Pak. j. sci. ind. res., vol. 32, no. 1, January 1989

ROLE OF WATER IN PROTEIN REACTION: VARIATION OF CHARGE ON METHEMOGLOBIN IN WATER AND ETHYLENE GLYCOL MIXTURES

Aladesanmi A. Oshodi

Department of Chemistry, The Federal University of Technology Akure, Nigeria

(Received July 20, 1988)

The net charge on methemoglobin in various water/ethylene glycol mixtures was determined at pH 6. The result showed that there was no significant change in the net charge of the methemoglobin upto 0.122 mole fraction of ethylene glycol in the solvent mixtures. This may be due to the suggestion that the hydrogen – bonding capacity of ethylene glycol and water, with respect to the peptide group, is sufficiently similar so that intrapeptide hydrogen-bonding occurred to about the same extent in the two solvents. Thus the methemoglobin may be predominantly in the aqueous forms. The significant change in net charge as from 0.245 mole fraction of ethylene glycol may be due to possible reversible exposition of an ionisable group that was burried at low concentration of ethylene glycol.

Key words: Charge, Methemoglobin, Water/ethylene glycol.

INTRODUCTION

The stability of the native conformation of proteins in water encourages the study of the solution properties of proteins and other biologically important macromolecules in aqueous media. In some ways, the situation can be compared with the study of simple electrolytes in which aqueous solutions are also important. Interestingly, a considerable knowledge into the properties of simple electrolytes and their aqueous solutions has come from the studies of their nonaqueous solutions [1]. These non aqueous solvents posses useful properties which may be utilised for the study of protein.

Proteins are generally not soluble or have very low solubilities in common non-polar solvents and some polar solvents such as monohydric alcohols and acetone [2] but some are readily soluble in some strongly protic solvents e.g. dichloroacetic acid [3], and some weekly polar solvents e.g. ethylene glycol [4].

The native conformation of a protein was previously believed to be a characteristic property of the macromolecule itself but it is now recognised that the solvent water plays an important role in stabilising the characteristic structure that a protein molecule assumes in an aqueous environment [4]. In this connection, the role of non-aqueous solvents in regulating macromolecular structure of protein has become important.

Monohydric alcohols are found to denature proteins when their volume compositions are more than 20 % in the alcohol – water mixtures and this is attributed to hydrophobic interaction [5]. Whereas, substances such as ethylene glycol, glycerol, sucrose and propylene glycol which have a higher content of hydroxyl groups, and when mixed with water they are less effective denaturing agents than the monohydric alcohols [5-8] and tends to stabilise the proteins [9-13]. In their presence the native conformation of many proteins remains stable even at high concentrations of the solvent.

In aqueous solutions, ion-pair bonds are not important in determining the native conformation of a protein molecule [14] whereas the repulsive interactions can destabilise the native conformation in water if the net charge on the protein becomes sufficiently great as occur at extreme of pH. Different solvents have different dielectric constants and change in dielectric constants can cause change in the net charge on the protein molecule. The dielectric constant of water-ethylene glycol mixtures vary with concentration of ethylene glycol [15], therefore, the present report is the result on the determination of the variation of net charge on methemoglobin in various water/ethylene glycol mixtures.

EXPERIMENTAL

The human methemoglobin A and the water-free ethylene glycol were obtained as described by Anusiom and Oshodi [16]. Methemoglobin solution in various percentages of water-ethylene glycol mixtures was titrated in a glass titration vessel surrounded by an outer jacket. The glass vessel is constructed in such a way that a combined electrode (Radiometer GK 2321C) can be inserted through one of the openings at the top. The two other openings serve as inlet and outlet for carbon dioxide free air, Methe-

moglobin is passed directly from the Dintzis column [17] into the vessel.

In a typical titration 15cm^3 of ethylene glycol is placed in the titration vessel containing an amount of sodium chloride which will finally give 0.05 ionic strength in the final solution. 5cm^3 of carbon dioxide free water was gradually added through a Dintzis column. Then 5cm^3 portion of methemoglobin was passed slowly (one drop in 20 seconds) through the Dintzis column directly into the titration vessel and carbon dioxide-free air obtained by blowing air through a long column packed with self indicating soda lime (10-16 mesh, obtained from British Drug House) was passed continuously into the vessel. The resulting methemoglobin solution was stirred mangnetically, but gently to prevent denaturation. The pH of the methemoglobin solution in the vessel was taken and this is noted as the isoionic point [18]. 5cm^3 of the solution was pipetted out for determination of concentration of the methemoglobin in the solution using the method of Okonjo [18]. The pH was raised to about 9 by adding some NaOH solution. 0.01 cm^3 portion of hydrochloric acid of known molarity was added by means of an Agla microsyringe, and stirred gently for about 20 seconds and stopped. After 2 minutes the pH of the solution was read. This process was repeated until the pH drops to about 5.60. The methemoglobin was titrated back following the same procedure, but this time adding 0.01cm^3 portion of sodium hydroxide of about the same molarity as the hydrochloric acid. All pH readings were taken with Radio-meter pH meter, model PHM 4d. The pH meter was standardised using two different standard buffers. All experiments were carried out at 20^o.

