The Adsorption of Water Vapor by Gelatin-Detergent Complexes

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In the previous investigation¹⁾ on the interaction of gelatin with detergent anions or cations, we have found that hydrophobic complexes are formed between them under appropriate conditions. Observing the sedimentation velocity as well as the sedimentation volume in both cases of gelatin-detergent anion complex and gelatin-detergent cation complex, it has been found that the sedimentation velocity of the former complex is greater than that of the latter, while the sedimentation volume of the former is smaller than that of the latter. This suggests that there must be some difference in the hydrophobic nature of these two types of complexes. It is the purpose of this paper to detect such a difference by measuring the adsorption of water vapor by these two types of complexes.

Experimental

Materials.—Gelatin, sodium dodecyl sulfate and dodecyl ammonium chloride used in this experiment are the same samples as used in the previous work¹⁾.

Samples for Adsorption Experiments.—The gelatin, purified by use of ion exchange resins, was precipitated by the addition of methanol to its aqueous solution, and the precipitate was dried in a vacuum desiccator. The detergent-gelatin complex was separated from the solution by centrifugation after the precipitate was formed, repeatedly washed with water and centrifugated, and then dried in a vacuum desiccator. The nitrogen content of the complex was determined by the Kjeldahl method. As the complex may be considered as a compound between gelatin molecule and dodecyl sulfate ion ($C_{12}H_{25}OSO_3^-=265.4$) or dodecyl ammonium ion ($C_{12}H_{25}NH_3^+=186.3$), the detergent content in the complex is calculated from the nitrogen percentage. The combined detergent, calculated in this way, was found to agree very well with that obtained by direct analysis in the previous investigation¹⁾. The data are given in Table I.

TABLE I

	Sample	pH of Solution	Nitrogen Content (%)	Detergent Content Cal. (mM/g. Gel.)	Detergent Content Exp. (mM/g. Gel.)	Gelatin Content (%)
No. 1	Pure Gelatin	4.8	16.26	0	0	100
2	Gelatin-Dodecyl	2.0	12.96	0.96	0.92	79.7
3	Sulfate Ion	3.6	13.80	0.67	0.68	84.9
4	Complex	4.0	14.70	0.40	0.40	90.4
5	Gelatin-Dodecyl	8.0	15.56	0.41	0.40	92.8
6	Ammonium Ion	9.3	15.20	0.65	0.63	89.2
7	Complex	10.6	14.80	0.94	0.98	85.0

Adsorption Experiment.—Adsorption experiment was carried out by the desiccator method similar to that adopted by H. Bull²⁾, who first studied in detail the adsorption of water vapor by several purified proteins. A definite quantity —between 0.5 g. and 0.9 g.—of each samples of complexes was taken in a weighing bottle, which was then placed in a desiccator containing sulfuric acid of a definite concentration. The desiccator was submerged in a thermostat maintained at 25±0.1°C. After the attainment of the equilibrium, the weighing bottle was weighed, and the density of the sulfuric acid was measured, from which the relative vapor pressure was calculated. The amount of the water vapor adsorbed by a given weight of protein was obtained from the increase in weight of the weighing bottle. In order to allow for the dilution effect of the added detergent, the amount of adsorbed water was calculated on the basis of protein portion.

According to Bull²⁾, it requires about two weeks for the attainment of equilibrium, but as Robinson³⁾, Benson et al⁴⁾. have remarked, the equilibrium can be attained in a shorter time, if the adsorption takes place under diminished air pressure. In our experiment, it was sufficient, to wait under the pressure of 10−20 mmHg for about twentyfour hours.

Fig. 1 shows some of the isotherms obtained, one with a sample of pure gelatin and two with samples of different type complexes.

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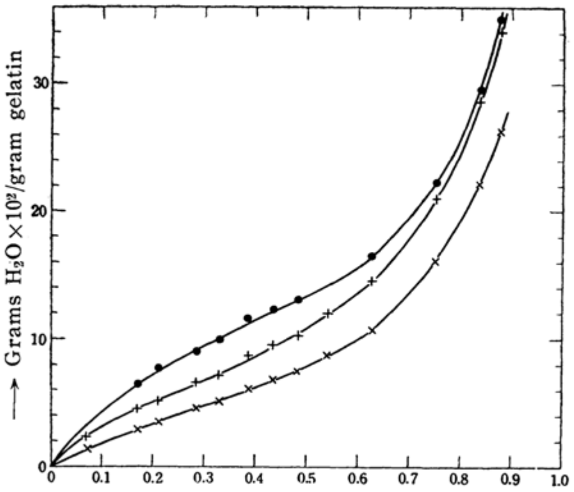
1) K. Tamaki and B. Tamamushi, This Bulletin, 28, 555 (1955).

2) H. Bull, *J. Am. Chem. Soc.*, 66, 1499 (1944).

3) R.A. Robinson, *J. Chem. Soc.*, 1948, 1083.

4) S.W. Benson, D.A. Ellis and R.W. Zwanzig, *J. Am. Chem. Soc.*, 72, 2102 (1950).



