[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY, NO. 1000]

The Adsorption of Water by Proteins

BY LINUS PAULING

Recent investigators of the adsorption of water by proteins[1,2] have applied the Brunauer–Emmett–Teller theory[3] of adsorption of gases in multimolecular layers to their data, and have compared the area covered by the water molecules adsorbed in the first layer, as found by application of this theory, with the total area of the protein when spread into a film about 10 Å. thick. The ratio of these areas varies from about 1:2.5 to 1:8 for different proteins, and no very reasonable interpretation of the values has been proposed.

It is generally believed that the polar side chains of amino-acid residues of proteins provide much of the attraction for the adsorbed water molecules. Accordingly a comparison of the amount of water adsorbed initially (that is, in the first layer) by proteins with the amino-acid composition of the proteins might be interesting. I have made this comparison, using the adsorption data of Bull[1] and Shaw[2] and the published analyses of the proteins,[4] and have found that initial adsorption takes place to the extent of one water molecule per polar side chain (capable of forming hydrogen bonds) for some proteins, and that interesting deviations from this simple relation also occur.

Sponsler, Bath, and Ellis[5] pointed out that there are two main types of hydrophilic groups in proteins: the polar side-chain groups of amino-acid residues such as glutamic acid, arginine, tyrosine, etc.; and the carbonyl and imido groups of the peptide bonds. The number of the latter is in general greater than that of the former; evidence indicating that the peptide carbonyl and imido groups usually have little attraction for water is, however, provided by Bull's data for nylon. This product of condensation of hexamethylenediamine and adipic acid contains 890 moles of carbonyl groups and 890 moles of imido groups per 10^5 g., but holds only about 100 moles of water per 10^5 g. in primary adsorption. It is likely that most of the carbonyl and imido groups are held together by hydrogen bonds in the configuration $\rangle N-H\cdots O=C\langle$, and that the hydrogen-bond-forming capacity of the groups is so sufficiently saturated in this way that the residual attraction for water is negligible. The observed adsorption of water by nylon indicates that about 6% of the carbonyl and imido groups fail to join together, presumably because of configurational accident, and that each of these odd groups then attracts a water molecule.[6]

The amino acids with polar side chains include serine, threonine, hydroxyproline, tyrosine, tryptophan, histidine, lysine, arginine, aspartic acid, glutamic acid, and hydroxyglutamic acid. On the assumption that a residue of each of these amino acids would hold one water molecule, there is given in the third column of Table I, as the first of the two numbers in the column, the number of moles of these amino acids reported by analysis for each protein. The second number in this column includes also the number of moles of proline and hydroxyproline (the latter thus being counted twice). This number is given because of the presence in the protein of a free carbonyl group, not involved in hydrogen bonding with

TABLE I

COMPARISON OF THE NUMBER OF WATER MOLECULES HELD BY PROTEINS IN INITIAL ADSORPTION AND THE NUMBER OF POLAR GROUPS IN THE PROTEINS

Protein	Water adsorbed in first layer, moles/10^5 g.		Number of polar groups,[a] moles/10^5 g.	Total reported amino acids[b]
Silk	226		219–228	107.0[c]
Ovalbumin,				
crystallized	329[d]	342 344[e]	277–313	75.7
lyophiled	314			
heat denatured	276			
Wool	366		303–341	71.4[g]
Gelatin, collagen	485	529	328–609	108.8[f]
C-Zein, B-zein	210	228	305–390	106.0[h]
Salmin	592[i]		611–707	110.5
Serum albumin	374		424–424[j]	86.8
β-Lactoglobulin,				
crystallized	370		472–508[k]	115.8
lyophiled	329			

