On the Molecular State of Water Bound to the Peptide Linkage

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According to A. M. Buswell *et al*(1) and K. Kuratani(2) the water molecules bound to gelatin and other proteins cause infrared absorption bands which range from 2.85 μ to 3.03 μ. As Sponsler, Bath, and Ellis(3) have pointed out, these water molecules must be of

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(1) A. M. Buswell, K. Krebs and W. H. Rodebush, *J. Am. Chem. Soc.*, **59**, 2603 (1937); *J. Phys. Chem.*, **44**, 1126 (1940).

(2) K. Kuratani, *Raports of the Radiation Chemistry Research Institute* (*Tokyo Univ.*), Vol. III, 14 (1949).

(3) O. L. Sponsler, J. D. Bath and J. W. Ellis, *J. Phys. Chem.*, **44**, 996 (1940).

two kinds—(1) those bound to the peptide linkages in the backbone of protein and (2) those bound to the polar groups in the side chains. In order to obtain information about the molecular state of water bound exclusively to the peptide linkage, the present writer made 3μ region infrared absorption measurements of a film of "amilan" (Poly ε-capramide),(4)

$$\ldots.—(CH_2)_5—CONH—(CH_2)_5—CONH—\ldots,$$

in dry as well as in wet state, and found that there exists a band at about $2.86\,\mu$ which is attributable to the water bound to the peptide linkage in this compound. The result obtained and its interpretation are given in this paper.

The dried specimen of amilan used here was prepared by keeping an amilan film for two weeks in a sealed tube specially made of silica glass with plane-parallel windows as shown in Fig. 1, which contained concentrated H_2SO_4 as is illustrated.(5) The dried film within the tube was subjected to the spectroscopic measurement without being taken out, by passing the infrared radiations through the film between the plane parallel windows. The result of this measurement is shown graphically by curve A in Fig. 2, where the infrared absorptions due to the silica glass plates of the windows have been eliminated. Curve B in Fig. 2 is obtained on the same amilan film, measured after it was taken out from the tube and exposed to the air of 76% relative humidity at 11°C. (*i. e.* 0.01 atmospheric pressure of water vapor). According to the private communication of Mr. H. Yumoto, amilan contains in the air of this humidity about 0.3 mole of H_2O per $-(CH_2)_5-CONH-$ unit (=113 g.) in equilibrium. As is seen in the figure, deviation of the absorption spectrum of wet amilan from that of dry amilan is conspicuous in 2.8-2.9μ region. Curve C in Fig. 2 represents the absorption spectrum due to the water bound to amilan as obtained by subtraction of the data of curves A from B (B—A). It shows an absorption peak at about 2.86μ, which may be associated to the O—H stretching vibrations in the bound-water molecules. It is noteworthy that, while bound-water of gelatin and other proteins always shows absorptions not only in 2.85-2.90μ region but also in 2.90-3.03μ region, the amilan-bound-water shows an absorption only at 2.86μ and not in the 2.90-3.03μ region. This fact may be taken as indicating that the water molecules which cause the absorption in the 2.90-3.03μ region are those bound to side chains of the proteins, and those bound to the peptide linkages cause the absorption at about 2.86μ.

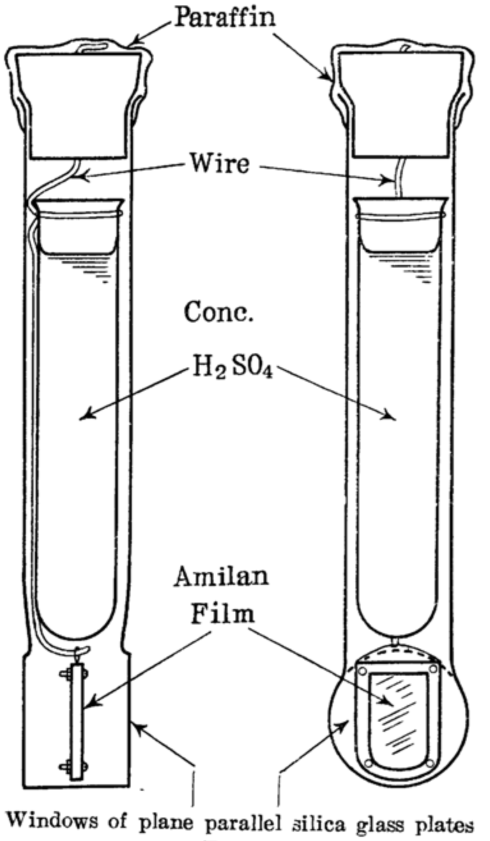

Fig. 1.

