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Titration of Proteins in Dimethyl Sulphoxide–Water Mixtures

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The acid–base behaviour of arginine, lysozyme and ovalbumin has been studied by potentiometric and catalytic thermometric titrimetry in a mixture of dimethyl sulphoxide–water, with acrylonitrile as the end-point indicator in the latter technique. It was observed that, with the exception of the SH groups, all the protonated groups, including the guanidine groups of lysozyme and ovalbumin, were titrated by catalytic thermometric titrimetry. By using potentiometric titrimetry, all the titratable groups of ovalbumin were determined, whereas the guanidine groups of lysozyme were not determined by this technique.

Keywords: *Protein; catalytic titrimetry; thermometric titrimetry; potentiometric titration; dimethyl sulphoxide*

An approximately equimolar mixture (80% m/m) of dimethyl sulphoxide (DMSO) and water has frequently been employed in potentiometric titrimetry (PT).[1] This mixture is convenient because it is not very hygroscopic, dissolves many organic and inorganic compounds and has a relative permittivity (78.5) close to that of water, but has very different acid–base properties from water.

The use of a DMSO–water–acrylonitrile (AN) mixture for the titration of a number of strong and weak acids, including some amino acids, by catalytic thermometric titrimetry (CTT) has been reported.[2] In this instance the rise in temperature caused by a cyanoethylation reaction and by the polymerization of AN catalysed by the excess of base is used to indicate the end-point of the titration. The influence of the solvent composition on the base-catalysed reactions of AN in the titration of benzoic acid in mixtures of some dipolar aprotic solvents, water and AN has also been reported.[3] In preliminary experiments involving the titration of acid groups of proteins in a DMSO–water–AN mixture, the influence of solvent composition has also been investigated.[4] By considering the shape of the titration curve and the solubility of proteins, it was decided to adopt a solvent composition of DMSO–water–AN of 7.0 + 0.8 + 4.0 ml for these titrations. It was observed that in addition to the carboxyl and α-amino groups of amino acids, the second carboxyl groups of glutamic and aspartic acids, the ε-amino group of lysine, the phenolic group of tyrosine and the imidazole group of histidine were titrated under the above conditions. However, the SH group of cysteine cannot be determined by this technique. This has been attributed to the cyanoethylation of this group, in agreement with Greenhow and Loo,[5] who reported the cyanoethylation of SH groups of thiols during an investigation of the determination of these compounds.

In this work, the behaviour of the amino acid arginine and the proteins lysozyme and ovalbumin has been investigated by PT in a DMSO–water mixture (7.0 + 0.8 ml) and by CTT in the solvent mixture DMSO–water–AN (7.0 + 0.8 + 4.0 ml).

Experimental

Reagents

Dimethyl sulphoxide was of laboratory-reagent grade and potassium hydroxide and propan-2-ol were of analytical-reagent grade. The solvents were dried over a molecular sieve 3A before use. The proteins employed were lysozyme, egg-white, from Aldrich (Cat. No. 85387-9, Lot No. 121567) and ovalbumin, from Sigma (Cat. No. A-5503, Lot No. 81F-8235). Potassium hydroxide solution, 0.1 mol dm^{-3} in propan-2-ol, was prepared and standardized against benzoic acid in ethanolic solution by employing phenolphthalein as indicator.

Apparatus

The apparatus employed to introduce the titrant at a constant delivery rate[6] and to detect the temperature change has been described elsewhere.[7]

In potentiometric titrations a glass electrode and a nichrome wire sealed in the syringe and immersed in the titrant were employed as indicator and reference electrode, respectively. The glass electrode was conditioned in the DMSO–water solvent mixture (7.0 + 0.8 v/v) for about 48 h before use. A derivative circuit constructed in this laboratory was used to obtain the first-derivative titration curves.

Procedure

For potentiometric titrations of protonated groups originally present in proteins and arginine, 20.00 mg of protein or 10.00 mg of arginine were weighed into a 50 ml beaker, dissolved in 0.8 ml of water and then 7.0 ml of DMSO were added. In the potentiometric titrations of the mixture of fully protonated proteins or arginine plus a strong acid, the same amounts of protein and arginine were dissolved in 0.8 ml of 0.1 mol dm^{-3} HCl instead of water. For thermometric titrations the same amount of protein or arginine was weighed into a 25 ml unsilvered Dewar flask, dissolved in 0.8 ml of water and then 7.0 ml of DMSO and 4.0 ml of AN were added. In both the potentiometric and thermometric techniques the titrant was added at a constant delivery rate of 0.40 ml min^{-1}. During the titration the solutions were stirred with a magnetic stirrer. The end-point of the thermometric titrations was taken as the point where the titration curve left the horizontal.

