results do not appear to follow any simple stoichiometry.

An acid such as HCl presents a very simple chemical behavior, forming permanent compounds with the strongly basic residue groups. Where it departs from simple stoichiometry, the results appear to be attributable to slow diffusion in the solid. BF_3 is a sufficiently strong Lewis acid to attack weakly basic groups. Here diffusion has become considerably more important and the results are much less simple to interpret than for HCl. Comparisons with the model substance Nylon are very interesting in showing that the peptide in Nylon is more reactive to BF_3 than the peptide in proteins. This would be expected on the basis of the α-helix model of the proteins proposed by Pauling and Corey.[20]

(20) L. Pauling and R. B. Corey, *Proc. Natl. Acad. Sci.*, **37**, 205 (1951).

LOS ANGELES, CAL.

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT, UNIVERSITY OF SOUTHERN CALIFORNIA, LOS ANGELES]

A Study of Hysteresis in the Sorption of Polar Gases by Native and Denatured Proteins[1]

BY SIDNEY W. BENSON AND RYDEN L. RICHARDSON[2]

RECEIVED DECEMBER 18, 1954

A modified McBain sorption balance has been used to measure the sorption–desorption isotherms of various polar gases, on native and heat denatured proteins at 25 and 40°. The relative sorptive capacities of egg albumin and bovine plasma albumin follow the same trend in binding the following vapors: H_2O > EtOH > Et_2O > EtCl. Apparently the amount of sorption decreases as the adsorbate becomes more restricted in its ability to form hydrogen bonds with the solid protein adsorbent. The same trend was observed with heat denatured egg albumin. The series MeOH, EtOH, and *i*-C_4H_9OH on egg albumin at 25° showed a marked decrease in the amount of sorption with increasing size of the adsorbate molecule. All of the isotherms showed hysteresis loops which could be approximately related to the total amount of sorption and the molar volume of the adsorbate. The hysteresis loops were quite reproducible, even after three successive cycles and sorption–desorption points showed negligible drift in 10–40 hour periods. The dissipation of free energy upon completion of a hysteresis loop was calculated with the aid of the Gibbs–Duhem equation. The values, expressed in kcal./mole protein, for egg albumin at 25° were: H_2O = 66; MeOH = 118; EtOH = 79 and EtCl = 38. Calculation of the heats of sorption and desorption by means of the Clausius–Clapeyron equation for EtOH in egg albumin showed quite normal behavior in contrast to the values reported on earlier for H_2O on bovine plasma albumin.

Introduction

There are marked differences in the interactions of polar and non-polar gases with solid proteins. For example, the adsorption isotherms of non-polar gases[3,4] are reproducible irrespective of path: the adsorption and desorption paths coincide and there is no hysteresis loop. This indicates that proteins have no fine pore structure which could give rise to capillary condensation. Moreover, equilibration rates are relatively rapid and the amount adsorbed can be related to the surface area of the protein on the basis of BET theory.[5] Such evidence emphasizes the fact that the adsorption of non-polar gases by proteins is a surface phenomenon and hence can give only indirect or restricted information concerning the internal structure of protein molecules.

The foregoing limitations do not apply to polar gases. This becomes evident when one considers the following observations: (1) polar gases have BET surface areas that are several orders of magnitude larger than those calculated for non-polar gases, (2) the sorption of polar gases is practically independent of particle size or surface area of the protein adsorbent, (3) the equilibration rates are low, suggestive of a rate-controlling diffusion of the adsorbate through the solid protein, and (4) the sorption isotherms have hysteresis loops. All these features point to interactions within the interior of the protein molecule and not on its surface only.[6-8]

In regard to the hysteresis effect, Seehof[9] has observed that the maximum hysteresis displacement in the water sorption isotherms of several proteins appears to correlate with the arginine, histidine and lysine groups of each protein. For example, the maximum displacement between the sorption and desorption branches of the 25° isotherm of H_2O on bovine serum albumin is 1.5 mmoles/g. This is in close agreement with 1.4 mmoles/g., the sum of the above-mentioned R groups in that particular protein. In view of the capacity for hydrogen bonding of both the adsorbate and the cited polar R troups, it was of interest to examine further the general problem of sorption hysteresis as it relates to a series of absorbates having decreasing capacity for hydrogen bonding (*i.e.*, H_2O, EtOH, Et_2O and EtCl). Another interesting comparison might arise from a series of polar adsorbates of equal capacity for hydrogen bonding but of increasing size (*i.e.*, MeOH, EtOH and *i*-BuOH)). The effects of temperature and denaturation also seemed worthy of study.

