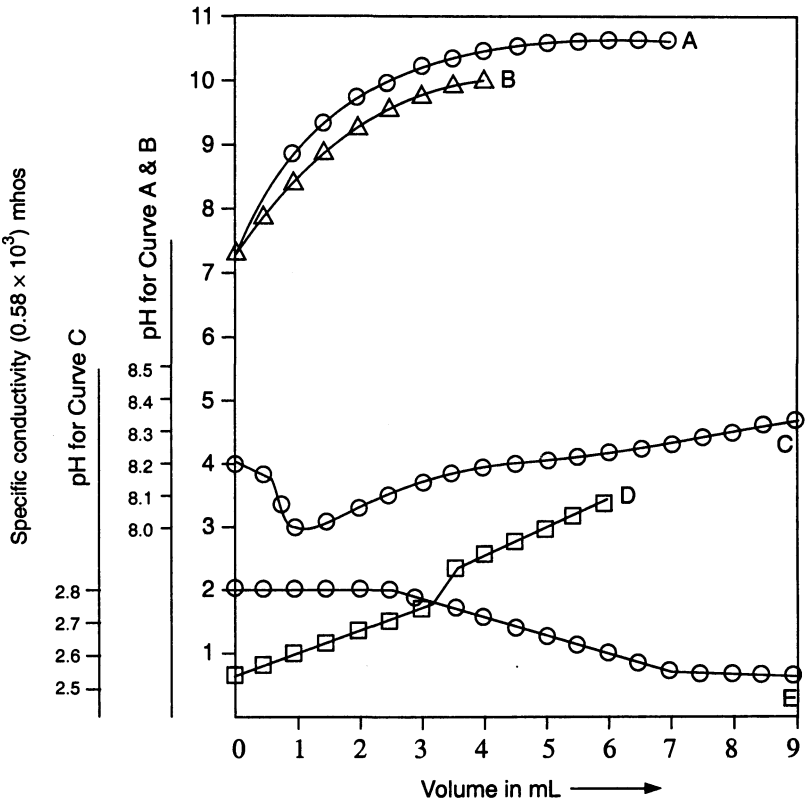
A. pH curve of blood serum in aqueous medium
B. pH curve of blood serum in KCl
C. pH curve of blood serum in urea
D. Cond. curve of blood serum in aqueous medium
E. Cond. curve of blood serum in urea

Fig. 1. Titration curves with HCl

The initial pH of solution A is 7.2 with the addition of only 1 mL the pH comes down to 2.7. After that the slopes of this curve is less and it is more or less smooth. After this it comes to pH 1.3 for 5 mL of acid addition. The corresponding KCl curve is also behaves same to the above curve in nature. Here the pH corresponding to 1 mL of acid addition is 3.0. This is little higher than earlier curve. In aqueous medium pH 2.0 is obtained by the addition of 5 mL of acid.

Table-1 gives the amount of N/20 HCl used at different inflexions in pH curves and breaks in conductometric titrations.

From Table-1 it is obvious that pH and conductometric titration are supporting each other 1st inflexions occur at 1.5 mL and the 2nd inflexions at 4.5 mL. The breaks are also at 1.0 and 4.0 mL. The fine features are also revealed by these curves. Two inflexions/breaks show two steps reaction whereas in KCl solution there is only one inflexion. Thus there is screening of some basic group by KCl solution.



A. pH curve of blood serum in aqueous medium
B. pH curve of blood serum in KCl
C. pH curve of blood serum in urea
D. pH. curve of blood serum in aqueous medium
E. pH curve of blood serum in urea

Fig. 2. Titration curves with NaOH

TABLE-1

Medium	Method	Volume of N/20 HCl in mL		
		1st Inf./break	2nd Inf./break	3rd Inf./break
Water	pH	1.5	–	–
	Cond.	1.0	4.0	–
KCl	pH	1.5	–	–
Urea	pH	0.5	5.5	9.7
	Cond.	0.5	4.0	10.0

In 8 M urea solution there are three inflexions and the corresponding three breaks. These are at 0.5 mL, 4.5 mL and 9.7 mL. The nature of the curve is also varying. This shows the change in the conformation of the protein chain such that

the number of basic groups have also increased and the nature of the curve has altered. The initial pH of blood serum rose from 7.2 to 7.9. (Fig. 1) showing there by the protons of the NH_3 cations in the zwitter ions might be immobilised by hydrogen bond formation as suggested by Donovan, Laskowshi and Scharage[9].

Table-2 shows the alkali titration of blood serum in aqueous salt and urea solution. The corresponding titration curve is given in Fig. 2.

Table-2 shows three inflexions/breaks taking place as in HCl titration. Alkali titration is also more or less the same in other respects. Thus the effect of urea and KCl solution in the titration behaviour of blood serum (Goat's) also follows the same pattern as in other protein molecules.

TABLE-2

Medium	Method	Volume of N/20 NaOH in mL		
		1st Inf./break	2nd Inf./break	3rd Inf./break
Water	pH	3.2	–	–
	Cond.	3.5	–	–
KCl	pH	3.0	–	–
Urea	pH	1.0	–	–
	Cond.	2.5	–	–

REFERENCES

1. C. Tanford, S.A. Swanson and W.S. Shore, *J. Am. Chem. Soc.*, **77,** 6414 (1955).
2. K. Linderstrom Lang, *Compt. Rend. Tran. Lab. Carlaber. Ser. Chem.*, **15,** 23 (1924).
3. M.D. Sterman and J.F. Foster, *J. Am. Chem. Soc.*,**78,** 3652 (1956).
4. C. Tanford, J.G. Bussell and D.A. Rands, *J. Am. Chem. Soc.*, **77,** 6421 (1955).
5. K.O. Pedrersen, *Diss. Farad. Soc.*, **13,** 49 (1953).
6. J.F. Foster and K. Aoki, *J. Phys. Chem.*, **61,** 1369 (1957).
7. G.E. Perimann, A. Oplatka and A. Katchalsky, *J. Biol. Chem.*, **245,** 5163 (1967).
8. J. Mazur, A. Oplatka and A. Katchaisky, *J. Polymer Sci.*, **35,** 43 (1959).
9. J.W. Downovan, M. Laskoski and H.A. Scherage, *J. Mol. Biol.*, **1,** 293 (1959).

(*Received:* 28 June 2000; *Accepted:* 4 September 2000) AJC-2129