Table 1 Results of the titration of protonated groups originally present in arginine, lysozyme and ovalbumin. Relative molecular masses of lysozyme and ovalbumin used in the calculations were 14307 and 42699, respectively

Compound	No. of groups found*		No. of groups found by another technique†	No. of guanidine groups
	PT	CTT		
Arginine	2.01 ± 0.01	1.98 ± 0.03	1	1
Lysozyme	11.4 ± 0.5	23.6 ± 0.7	10.3	11‡
Ovalbumin	52.3 ± 1.5	47.6 ± 0.6	39.1	15§

* ± Standard deviation.
† Titration of denatured protein in 8.0 mol dm^{-3} aqueous urea solution (reference 8).
‡ Reference 9.
§ Reference 10.

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Results and Discussion

Results from the titration of arginine (Table 1) indicate that although the guanidine group is a weak acid (pK_a = 12.5 in water) it can be titrated by both CTT and PT in the medium employed. This observation is expected as DMSO is a protophilic dipolar aprotic solvent and hence it favours the titration of very weak acids.[11]

Thermometric titration curves of arginine, lysozyme and ovalbumin are shown in Fig. 1. The results of the titration of protonated groups originally present in lysozyme and ovalbumin by both PT and CTT are shown in Table 1. The results

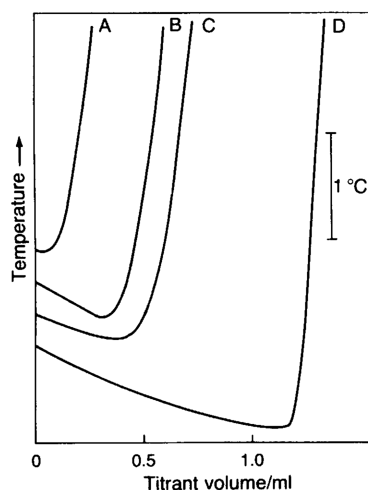

Fig. 1 Catalytic thermometric titration of protonated groups originally present in arginine hydrochloride and in some proteins in a solvent mixture of DMSO–water–AN (7.0 + 0.8 + 4.0 ml): A, blank sample; B, 20.70 mg of ovalbumin; C, 21.11 mg of lysozyme; and D, 12.08 mg of arginine hydrochloride. Titrant, 0.0995 mol dm^{-3} KOH in propan-2-ol

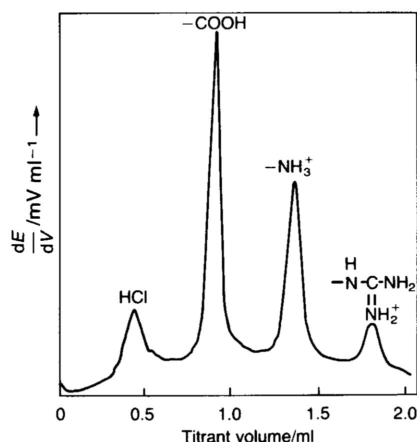

Fig. 2 First derivative of the potentiometric titration curve of arginine plus excess of a strong acid: 9.11 mg of arginine hydrochloride in 0.809 ml of 0.1018 mol dm^{-3} HCl, followed by the addition of 7.0 ml of DMSO. Titrant, 0.0995 mol dm^{-3} KOH in propan-2-ol

are compared with those obtained by the potentiometric titration of these proteins denatured in urea[8] when the guanidine groups are not determined. For lysozyme[9] it can be seen that although the guanidine groups are determined by CTT they are not determined by PT. The observation that the guanidine groups of lysozyme are not determined by PT might be explained by considering that the protein is not completely denatured in this solvent mixture and hence these groups are not accessible for titration. The cysteine groups of lysozyme are not determined because they form interlinking disulphide bridges.

For ovalbumin the guanidine groups are determined by both techniques. However, the number of protonated groups obtained by CTT is about four less than that obtained by PT. This discrepancy is ascribed to the cyanoethylation of the SH groups of cysteine residues during the catalytic titration by analogy with the results obtained for the amino acid cysteine.[4] This explanation is consistent with the fact that four cysteine residues are present per mole of ovalbumin.