Experimental

Materials and Reagents.—The various proteins and polar adsorbates used in this research are briefly described as follows: (1) egg albumin, 2% spray frozen, area 18 m.²/g.; (2) egg albumin, crystalline powdered, Armour and Co., Lot E 90115; (3) heat denatured egg albumin, coagulated

(1) This work has been supported by a grant (G-3541) from the U. S. Public Health Service of the National Institutes of Health.

(2) Part of the material presented in this paper has been included in a dissertation submitted by R. L. Richardson to the Graduate School of the University of Southern California in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(3) S. W. Benson and D. A. Ellis, THIS JOURNAL, **70**, 3563 (1948).

(4) S. W. Benson and D. A. Ellis, *ibid.*, **72**, 2095 (1950).

(5) S. Brunauer, "The Adsorption of Gases and Vapors," Vol. I, Princeton University Press, Princeton, N. J., 1943.

(6) S. W. Benson, D. A. Ellis and R. W. Zwanzig, THIS JOURNAL, **72**, 2102 (1950).

(7) L. Pauling, *ibid.*, **67**, 555 (1945).

(8) J. M. Seehof, B. Keilin and S. W. Benson, *ibid.*, **75**, 2427 (1953).

(9) J. M. Seehof, B. Keilin and S. W. Benson, *ibid.*, **75**, 2428 (1953).

upon 5 min. boiling, evacuated overnight, source; Armour and Co., Lot E 90115; (4) gelatin, 2% spray frozen, California Institute of Technology; (5) bovine plasma albumin, 2% spray frozen, Armour and Co.; (6) water, deionized, boiled, several cycles of freezing, pumping and melting; (7) methyl alcohol, Baker and Adamson, reagent grade, meets ACS specs., Code 1212; (8) ethyl alcohol, U.S. Industrial Chemicals Co., absolute, U.S.P.; (9) isobutyl alcohol, The Matheson Co., Inc., "For laboratory or manufacturing use only," Code 2858; (10) diethyl ether, Baker and Adamson, reagent grade, anhydrous, meets ACS specs., Code 1700; (11) ethyl chloride, Eastman Organic Chemicals, Code 1075; (12) hydrogen chloride, The Matheson Co., Inc., commercial cylinder. Subsequent batchwise distillation and degassing in high vacuum system.

Apparatus.—A modified McBain sorption balance containing a quartz helix of high sensitivity (25.59 mg./cm.) was used in this research. The $\overline{\S}$ joints were usually sealed with Shawinigan resin and stopcocks were greased with Apiezon N or Silicone. The entire sorption balance, adsorbate supply bulb and mercury manometer were submerged in a large water-bath held within ± 0.005°. Both temperatures were set by a calibrated thermometer (U.S. Bureau of Standards). Pressure readings and elongations of the quartz helix were measured with a Gaertner cathetometer mounting a vernier for measurements to 0.005 cm. Calibration of quartz helix revealed a conformance to Hooke's law and the probable error of weighing amounted to ±0.2 mg. Dry sample weights normally ranged between 300 and 500 mg. Blank runs with empty balance contacting H_2O or EtOH vapor at room temperature proved that adsorption on helix and sample bucket was negligible. For the small sample sizes and low pressures involved buoyancy corrections were negligible. A preliminary run was concerned with the 25.0° isotherm for H_2O on powdered egg albumin (crystalline). The resulting data agreed closely with those reported by Bull[10] for the same system and also workers in our own laboratories.[8]