[a] Of each pair of numbers, the first number represents the polar residues not including the carbonyl groups of proline and hydroxyproline, and the second includes these. [b] The percentages include the water added to the residues on hydrolysis of the proteins; complete analysis corresponds to 110 to 116%. [c] Analysis for silk fibroin. [d] Value found by Shaw. [e] Value calculated by Shaw from data obtained by Barker. [f] Analysis for wool keratin. [g] Analysis for gelatin. [h] Analysis for zein. [i] Water adsorbed in first two steps—see text. [j] Analysis by E. Brand, B. Kassell, and L. J. Saidell, *J. Clinical Inv.*, **23**, 437 (1944); the analysis is for bovine serum albumin, whereas the adsorption data are for horse serum albumin. [k] E. Brand and B. Kassell, *J. Biol. Chem.*, **145**, 365 (1942), and private communication from Professor Erwin Brand.

(1) H. B. Bull, THIS JOURNAL, **66**, 1499 (1944).

(2) T. M. Shaw, *J. Chem. Phys.*, **12**, 391 (1944).

(3) S. Brunauer, P. H. Emmett, and E. Teller, THIS JOURNAL, **60**, 309 (1938).

(4) Mainly as summarized by E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids, and Peptides," Reinhold Publishing Corporation, New York, N. Y., 1943.

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

(6) It has been pointed out by a Referee that the small observed adsorption of water by nylon might result from a very compact structure of the fibers, into which penetration by the water would take place only with difficulty.

an imido group, for every proline or hydroxyproline residue, whose peptide nitrogen atom does not carry a hydrogen atom.

It may be seen from Table I that there is encouraging agreement between the number of molecules of water initially adsorbed by the proteins (as given by Bull except where otherwise noted) and the number of polar groups in the proteins. The agreement is especially satisfactory for silk, for which the amino-acid composition is reliable. The comparison for ovalbumin and wool, for which the analyses are incomplete, indicates that not all of the polar amino acids have been reported.

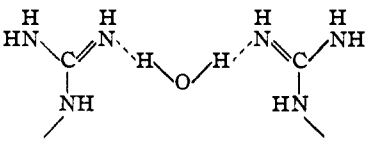
The amount of water adsorbed initially by gelatin and collagen, 485 and 529 moles per 10^5 g., is much larger than the number of polar side chains, 328, and somewhat smaller than the number of polar groups including the proline- and hydroxyproline-liberated peptide carbonyl groups, 609. This fact supports the suggestion that these carbonyl groups are capable of binding water sufficiently strongly to be classed with the polar side-chain groups. Whether the failure of the amount of initially adsorbed water to reach the value 609 is due to the relative weakness of the attraction by the liberated peptide carbonyl groups (as indicated by the low value of the Brunauer–Emmett–Teller constant c for nylon—6, as compared with 10 or more for proteins) or perhaps to some such cause as interaction of some of the carbonyl groups with the hydroxyl groups of the hydroxyproline residues (the sum of polar side chains and proline residues alone is 499) may be decided by further experiment.

It is to be expected that the different polar groups of a protein would attract water molecules with different avidity, and that the amount of water adsorbed, a, would be related to the amount initially adsorbed, a_0, and the activity of the water vapor, x (expressed as the ratio of partial pressure to vapor pressure at saturation), not by the simple Brunauer–Emmett–Teller equation

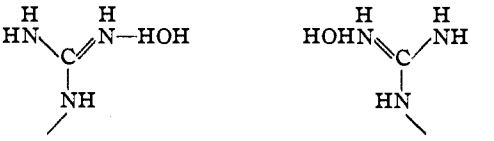
$$a = a_0x/(1 - x)\{1 + (c - 1)x\}$$

but by a sum of these terms, with different values of a_0 and c for the different polar groups. Some evidence for this is given by the data reported by Bull; however, the deviations of these data from the simple Brunauer–Emmett–Teller equation are not great enough to permit the reliable evaluation of the constants a_0 and c for the several polar groups present in the proteins.