Potentiometric Titration of Fully Protonated Arginine and Proteins

The first derivative of the potentiometric titration curve of arginine plus a strong acid is shown in Fig. 2 and the results are presented in Table 2. Four peaks corresponding to the titration of hydrochloric acid, carboxylic, amino, and guanidine groups are observed.

Similarly, first derivatives of the potentiometric titration curves of total protonated groups of proteins in the presence of a strong acid are shown in Fig. 3 and the results are presented in Table 2. By analogy with the results obtained for arginine and other amino acids,[4] the first peak is related to the titration of the strong acid. The second and third peaks are related to the titration of acid and basic groups of proteins, respectively. Groups named as 'acid groups' can be identified as carboxylic groups by comparison with the potentiometric titration curves of arginine and other amino acids.[4] On the other hand, groups named as 'basic groups' might include protonated imidazole, amino, phenolic, SH and guanidine groups. In fact the potentiometric titration curves of proteins do not exhibit discrete inflections corresponding to each of these basic groups.

The number of acid groups, which is related to the number of carboxylic groups, and the total number of groups are shown in Table 2. For lysozyme, 10–11 carboxylic groups are expected but only 8.7 were determined, although ovalbumin has 47 carboxylic groups only 42.2 were determined. It is assumed that because of electrostatic effects[12] the first carboxylic group being titrated has a pK_a sufficiently low for it to be titrated as a strong acid.

By considering the total number of groups titrated for lysozyme it was deduced that the guanidine groups were not determined in this protein. In fact 22.7 groups were determined and the total number of groups present is 21, if the guanidine groups are excluded. This conclusion is in agree-

Table 2 Results obtained by PT for total protonated groups of arginine, lysozyme and ovalbumin

	No. of carboxylic groups		Total No. of groups		No. of Guanidine groups present
Compound	Found*	Present	Found*	Present†	
Arginine	1.01 ± 0.01	1	2.96 ± 0.03	2	1
Lysozyme	8.7 ± 0.8	10–11	22.7 ± 0.8	21–22‡	11
Ovalbumin	42.2 ± 2.2	47	97.0 ± 2.1	88§	15

* ± Standard deviation.
† Not including guanidine groups.
‡ 10–11 Carboxylic, 1 imidazole, 1 α-amino, 6 ε-amino and 3 phenolic groups (reference 9).
§ 47 Carboxylic, 7 imidazole, 20 ε-amino, 10 phenolic and 4 SH groups (reference 10).

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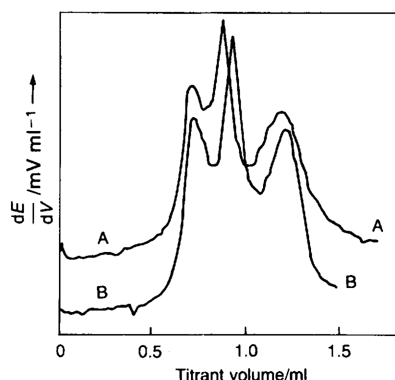

Fig. 3 First derivative of the potentiometric titration curves of lysozyme and ovalbumin plus excess of a strong acid. Protein dissolved in 0.809 ml of 0.1018 mol dm^{-3} HCl, followed by the addition of 7.0 ml of DMSO: A, 25.69 mg of lysozyme; and B, 19.09 mg of ovalbumin. Titrant, 0.0995 mol dm^{-3} KOH in propan-2-ol

ment with the results obtained for the titration of the protonated groups originally present in lysozyme (Table 1).

On the other hand, for ovalbumin a total of 103 protonated groups are present, if the 15 guanidine and 4 SH groups are included. Ninety-seven groups were found in the titration. This fact might be explained by considering, as mentioned above, that 4–5 carboxylic groups are titrated as if they were a strong acid and hence their effect is added to that of the hydrochloric acid. In this way it can be assumed that for ovalbumin the guanidine and SH groups are titrated. This assumption is in agreement with that based on the potentiometric titration of the protonated groups originally present in the protein, as shown in Table 1.

The results presented here offer some interesting possibilities: for example, the determination of cysteine in the presence of other amino acids, or even of cysteine residues in proteins, by comparing the results obtained by PT in DMSO–water with those of CTT in DMSO–water–AN. Similarly, it might be possible to determine the amino acid arginine or arginine residues in proteins by comparing the results obtained by PT in DMSO–water or CTT in DMSO–water–AN with the results of a titration in water where the guanidine groups are not determined. However, these methods needs to be applied to other proteins or to a mixture of amino acids resulting from the hydrolysis of proteins in order to establish their applicability.

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