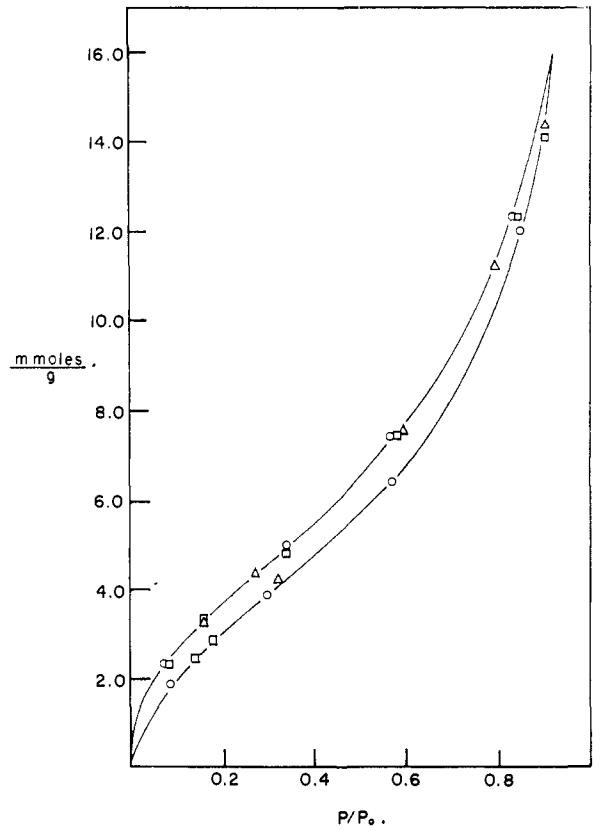

Fig. 1.—Showing reproducibility of sorption-desorption cycles of water on egg albumin at 25° for 3 successive cycles: first cycle, O; second, △; third, □.

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

Data and Discussion

A. Reproducibility of Hysteresis Loop.—The reproducibility of the hysteresis loop was examined by carrying out three sorption–desorption cycles with H_2O on egg albumin (hereafter designated as EA) at 25.0°. Figure 1 shows that the hysteresis effect is reproducible within the limits of experimental error. The same result was obtained with EtOH on EA at 25.0°. This persistence of the hysteresis effect, despite repeated sorptions, has also been reported for H_2O on cotton[11] and H_2O on spruce.[12] However, Rao reports[13] that the loop decreases upon the second cycle and disappears upon the third cycle for H_2O on casein, denatured casein and egg albumin at 30°.

B. Kinetic Studies.—One of the main questions to be considered as part of the hysteresis problem is whether or not the hysteresis loop represents a failure to reach equilibrium. Moreover, if the hysteresis loop is rate dependent, how does the area of the loop vary as longer periods of equilibration are afforded for the isotherm determination? The rate curves of Fig. 2 indicate that H_2O is sorbed very quickly by EA at 25.0° and that after 3 hours of equilibration the amount of sorption becomes essentially constant. A much longer period of time is required by EtOH at this temperature.[14]

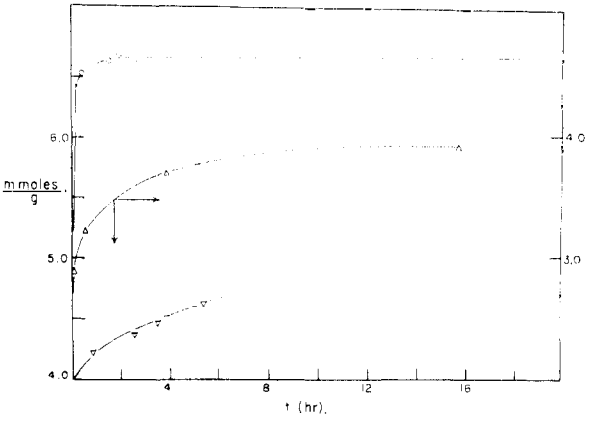

Fig. 2.—Differential rates of adsorption on egg albumin of H_2O and C_2H_5OH at 25°: open circles, H_2O; p/p_0 = 0.56 (sample initially saturated at p/p_0 = 0.32); erect triangles, △, C_2H_5OH, p/p_0 = 0.82 (sample initially saturated at p/p_0 = 0.62); use right hand ordinate; inverted triangles, ▽, C_2H_5OH, p/p_0 = 0.98 (sample initially saturated at p/p_0 = 0.82).

The data of these and other rate measurements could not be satisfactorily treated by first- or second-order plots. However, a special type of second-order plot having the form

$$\frac{1}{x} = \frac{k}{t} + \frac{1}{x_0}$$

fits reasonably well, an example of such a plot appearing in Fig. 3. The amount of sorption in millimoles of adsorbate per gram of dry protein is represented by x and the time in hours is represented

(11) A. R. Urquhart, *J. Text. Inst.*, **20**, T125 (1929).

(12) A. J. Stamm and S. A. Woodruff, *Ind. Eng. Chem., Anal. Ed.*, **13**, 836 (1941).

(13) G. N. Subba Rao, *Proc. Indian Acad. Sci.*, **25**, 221 (1947).

(14) The rate is much faster at higher temperatures.

by t. Without plotting all the available data, it may be reported that: (1) adsorbates of large molar volume diffuse relatively slowly through solid proteins and hence equilibrate at a slow rate, (2) temperature and nature of adsorbent both influence the sorption rate of a given adsorbate and (3) desorption rates are generally slower than sorption rates.