Bull pointed out that the data for salmin deviate strikingly from the simple BET theory, and that, in fact, two values, 293 and 592 moles/10^5 g., can be derived for a_0 from the first and second approximately linear segments of the BET plot of $x/a(1 - x)$ against x. The data for salmin cannot be simply represented by a sum of BET terms, but can be represented by another equation which has a reasonable physical basis. Salmin contains very many polar groups, and these groups may well be so close together than the first process of adsorption of water is the attachment of one water molecule to two polar groups, which are mainly the side-chain groups of arginine



A second water molecule would then be added



Additional water molecules would then attach themselves, corresponding to the third layer and successive layers in the BET treatment. If the BET theory is extended to apply to this case, with the assumptions that a_0 is the number of moles of water held by the first process, a_0 is also held by the second process, αa_0 is held in each of the succeeding layers, and only the first process holds the water molecules more tightly than in liquid water, there is obtained the equation

$$a = \frac{a_0x\{1 + (\alpha - 1)x^2\}}{(1 - x)\{1 + (c - 1)x\}}$$

for the total amount of adsorbed water. This equation fits Bull's data for salmin out to $x = 0.6$; a_0 and c have essentially the values assigned by Bull for the first linear segment of the BET plot, and α has the value 3.25. It is interesting that this value of α corresponds to an area of 615 square meters per gram of protein for each layer of water after the first two, and that the specific area of salmin in layers 11.5 Å. thick (the estimated side-chain spacing for this protein) is also 615 sq. m./g.

We conclude that the process of adsorption of water by salmin is the following: one molecule is first bound tightly by two coöperating polar groups; a second molecule then is added, giving a total of one water molecule per polar group; next a complete layer of water is sandwiched into each space between the protein layers; and then other layers of water are similarly introduced. At 95% relative humidity six or seven of these water layers have been introduced between each protein layer and each of its neighboring layers.

The circumstances under which polar groups may coöperate in holding water molecules await study; possibly the small amount of water adsorbed by zein is the result of this phenomenon—the glutamic acid residues, which constitute most of the polar groups in this protein, may be arranged in pairs in such a way as to facilitate their coöperation.

Summary

The data published by Bull and other investigators on the adsorption of water by proteins can

be in considerable degree interpreted on the assumption that the initial process is the attachment of one water molecule to each polar amino-acid side chain. The data also indicate that peptide carbonyl and imido groups usually do not bind water, because of their mutual interaction by hydrogen-bond formation, but that water is bound by carbonyl groups which are not coupled by hydrogen bonds with imido groups. In salmin, in which most of the amino-acid residues are polar, these polar residues coöperate to attach one water molecule jointly to two polar groups in the initial process of hydration.

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Catalytic Dehydration of 1-Hexanol and 1-Octanol

BY V. I. KOMAREWSKY, S. C. UHLICK AND M. J. MURRAY

The recent publication of Appleby, Dobratz and Kapranos[1] on vapor phase dehydration of 1-heptanol prompts us to report similar results on catalytic, vapor phase dehydration of 1-hexanol and 1-octanol. In line with the work of the above-mentioned authors, we have found that 1-hexanol and 1-octanol when dehydrated over aluminum oxide catalyst yield as a main product 1-hexene and 1-octene, respectively. The purity of the olefins was checked by boiling points, indices of refraction, specific gravities and Raman spectra. In addition, the hydrogenation to corresponding paraffins was carried out.

Experimental Part

Apparatus and Procedure.—The apparatus consisted of a Pyrex glass reaction tube of 12-mm. diameter placed in a tubular, electrically heated, bronze block furnace with automatic temperature control (±1°). The catalyst bed was 40 cm. long. It was preceded by a layer of glass beads 5 cm. in length which served as a preheater. Alcohols were passed over the catalyst at a constant rate of 35 ml. per hour (space velocity, 0.5) at 350°. There was practically no gas evolution nor carbon deposition on the catalyst.

