Studies of the Hydration and the Structure of Water and Their Roles in Protein Structure. IV. The Hydration of Amino Acids and Oligopeptides[1]

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To what degree is hydration formed around the molecules of proteins and nucleic acids? How does the hydration contribute to the maintenance of their native structure? What structure may be considered for the hydration?

It seems that solutions to these problems are essential for an understanding of the behavior of biological macromolecules in aqueous solutions. Nevertheless, we have as yet little information about such problems. However, since the molecules of proteins or nucleic acids are hydrated, it is natural to consider that the structure of the solvent water affects the state of the hydration around the macromolecules and, correspondingly, affects the configurations to a great extent. The possibility that the structure of the solvent water and the structure and degree of hydration around protein molecules play important roles in maintaining the native structure can be inferred from a study of the denaturation processes. Facts which suggest the dependence of the configuration of proteins or nucleic acids on the structure of the solvent water have been accumulated recently.[2-4]

In the preceding papers of this series[6-7] we reported on the states of water in aqueous electrolyte solutions and proposed that there are clathrates around various ions. The present paper will report on the numbers of hydration for various amino acids, oligopeptides and saccharides, as measured by the method of ultrasonic interferometry.

Experimental

The methods used in this study have been described in a previous paper.[6] The amino acids and oligopeptides used were supplied by the Peptide Center of the Institute for Protein Research, Osaka University.

Results and Discussion

The following equation, described in a preceding paper[6] as Eq. 2, was used for the determination of the number of hydration:

$$\frac{\beta_0-\beta}{c}-\bar{v}\beta_0=-\bar{v}\beta_1+\frac{v_2}{c}(\beta_0-\beta_2)\equiv K$$

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1) Presented at the 15th Annual Meeting of the Chemical Society of Japan, Kyoto, April, 1962.

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2) I. M. Klotz, *Science*, **128**, 815 (1958).

3) G. Nemethy and H. A. Scheraga, *J. Phys. Chem.*, **66**, 1773 (1962).

4) K. Hamaguchi and E. P. Geiduschek, *J. Am. Chem. Soc.*, **84**, 1329 (1962).

5) S. Goto, This Bulletin, **37**, (1964).

6) T. Isemura and S. Goto, ibid., **37**, 1690 (1964).

7) S. Goto and T. Isemura, ibid., **37**, 1693 (1964).

where β, β_1, β_0 and β_2 are the compressibilities of the solution, solute, solvent and hydrated water respectively; v, the partial specific volume of the solute; c, the weight concentration of the solute, and v_2, the volume fraction of the hydrated water. From the results reported by Shiio,[8,9] it can be assumed that the compressibilities of the solutes, amino acids and saccharides are zero. Therefore, as in the previous study, for the purpose of comparing various amino acids, oligopeptides and saccharides we temporarily assumed that the compressibilities of both solute and hydrated water were zero and determined the numbers of hydration on the basis of this assumption.

As has been described in the preceding paper, in aqueous 1-1 electrolyte solutions the numbers of hydration of the electrolytes differ from each other at any given temperature, but decrease in parallel with an increase in the temperature. As may be seen from Fig. 1, the number of hydration of glycine decreases in parallel with those of 1-1 electrolytes with an increase in the temperature. This suggests that both the cation and the anion of the amino acid resemble those of strong electrolytes in the mechanism of hydration and that the hydration of the ions is strong and depends only slightly on the temperature.

From Fig. 2 it is clear, from a comparison of the numbers of hydration of aspartic acid and asparagine or glutamic acid and glutamine, that the dependence on the temperature of the acid type is greater than that of the amide type. This finding is similar to that seen in the comparison of 1-1 and 1-2 electrolytes described in a preceding paper. This also suggests that the state of hydration of the amide group is different from that of ionic groups. On the other hand, histidine, a basic amino acid, in an aqueous solution has a number of hydration which is larger, but its dependence

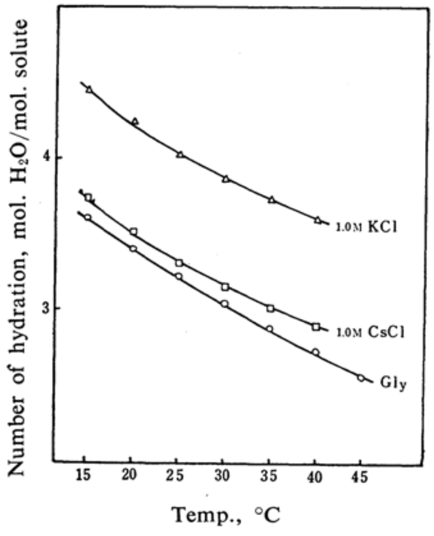

Fig. 1. Numbers of hydration and their temperature dependence.