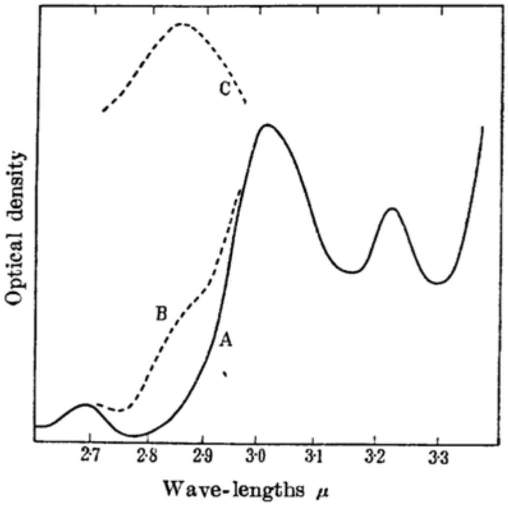

Fig. 2.—Infrared absorption curves for (A) conc. H_2SO_4-dried amilan film, (B) amilan film under water vapor of 0.01 atm., and (C) water bound to amilan.

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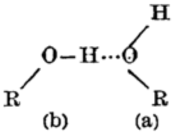
(4) Material of synthetic fibers made by the Toyo Rayon Co.

(5) The writer owes to Mr. H. Yumoto's suggestion in devising this drying apparatus.

Table 1

Molecules and their states	Number of perturbing hydrogen bonds versus maximum possible number of perturbing hydrogen bonds	Positions of O−H bands μ	Observers
H_2O in CCl_4	0/4	2.70	Fox and Martin(7)
in amilan	2 ? /4	2.86	Tsuboi
pure water	4/4	2.95	Fox and Martin(7)
ice	4/4	3.08	Fox and Martin(7)
gelatin		2.85—3.03	Buswell, Krebs, and Rodebush(1); Kuratani(2)
ROH in CCl_4 (dil.)	0/2	2.74	Fox and Martin(5) Smith and Creitz(9)
in 	1/2	(a) 2.76	Fox and Martin(8) Smith and Creitz(9)
		(b) 2.86	Smith and Creitz(9)
$ROH\cdots O(CH_3)C_6H_5$	1/2	2.80	Tsuboi(10)
$ROH\cdots O(C_2H_5)_2$	1/2	2.85	Tsuboi(10)
pure liquid	2/2	2.95	Smith and Creitz(9), and others
pyridine	1/2	3.00	Errera and Sack(11)

It is also noticed that the O−H band of amilan-bound-water or peptide-bound-water is situated at a wave-length much shorter than the O−H band of pure water (at 2.95μ). This indicates that the water molecule in peptide linkage is in a less distorted state than the water molecule in pure water.

Further details of the molecular state of the peptide-bound-water may be conjectured from the position of its O−H band in the following way.

The positions of the O−H bands of H_2O as well as of other hydroxy molecules (ROH) depend upon how far O−H vibrations in these molecules are perturbed through hydrogen bondings by other molecules. Each of these molecules has two possible types of perturbing hydrogen bonds—*i. e.* (I) hydrogen bonds through the hydrogens of the molecule under consideration and (II) hydrogen bonds through