TABLE I

PHYSICAL CONSTANTS OF 1-HEXENE AND 1-OCTENE

Olefin			1-Hexene	1-Octene
Present authors	B. p.	°C.	62.8–63.1	121.1–121.4
		Mm.	743	750
	$n^{20}D$		1.3880	1.4095
	d^{20}_4		0.6740	0.7152
Egloff[a]	B. p.	°C.	63.5	122.5
		Mm.	760	760
	$n^{20}D$		1.3886	1.4103
	d^{20}_4		0.6747	0.7159
Doss[b]	B. p.	°C.	63.7	121.6
		Mm.	760	760
	$n^{20}D$		1.3880	1.4090
	d^{20}_4		0.6733	0.7150

[a] Egloff, Physical Constants of Hydrocarbons, Vol. 1. Reinhold Publishing Corporation, New York, N. Y., 1939.

[b] Doss, Physical Constants of the Principal Hydrocarbons, Technical and Research Division of the Texas Company, Third Edition, New York, N. Y., 1942.

(1) Appleby, Dobratz and Kapranos, THIS JOURNAL, 66, 1938 (1944).

Alcohols.—1-Hexanol, Carbon and Carbide Chemical Corporation; 1-octanol, E. I. du Pont de Nemours and Company: both alcohols were purified by careful distillation with a 100-plate Podbielniak column. Boiling points of the purfied alcohols were: 1-hexanol, 157°; 1-octanol, 192–193°.

Catalyst.—Alumina catalyst was prepared by precipitation of aluminum hydroxide from a clear solution of sodium aluminate by ammonium chloride. The precipitate was washed ion-free, dried at 105–110° in an oven, screened to 8–10 mesh particle size and finally dried in the catalytic tube in a slow stream of dry nitrogen at 350°.

Analysis of the Product.—The products, after separation of water and drying over anhydrous sodium sulfate, were distilled in a 100-plate Podbielniak column with heligrid packing. In each case more than 90% of the product boiled in the range of the 1-olefin. The distillation bot-

TABLE II

RAMAN SPECTRA OF 1-HEXENE AND 1-OCTENE

1-Hexene						1-Octene			
Present work: Dehydration of 1-hexanol		Present work: Synthetic		Literature[a]		Present work		Literature[b]	
cm.$^{-1}$	I^c	cm.$^{-1}$	I^c	cm.$^{-1}$	I	cm.$^{-1}$	I	cm.$^{-1}$	I^d
312	1	312	1			291	2	289	10
357	2	357	3	358	2				
395	1	398	1			430	1	433	vw
626	1	627	1			633	1	633	vw
819	3	819	3	818	2	812	2	813	4
872	1	871	1	873	1	854	1	850	5
890	1	889	1			886	2	886	11
910	3	912	2	912	2	911	2	909	11
						970	0	966	vw
984	1	984	0b					989	vw
						1013	1	1014	6
1056	3	1057	2	1056	2	1061	2	1067	12
						1076	2	1079	13
1102	2	1102	2			1111	2	1114	8
1216	1	1216	0						
1293	6	1293	7vb	1296	7	1292	5	1293	94
1417	5	1416	5	1417	8	1416	4	1415	42
1449	6b	1448	5b	1448	7	1443	5	1440	79
						1457	5	1456	63
1643	10	1641	10	1642	8	1644	7	1641	134
						1671	0		
						2729	1	2727	4
2861	8b	2863	8b	2859	5	2856	10	2853	544
				2876	8			2873	778
2911	9	2913	8	2909	8	2891	10	2899	1000
2935	6	2937	7			2934	8	2925	535
2963	4	2961	3	2963	5	2967	3	2961	98
3003	8	2999	9	3002	8	3006	5	2999	124
3081	5	3082	4	3076	5	3087	4	3078	23

[a] See ref. 2. [b] Forrest F. Cleveland, see ref. 4. Spectrum of an A. P. I. sample. [c] Intensity visually estimated on a scale of 10. [d] Intensity on a presumed scale of 1000.