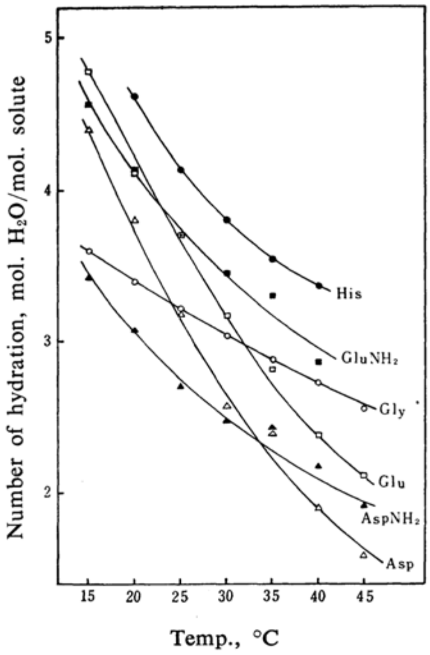

Fig. 2. Numbers of hydration and their temperature dependence.

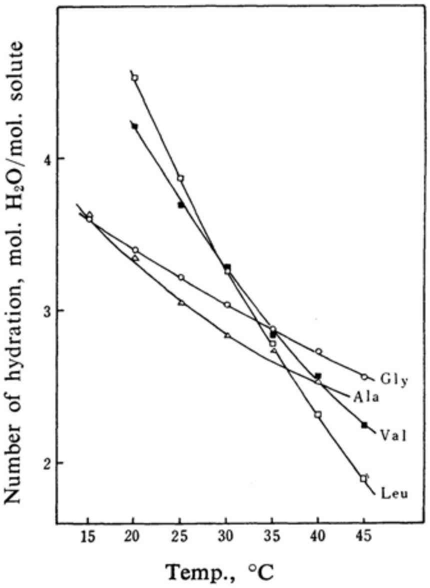

Fig. 3. Numbers of hydration and their temperature dependence.

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[8] H. Shiio, *J. Am. Chem. Soc.*, **80**, 70 (1958).

[9] K. Ito and H. Shiio, This Bulletin, **30**, 745 (1957).

on the temperature is less than those of acidic amino acids.

Figure 3 shows the numbers of hydration of some aliphatic amino acids and their temperature dependence. At lower temperatures the numbers of hydration increase with an increase in the hydrophobic side chains, as may be seen by a comparison of the numbers of hydration for alanine, valine and leucine. This apparently indicates that there is hydration around the non-polar side chain. Frank and Evans[10] suggested the existence of icebergs around non-polar substances judging from the thermodynamic properties of their solutions. Also, from the results of X-ray studies, Jeffrey et al.[11-13] proposed that the structure of water around tetraalkylammonium salts consists of molecules of water mutually bound by hydrogen bonds and forming clathrates. From the data given in Table I, it will be seen that at 20°C the difference in the numbers of the hydration water of alanine and valine is about 1 mol. H_2O/mol. solute. Their numbers were determined assuming that the compressibility of the hydrated water was zero. However, as was mentioned in the preceding paper, compressibility of the water of this hydration clathrate may not be negligible. Therefore, it may be that the number of molecules of the water of hydration around non-polar side chains is quite large.

Another point of interest is that the hydration of the hydrophobic side chains of amino acids depends greatly on the temperature, although the hydration of strong electrolytes and the ionic sites of amino acids depends only slightly on the temperature, as has been mentioned above. As may be seen from Table I, the difference in the number of hydration water at 20°C and at 40°C increases in the following order: glycine, alanine, valine and leucine. This phenomenon is to be regarded as additional evidence for the hypotheses that the icebergs melt with an increase in the temperature or that the structure of the clathrates is broken down at higher temperatures.[10,14,15]

Similarly, as may be seen from Fig. 4, the hydration of the side chain of methionine, containing a sulfur atom, is similar to that of the aliphatic hydrocarbon chain in such an amino acid as valine.

TABLE I. NUMBERS OF HYDRATION OF VARIOUS COMPOUNDS AND THEIR TEMPERATURE DEPENDENCE

The numbers were determined assuming the compressibilities of both the solute and the hydrated water to be zero