(type I) O—H....X
X−H....O−H (type II)

the hydrogens of molecules other than the one under consideration.(6) In Table 1 are listed the positions of some of the O−H bands known for water and alcohol molecules perturbed in various ways. As may be seen from the table, the number of the perturbing hydrogen bonds of each type is a factor determining the degree of perturbation; and the strength of the proton-accepting or proton-donating powers of molecules with which the molecule under consideration is hydrogen-bonded is another factor. Thus, an alcohol molecule perturbed through the single hydrogen bond of type II by another alcohol molecule gives an O−H band at 2.76μ, and that perturbed through the single hydrogen bond of type I by another alcohol molecule (or ether molecule) gives an O−H band at about 2.86μ. While, an alcohol molecule perturbed through hydrogen bonds of both types (I and II) by other alcohol molecules gives an O−H band at 2.95μ. The carbonyl O in the peptide linkage is considered to be a stronger proton-acceptor than the ether or hydroxy O, because there is the electron migration from NH group towards the carbonyl group in the peptide linkage. It is expected, therefore, that the hydrogen bond O−H....O between the peptide C=O and the alcohol molecule, if it exists, should give O−H band at a longer wave-length than 2.86μ (Let its position be $2.86+\alpha\mu$).

Since the perturbation through the hydrogen bond of type II is weak as shown above, it is unlikely that the N−H....O bonding between the peptide N−H group and the bound-water molecule, which is one of the possible perturbing hydrogen bonds, is responsible by

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(6) P. C. Cross, J. Burnham and P. A. Leighton, *J. Am. Chem. Soc.*, **59**, 1134 (1937).

(7) J. J. Fox and A. F. Martin, *Proc. Roy. Soc.* (*London*), **A 174**, 234 (1940).

(8) J. J. Fox and A. F. Martin, *Trans. Faraday Soc.*, **36**, 897 (1940).

(9) F. A. Smith and E. C. Creitz, *J. Resear. Nat. Bur. Stand.*, **46**, 145 (1950).

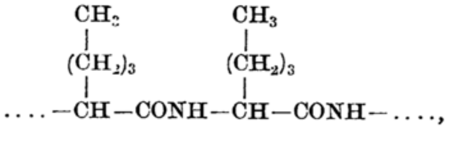
(10) M. Tsuboi, This Bulletin **25**, 60 (1952).

(11) J. Errera and H. Sack, *Trans. Faraday Soc.*, **34**, 728 (1938).

itself for shifting the water band from 2.70μ (due to free H_2O) to 2.86μ. On the other hand, simultaneous perturbations of two types (I and II) as those in pure water may be ruled out for the bound-water molecule, because the O—H band under consideration is situated at a much shorter wave-length than that of pure water. The simple perturbation through hydrogen bonds of type I is the probable one for the present case. Calculations of the vibration frequencies of H_2O molecule(12) show that, if one of the two O—H's of a water molecule is perturbed through the hydrogen bond of type I by a peptide oxygen atom and the other O—H is left free, the molecule will give two O—H bands at about 2.7μ and at $2.86+\alpha\mu$, while, if both of its two O—H's are perturbed by peptide oxygen atoms, it will give two O—H bands, at a little longer wave-length than $2.86+\alpha\mu$ (corresponding to the symmetric stretching vibration) and at a little shorter wave-length than $2.86+\alpha\mu$ (corresponding to the antisymmetric stretching vibration). In the case now at issue no band attributable to the peptide-bound-water has been observed in the vicinity of 2.7μ,(13) showing that the bound-water molecule has no free O—H. Then, a view may be set forth here tentatively that the peptide-bound-water molecule has both of the two O—H's perturbed through O—H....O bondings by two peptide oxygen atoms (located near to each other),(14) and that the 2.86μ band corresponds to the antisymmetric vibration of that water molecule. The band due to the symmetric vibration, which, according to the above view, should appear at a little longer wave-length than $2.86+\alpha\mu$ has not been observed. This, however, may not necessarily be taken as vitiating the above view, for it is conceivable that this band is too weak to be observed, the dipole-moment-oscillation due to the symmetric vibration being smaller in amplitude than that due to the antisymmetric vibration.(15)

The bound-water band in the 2.90-3.03μ region observed on proteins may be attributed either to water molecules bound by much stronger proton-acceptor groups in the side chains such as ionized carbonyl groups, or to water molecules forming an aggregate around the polar groups just as those in liquid water.