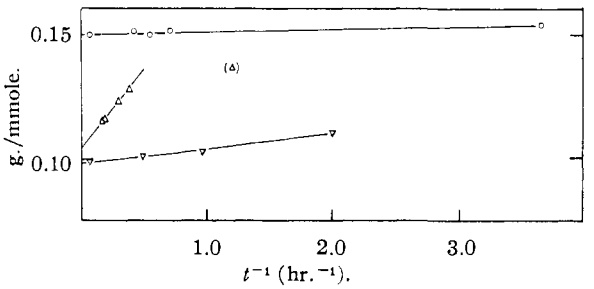

Fig. 3.—Empirical treatment of the sorption rate data on egg albumin by the equation $1/x = k/t + 1/x_0$: open circles, H_2O at 25°; erect triangles, C_2H_5OH at 25°; inverted triangles, ∇, C_2H_5OH at 40.2°.

Although equilibration periods were seldom extended beyond 24 hours, the extrapolation of the foregoing plots to infinite time gave sorption values (x_0) which still delineated a hysteresis loop. While the authors will readily concede the advisability of checking the predicted extrapolations with data pertaining to equilibration periods of several weeks or months, it should be pointed out that other work has been started in this direction and similar results have been obtained.[15,16]

Hence it appears that if the hysteresis loops manifested by the sorption of polar gases on proteins are due to failure to reach equilibrium, the metastable states involved have life-times too long to observe under any reasonable laboratory conditions.

C. Sorption Isotherms of Polar Gases on Native Proteins.—Figure 4 shows the sorption isotherms of H_2O, EtOH, Et_2O and EtCl on bovine plasma albumin (hereafter BPA) at 25.0°. Adsorbates of greater hydrogen-bonding ability (H_2O and EtOH) develop large hysteresis loops but those of little or no hydrogen-bonding ability (Et_2O and EtCl) give very small loops. A further examination of the isotherms reveals that the loops of large area occur for those gases sorbed in considerable amounts. This suggests that the hysteresis effect might be caused by a deformation of the polypeptide chains within the protein molecule as the polar adsorbates settle into suitable positions for hydrogen bonding or ion–dipole interaction.[17]

Another set of data appear in Fig. 5. These isotherms pertain to H_2O, EtOH, EtCl and Et_2O on

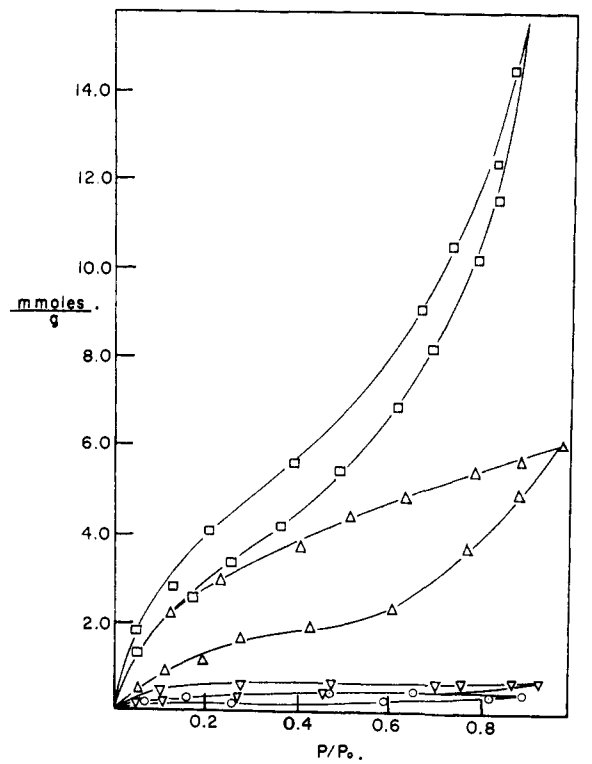

Fig. 4.—Hysteresis cycles of different sorbates on bovine plasma albumin (BPA) at 25°: square, □, H_2O; erect triangles, △, C_2H_5OH; inverted triangles, ∇, C_2H_5Cl; open circles, O, diethyl ether.