Solute (2%)	Number of hydration (mol. H_2O/mol. solute) 20°C	40°C	Difference in numbers at 20 and 40°C
Gly	3.40	2.73	0.67
Ala	3.35	2.53	0.82
Val	4.22	2.56	1.66
Leu	4.53	2.31	2.22
Ser	4.04	3.14	0.90
Thr	4.06	2.93	1.13
Met	4.53	2.60	1.93
Asp[a]	3.80	1.90	1.90
AspNH$_2$	3.07	2.17	0.90
Glu[b]	4.11	2.38	1.73
GluNH$_2$	4.13	2.86	1.27
His	4.62	3.37	1.25
Phe	4.48	2.10	2.38
Pro	3.26	1.85	1.41
(Gly)$_2$	4.70	3.70	1.00
(Gly)$_3$	5.66	4.22	1.44
(Gly)$_4$[c]	6.38	4.79	1.59
Sucrose	3.42	1.60	1.82
Mannite	2.39	1.33	1.06

a) 0.50%
b) 0.76%
c) 0.95%

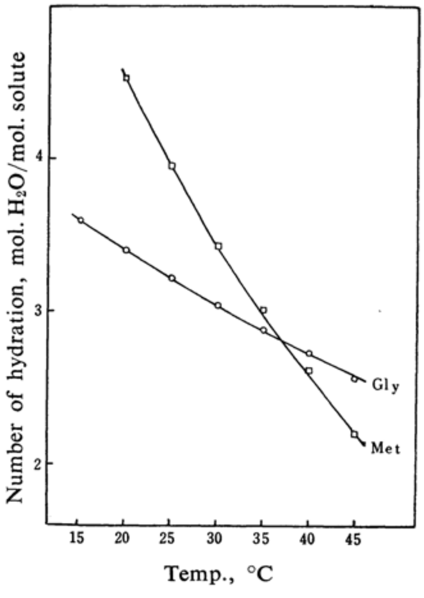

Fig. 4. Numbers of hydration and their temperature dependence.

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10) H. S. Frank and M. W. Evans, *J. Chem. Phys.*, **13**, 507 (1945).

11) D. Feil and G. A. Jeffrey, ibid., **35**, 1863 (1961).

12) M. Bonamico, G. A. Jeffrey and R. K. McMullan, ibid., **37**, 2219 (1962).

13) G. A. Jeffrey and R. K. McMullan, ibid., **37**, 2231 (1962).

14) H. S. Frank and W. Y. Wen, *Discussions Faraday Soc.*, **24**, 133 (1957).

15) F. S. Feates and D. J. G. Ives, *J. Chem. Soc.*, **1956**, 2798.

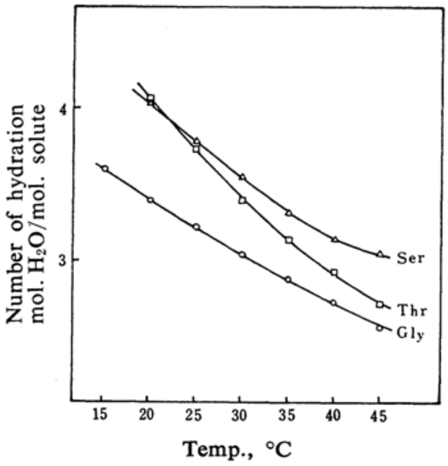

Fig. 5. Numbers of hydration and their temperature dependence.

On the other hand, as may be seen from Fig. 5 and Table I, the side chains of serine and threonine, in which there is a hydroxyl group, cause a slight increase in the numbers of hydration, but the numbers for serine and threonine decrease in parallel with that of glycine with an increase in the temperature. This suggests that the hydration of hydrophilic groups, such as the hydroxyl group, is comparatively small and acts competitively with that of hydrophobic groups, and that the former is stronger. The evidence for these conclusions is given in Fig. 6. The hydration of the hydroxyl group is suggested more strongly from the results for sucrose and mannite. These saccharides have several hydroxyl radicals, but their numbers of hydration are comparatively small. Also, Table I indicates that the number of hydration of sucrose decreases greatly with an increase in the temperature.

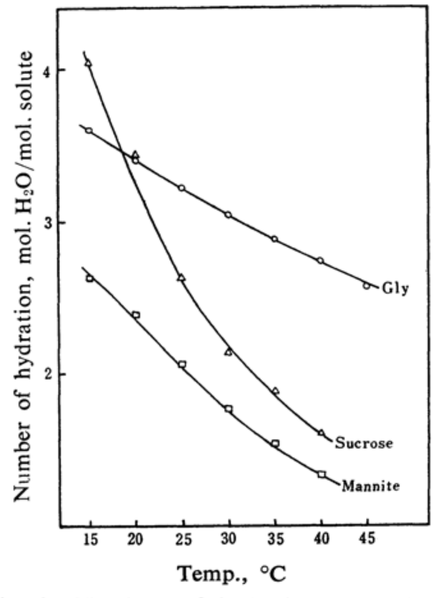

Fig. 6. Numbers of hydration and their temperature dependence.