A few words may be added here concerning the bound-water band at 2.86μ. In the writer's previous measurements of the 3μ region infrared absorption on egg albumin and horse serum albumin,(16) remarkable peaks at about 2.86μ were observed. This fact suggests that the water molecule bound to the carbonyl O in the backbone in the manner shown above are abundant in these proteins. On the other hand, the present writer's recent study on a film of poly-D, L-norleucine(17) (an isomer of amilan),



$$\begin{array}{cccc} & CH_3 & & CH_3 \\ & | & & | \\ & (CH_2)_3 & & (CH_2)_3 \\ & | & & | \\ \cdots\cdots & -CH- & CONH & -CH-CONH-\cdots\cdots, \end{array}$$

indicated that the film shows no absorption band at 2.86μ in the air of equal humidity as the air in which the measurement corresponding to the curve B for amilan (Fig. 2) was made. The crowded hydrophobic side chains ($-CH_2-CH_2-CH_2-CH_3$) seem to prevent the water molecule from reaching the backbone.

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(12) In order to get knowledge about the coupling between the vibrations of two O-H's in a water molecule, the normal vibration frequencies of the water molecule were calculated, assuming the potential energy (V) of the molecule as,

$$V=\frac{1}{2}f_1(\Delta r_1)^2+\frac{1}{2}f_2(\Delta r_2)^2+\frac{1}{2}f_3(r_0\Delta\alpha)^2,$$

where r_1 and r_2 are the bond lengths of the two O-H's, r_0 the equilibrium O-H distance, α the angle HOH, and f_1 etc. the force constants. If we put $f_1=f_2=7.54655\times10^5$ dyne/cm, $f_3-0.70195\times10^5$ dyne/cm; we obtain three normal vibration frequencies: 3702, 3648, and 1600 cm^{-1}, which are close to the frequencies observed for water vapor (3750, 3650, and 1595 cm^{-1}). Fig. 3 and 4 show the two O-H stretching frequencies of H_2O molecule, as calculated by assuming that $f_1=f$ (variable), $f_2=7.54655\times10^5$ dyne/cm., $f_3=0.70195\times10^5$ dyne/cm. (Fig. 3); and that f_1-f_2-f (variable), $f_3=0.70195\times10^5$ dyne/cm. (Fig. 4). The dotted lines in the figures indicate the frequencies calculated for the case when the two O-H's vibrate independently of each other.

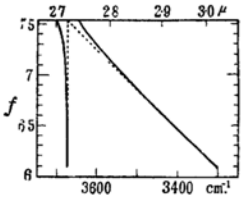

Fig. 3.

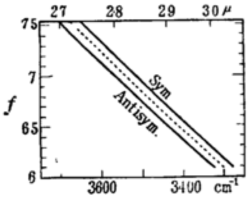

Fig. 4.

(13) Amilan shows a weak band at 2.7μ, but its intensity remains unchanged even when amilan is dried.

(14) The heat of adsorption of water into amilan from liquid water is, according to K. Hoshino and H. Yumoto (*J. Chem. Soc. Japan*, **70**, 104 (1949)), about -1 kcal/mol. From this the heat of adsorption from water vapor is calculated to be about -11 kcal/mol. This value is just as may be expected from the molecular state of the bound water here assumed.

(15) For instance, the symmetric O-H stretching vibration of H_2O gives much weaker infrared absorption band than the antisymmetric one in the CCl_4 solution (see reference (7)).

(16) M. Tsuboi, This Bulletin **22**, 255 (1949).

(17) This material was kindly placed by Dr. H. Tani (Osaka Univ.) at the writer's disposal.

In conclusion the writer wishes to express his sincere thanks to Professor San-ichiro Mizushima and Dr. Takehiko Shimanouchi for their kind guidance in this work, and to Mr. Hirosuke Yumoto for his valuable suggestions and for his placing the amilan film at the writer's disposal. The cost of this research has been partly defrayed from a Grant in Aid for Scientific Research from the Ministry of Education.

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