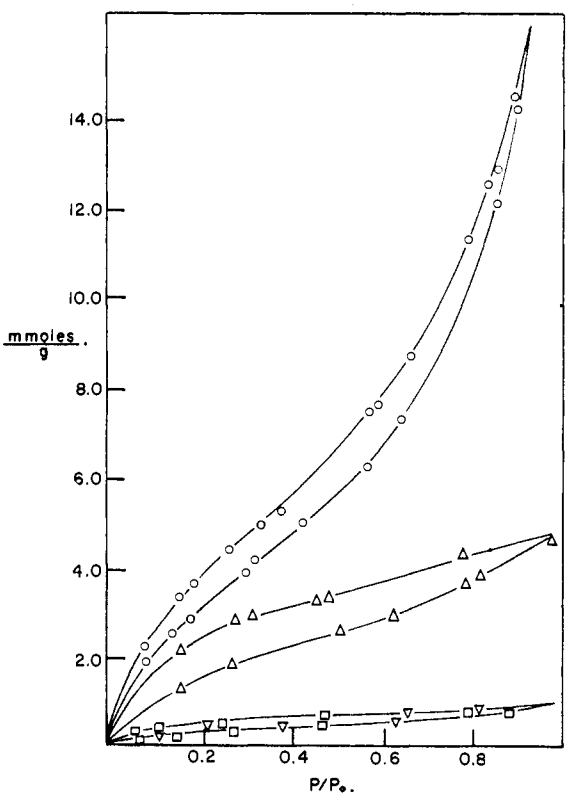

Fig. 5.—Hysteresis cycles of different sorbates on egg albumin at 25°: open circles, O, H_2O; erect triangles, △, C_2H_5OH; squares, □, diethyl ether; inverted triangles, ∇, C_2H_5Cl.

(15) (a) R. A. Robinson, *J. Chem. Soc.*, 1083 (1948); (b) P. A. Marshall and W. J. Moore, THIS JOURNAL, **74**, 4779 (1952).

(16) A. R. Urquhart and A. M. Williams, *J. Textile Inst.*, **15**, 48T, 138 (1924).

(17) The low partial molal heats of sorption calculated by Keilin[8] for H_2O on bovine serum albumin at 20–25° may be due to this energy absorbed in swelling (*i.e.*, deforming) the protein structure.

EA at 25.0°. Again one notes trends similar to those of the preceding systems. An adsorbate sorbed in relatively large amounts would cause a greater total deformation of the protein network and hence hysteresis loops of larger areas. The versatile and strong hydrogen-bonding adsorbate, H_2O, would best typify this situation. On the other hand, the sizable loop for EtOH may be due to the larger molar volume of this substance. Although there are comparatively fewer EtOH molecules sorbed per gram of protein, the network deformation per molecule is larger for EtOH than H_2O and accordingly the former is still able to produce considerable deformation and hence large hysteresis loops. In the case of Et_2O, the small loop could be attributed to the very slight amount of sorption involved. Probably the considerable bulk of the Et_2O molecule causes too large local deformation, making sorption energetically unfavorable. Another factor involved in the small amount of Et_2O sorbed may be the inability of this molecule to donate a hydrogen bond. The great difference between the amount of sorption of EtOH and EtCl also appears quite illustrative of this factor. (These molecules are about equal in size and their dipole moments[18] in Debye units are not very different: EtCl = 1.99, EtOH = 1.70.)

A further study of the relation of molecular size to the extent of sorption was made with a group of alcohols on EA at 25.0°. The alcohol adsorbates involved R groups of increasing size: MeOH, EtOH and i-C_4H_9OH. Figure 6 shows that the larger the R group, the smaller the amount of sorption. This is in accord with the earlier suggestion that energetically the sorption process becomes less favorable because of the increasing work required by larger deformations of the larger adsorbates. As to the size of the hysteresis loops, it again appears that not only the amount of sorption but also the size of the adsorbate molecule must be considered to account, for example, the larger loop of MeOH compared to H_2O (broken line indicates H_2O). The small loop for i-C_4H_9OH also fits this viewpoint. The maximum hysteresis displacement, however, shows no uniform trend with any parameters considered, being greatest for MeOH.

D. Sorption Isotherms of Polar Gases on Heat Denatured Protein.—Figure 7 reveals that the same trend in area if the hysteresis loop with hydrogen-bonding ability holds for heat denatured as well as for native EA. But the size of a hysteresis loop for a given gas appears to change upon denaturation. For example, native EA gives a H_2O hysteresis loop which has an area of 20 (areas measured by a planimeter and expressed in arbitrary units). But heat denatured EA gives a H_2O hysteresis looping having an area of 26. The increase in loop area is far more pronounced in the case of EtOH. The hysteresis loop for EtOH on native EA has an area of 16: upon heat denaturation the area practically doubles, increasing to a value of 30.