The numbers of the hydration of proline, a cyclic imino acid, and phenylalanine, containing a benzene ring, are shown in Fig. 7. As may be seen from this figure, the hydration of phenylalanine is very similar to that of leucine. This indicates that the phenyl radical is also hydrated. On the other hand, the hydration of proline is nearly the same as that of glycine, but at a higher temperature it is nearly the same as that of leucine and phenylalanine. From the results for proline and sucrose, it seems that the hydration of cyclic compounds is very dependent on the temperature.

As may be seen from Fig. 7, the numbers of the hydration of leucine and phenylalanine at higher temperatures are much smaller than that of glycine. It can be supposed that the hydration of hydrophobic regions is not only broken at higher temperatures, but that it also affects the hydration of ionic sites, giving mutual compensation.

The hydration of peptide bonds was also investigated. Pauling[16] reported that there is no adsorption of water on the peptide bond.

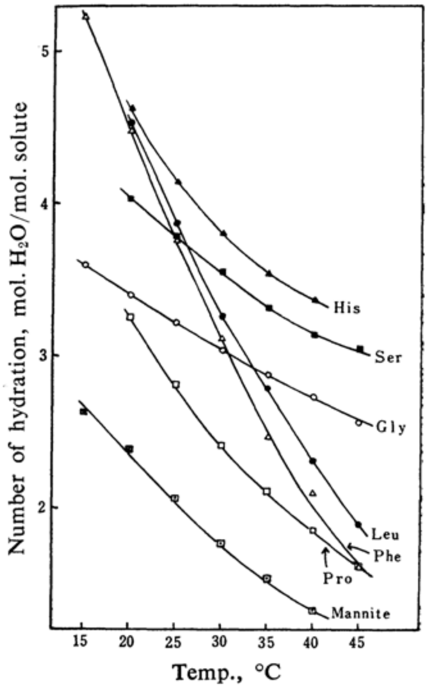

Fig. 7. Numbers of hydration and their temperature dependence.

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[16] L. Pauling, *J. Am. Chem. Soc.*, **67**, 555 (1945).

On the other hand, Mellon et al.[17] reported the hydration of peptide bonds. As may be seen from Fig. 8, the results for glycine, diglycine, triglycine and tetraglycine indicate that the peptide bond is hydrated. The stoichiometry of their numbers of hydration and their temperature dependence are good. Therefore the hydration of the peptide bond is probably like that of hydrophilic groups.

In conclusion, the hydration of various amino acids and the effect of various conditions on these hydrations differ. However, the hydration of amino acids can be classified roughly into the following three types: the hydration of (1) ionic sites, (2) hydrophilic sites, and (3) hydrophobic sites. Further, the hydrations of the hydrophobic sites of various amino acids differ in number and in temperature dependence. These properties may effect various physicochemical properties of proteins, such as their secondary and tertiary structures, the stability of their hydrophobic bonds, and the processes of their denaturation. Further studies of these problems will be reported in a following paper.

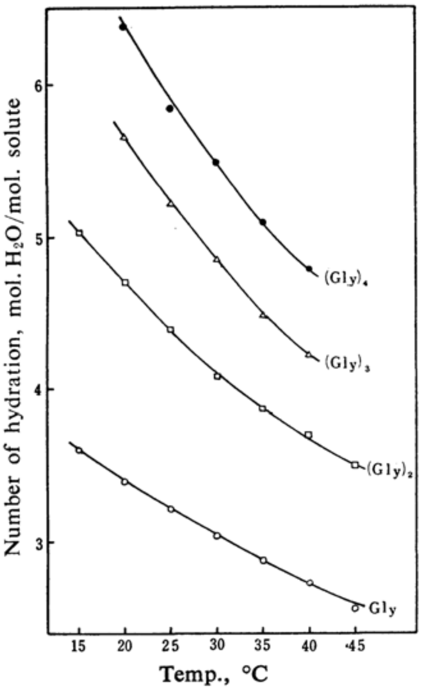

Fig. 8. Numbers of hydration and their temperature dependence.

It is very interesting that the results obtained by Whitney and Tanford[18] on the solubilities of amino acids in aqueous urea solutions agree with our results on the temperature dependence of the hydrations of amino acids. Therefore, the solubility of various compound must depend on their states and numbers of hydration.

Summary

The numbers of hydration of various amino acids, oligopeptides and saccharides in aqueous solutions and their dependence on the temperature have been determined by the method of ultrasonic interferometry. The hydration on ionic, hydrophilic and hydrophobic sites has been discussed. Particularly, evidence for the hydration around hydrophobic groups has been obtained. Also, we have established the hydration of the peptide bond.

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[17] E. F. Mellon, A. H. Korn, E. L. Kokes and S. R. Hoover, ibid., 73, 1870 (1951).

[18] P. L. Whitney and C. Tanford, *J. Biol. Chem.*, 237, PC1735 (1962).