→ relative vapor pressure (p/p_0)

Fig. 1. Water vapor adsorption isotherms
·No. 1; ×No. 2; +No. 7

Discussion

As Fig. 1 indicates, the amount of water vapor adsorbed by gelatin is markedly reduced by the combination of detergent ion with it. However, the amount adsorbed by the gelatin-dodecyl sulfate ion complex is less than that by gelatin-dodecyl ammonium ion complex over the whole range of relative vapor pressures. From this fact, it is concluded that the former is more hydrophobic than the latter, which is in accord with the experimental results obtained in the measurement of sedimentation volume and sedimentation velocity. For both types of complexes, it was found that the complexes containing detergent in greater amount adsorb water vapor in less degree.

As has been reported by many investigators, adsorption isotherms of water vapor on proteins are generally S-shaped, and these are represented by the Brunauer-Emmett-Teller (B.E.T.) equation[5] in the range of relative vapor pressure from 0.05 to 0.5. The isotherms obtained in our experiment have been found too in accord with the B.E.T. theory as shown by Fig. 2. The amount of the first monolayer coverage V_m as well as the constant C in in the B.E.T. equation are listed in Table II.

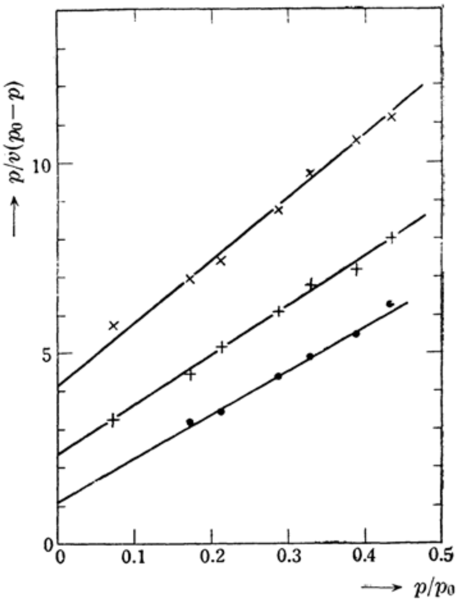


Fig. 2. B.E.T. plot.
·No. 1; ×No. 2; +No. 7

According to Dunford and Morrison[6], the isotherms obtained by Bull[2] are represented by the Harkins-Jura equation[7], while the isotherms obtained in our experiment do not obey that equation except the isotherm for pure gelatin.

Many workers have discussed the mechanism of the adsorption of water on proteins. Pauling[8] suggested that the amount of the first monolayer adsorption V_m could be interpreted in terms of the number of polar

TABLE II

	Sample	($g.H_2O$/g. Gel.) V_m	C	Decrease in V_m (mM/g. Gel.)	Detergent Combined (mM/g. Gel.)
No. 1	Pure Gelatin	0.0821	9.54		
2	Gelatin-Dodecyl	0.0493	4.85	1.82	0.96
3	Sulfate Ion	0.0503	5.73	1.76	0.67
4	Complex	0.0512	6.13	1.71	0.40
5	Gelatin-Dodecyl	0.0654	7.66	0.93	0.41
6	Ammonium Ion	0.0648	7.45	0.96	0.65
7	Complex	0.0632	7.00	1.05	0.94

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[5] S. Brunauer, P.H. Emmett and E. Teller, ibid., **60**, 309 (1938).

[6] H.B. Dunford and J.L. Morrison, *Can. J. Chem.*, **32**, 558 (1954).

[7] W.D. Harkins and G. Jura, *J. Am. Chem. Soc.*, **66**, 1366 (1944).

[8] L. Pauling, ibid., **67**, 555 (1945).

amino-acid side-chains in protein molecules. Benson et al[4]. inferred from their experimental result that the adsorption of water vapor on proteins should be qualitatively different from that of non-polar gases such as nitrogen, and the adsorption of water would take place at specific sites on protein molecules. According to the work of Mellon, Korn and Hoover[9], a reduction in the amount of adsorption takes place over the whole range of relative vapor pressures, when the amino-groups of casein are benzoylated. By the B.E.T. treatment of the data, they have shown that the amount of reduction in V_m roughly correspnds to the amounts benzoylated on the protein molecule. On the other hand, Green and Ang[10] came to the conclusion, on the basis of their study of the adsorption of water vapor by acetyl derivatives of collagen and of silk fibroin, that the change in V_m caused by acetylation is independent of number the of acetyl groups introduced.

As indicated in Table II, there is a certain parallel correlation, but no stoichiometrically definite relation between the reduction in V_m and the amount of the combined detergent in our experimental data. For both complexes, the decrease in V_m is always greater than the amount of combined detergent.

Summary

1. The adsorption of water vapor by gelatin-dodecyl sulfate ion complexes and gelatin-dodecyl ammonium ion complexes has been measured.
2. The adsorption of water vapor by both those kinds of complexes has been found to be less than that by pure gelatin.
3. The amount of adsorption on the gelatin-dodecyl sulfate ion complexes is always smaller than on the gelatin-dodecyl ammonium ion complexes, and this fact indicates that the former complexes are more hydrophobic than the latter.
4. The adsorption isotherms have been interpreted by the B.E.T. theory and the amount of the first monolayer adsorption has been calculated.
5. In both types of complexes, there is a certain parallel correlation but no stoichiometric relationship between the reduction in the amount of the first monolayer adsorption and the amount of combined detergent.

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[9] E.F. Mellon, A.H. Korn and S.R. Hoover, ibid., 70, 3040 (1948).

[10] R.W. Green and K.P. Ang, ibid., 75, 2733 (1953).