The net charge on methemoglobin in various percentages of ethylene glycol at pH 6 was carried out as follows [18]. The isoionic point of the methemoglobin in a mixture containing 20 % ethylene glycol by volume is determined to be 7.50. During the experiment, the volume of acid required to change the pH from 6.35 to 5.73 was found to be 0.03cm^3. Therefore the volume of acid required to titrate the protein from the isoionic point to pH 6.00 is;

$$\frac{7.50 - 6.00}{6.35 - 5.73} \times 0.03 = 0.0726\text{cm}^3$$

The concentration of the acid used was 0.0989M, the number of moles of acid required to shift from the isoionic point to pH 6.00 is;

$$\frac{0.0726 \times 0.0989}{1000} = 7.180 \times 10^{-6} \text{ mole}$$

The concentration of the methemoglobin was 8.6798×10^{-5} mole Fe dm^{-3} hence in 20 cm^3, the quality of methemoglobin from to be;

$$8.6789 \times \frac{20}{1000} \times 10^{-5}$$

$$= 1.7358 \times 10^{-6} \text{ mole Fe dm}^{-3}$$

The charge per monomer of the methemoglobin is given by

$$\frac{\text{No of mole of acid}}{\text{No of mole of iron}} = \frac{7.180 \times 10^{-6}}{1.7358 \times 10^{-6}}$$

$$= +4.136$$

the charge per tetramer is four times this amount i.e.

$$Z_{Hb} = 4 \times (+4.136)$$
$$= +16.55$$

The results are shown in Table 1:

RESULTS AND DISCUSSION

Table 1 shows the variation of net charge (Z_{Hb}) on methemoglobin as a function of mole fraction of ethylene glycol – at pH 6. AT this pH in aqueous solution, the value obtained for Z_{Hb} in the present work is + 16.96 while Beetle-stone and Irvin [19] reported a value of + 17.20. Table 1 further shows that at a mole fraction of 0.245 of ethylene glycol, the net charge on the methemoglobin shows a significant increase from the + 16.96 in aqueous solution to + 18.26 and + 20.98 at 0.744 mole fraction of ethylene glycol.

Table 1. Variation of charge on methemoglobin as a function of ethylene glycol concentration at pH 6.

Mole fraction of ethylene glycol	Net charge (Z_{Hb})	Dielectric constant
0.000	16.96	80.35
0.035	16.76	75.60
0.075	16.89	72.80
0.122	17.04	69.80
0.178	17.52	66.60
0.245	18.26	63.20
0.327	19.82	59.40
0.430	20.26	54.70
0.564	20.96	49.30
0.744	20.98	43.70

Dielectric constant values are from ref. 15.

Figure 1 is a plot of net charge (Z_{Hb}) at pH 6 versus dielectric constant of ethylene glycol/water mixtures which gives a sigmoid curve. This figure shows that at low concentration of ethylene glycol up to about 0.122 mole fraction, there is no significant change in net charge on the methemoglobin compared with its value in aqueous solution. This may be due to the suggestion that the hydrogen-bonding capacity of ethylene glycol and water, with respect to the peptide group, is sufficiently similar so that intrapeptide hydrogen bonding occurs to about the same extent in the two solvents [6,20]. It is also known that usually no significant conformational change is observed for most proteins in water-ethylene glycol mixtures until the mole fraction is about 0.245 [6,8]. Thus, the insignificant change in net charge on methemoglobin in the solvent mixtures up to 0.122 mole fraction of ethylene

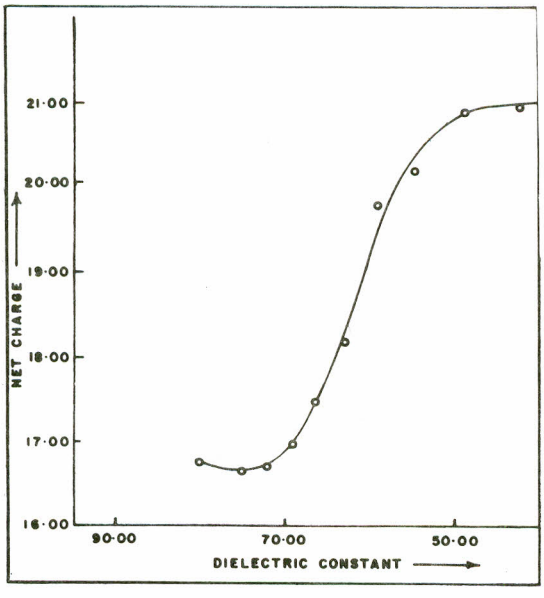

Fig. 1. Plot of dielectric constant of water/ethylene glycol mixtures vs. net charge on methemoglobin in the mixtures.

glycol may be because the methemoglobin is predominantly in the aqueous form [16] and there is no significant conformational change in this concentration range of ethylene glycol [16,18]. The small but significant change in net charge as from 0.245 mole fraction of ethylene glycol with decrease in dielectric constant may be due to possible partial exposition of an ionisable group that was burried at low concentration of ethylene glycol but exposed at high concentration of ethylene glycol i.e. at low dielectric constant. Such exposition is a reversible one since an exhsustive dialysis of sample of methemoglobin in ethylene glycol with buffer yields a sample which shows spectra characteristics identical to those in aqueous solution [16,21]. Optical rotatory dispersion and circular dichroism measurements on methemoglobin samples in ethylene glycol showed exactly the same characteristics as in aqueous solution [16], indicating that there may be no gross unfolding of the molecule in the presence of ethylene glycol. This kind of partial but reversible exposition has been observed in the titration of pancreatic ribonulease in ehtylene glycol/water mixtures studied by sage and singer [4]. It is also possible that a new helical region might be formed in high concentration of ethylene glycol which is reversible to its native form in aqueous system. Thus during the partial formation of the equivalent helical regions, a chargeable group may be exposed or partially exposed as suggested by singer [2], sage and singer [4]. A more plausible explanation could be due to the changes in the extent of hydrogen bonding that would occur when ethylene glycol replaces water in the hydration structure of the protein, However, a combination of these two effects may not be unequivocally ruled out.

The sigmoid shape of Fig. 1 further supports the earlier suggestion that in presence of ethylene glycol, methemoglobin exists in two forms [16,21] the 'aquo-form' which is predominant at low concentration of ethylene glycol and the 'imidazole-form' which is predominant at high concentration of ethylene glycol and that the two forms are in equilibrium. These two forms of methemoglobin, are known to exist in equilibrium even in water [22,23]. The present report further shows that the imidazole-form which is believed to be predominant at high concentration of ethylene glycol [16,21] may be more positively charged than the aquo-form at pH 6.

REFERENCES

1. E.C. Evers and R.L. Kay, Ann. Rev. Phy. Chem. **11**, 21 (1960).
2. S.J. Singer, Advances in Protein Chemistry, **17**, 1 (1962).
3. J.Y. Yang and P. Doty, J. Am. Chem. Soc., **79**, 761 (1957).
4. H.T. Sage and S.J. Singer, Biochemistry., **1**, 305 (1962).
5. F. Franks and D.J.G. Ives, Qt. Rev., **20**, 1 (1966).
6. C. Tanford, C.E. Bunckley, P.K. De and E.P. Lively, J. Bid. Chem., **237**, 1168 (1962).
7. C. Tanford, *Advances in Protein Chemistry*, **23**, 121 (1968).
8. Y. Nozaki and C. Tanford, J. Biol. Chem., **240**, 3568 (1965).
9. S.Y. Gerslma, J. Biol. Chem., **243**, 957 (1968).
10. I.I. Herskovits, B. Gadegbeku and H. Jaillet, J. Biol. Chem., **245**, 2588 (1970).

11. S.Y. Gerslma and E.R. Stuur, Int. J. Peptide. Protein Res., **4**, 377 (1972).
12. J.S. Myers and W.B. Jakoby, Biochem. Biophy. Res. Commun., **51**, 631 (1973).
13. P. Maurel, G.H.B. Hoa and P. Douzou, J. Biol. Chem., **250**, 1376 (1975).
14. C.F. Jacobsen and K. Linderstom-Lang, Nature, **164**, 411 (1949).
15. H.S. Harn and B.B. Owen, *Physical Chemistry of electrolyte Solutions*, (Reinhold, New York, 1958), 3rd ed.
16. A.C.I Anusiem and A.A. Oshodi, Arch. Biochem. Biophy., **189**, 392 (1978).
17. H.M. Dintzis, Ph.D. Thesis, Havard Univ. Cambridge, Mass (1952).
18. K. Okonjo, Ph.D. Thesis, Univ. Ibadan, Nigeria (1972).
19. J.G. Beetlestone and D.H. Irvine, J. Chem. Soc., **(A)**, 951 (1968).
20. A. Ben-Naim, J. Phy. Chem., **68**, 3245 (1965).
21. A.C. Anusien and A.A. Oshodi, J. West Africa. Sc. Ass. (In press).
22. A.C.I. Anusien, J.G. Beetlestone, J.B. Kushimi and A.A. Oshodi, Arch. Biochem. Biophy., **175**, 138 (1976).
23. A. Levy and I.M. Rifkind, Biochemistry, **24**, 6050 (1985).