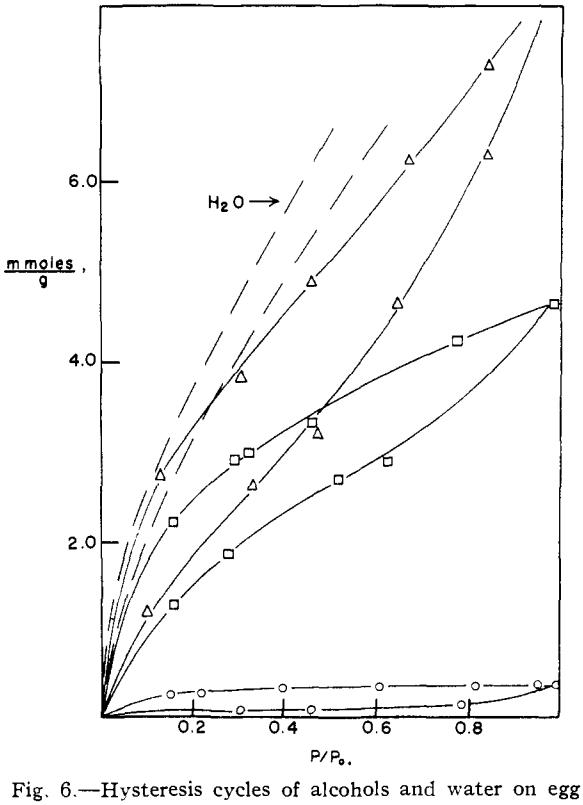

Fig. 6.—Hysteresis cycles of alcohols and water on egg albumin at 25°: dotted line, H_2O; erect triangles, Δ, CH_3OH; squares, □, C_2H_5OH; open circles, O, i-C_4H_9OH.

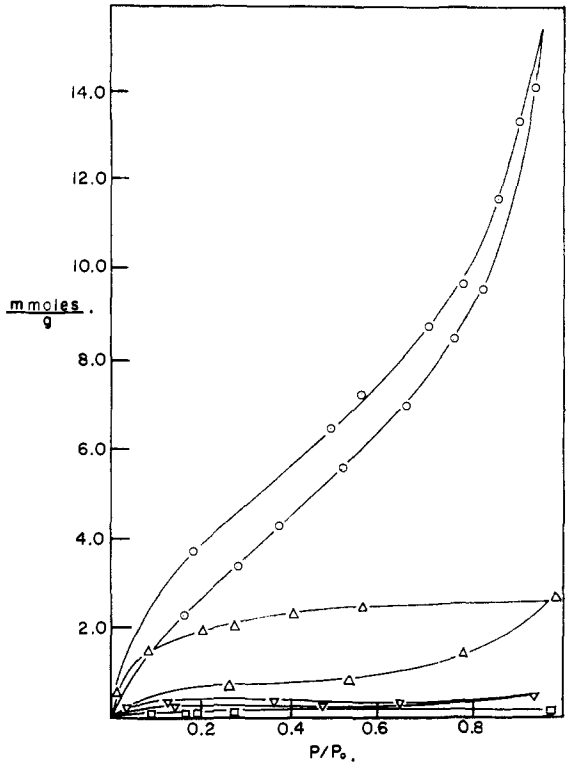

Fig. 7.—Hysteresis cycles on denatured egg albumin at 25°: open circles, O, H_2O; erect triangles, Δ, C_2H_5OH; inverted triangles, ∇, C_2H_5Cl; squares, □, diethyl ether.

Another effect observed upon denaturation is the decrease in sorptive capacity. For H_2O it is slight, as evidenced by the small downward dis-

(18) C. P. Smyth, "Dielectric Constant and Molecular Structure," The Chemical Catalog Co., Inc., New York, N. Y., 1931, pp. 87, 102.

placement of the sorption branch. However, the effect is severe for EtOH. The sorption of both EtCl and Et_2O appears to be decreased somewhat by heat denaturation. The absolute values for these last two are, however, too small to be measured very accurately.

Recent ideas on protein denaturation[19] indicate that the unfolding of the polypeptide chains might be the general process involved in all types of denaturation. If this is so, one might expect the amount of sorption to increase upon denaturation because the unfolding of chains should make the sorption sites within the protein more accessible. An unfolded or open net-work presumably could sorb polar gases with little or no deformation and, on the basis of ideas already developed, there should be little or no hysteresis loop for polar gases on denatured proteins. But this is not observed. The loop for H_2O is approximately the same as for the native sample, and the loop for EtOH is twice as large as that for the native adsorbent—even when EtOH is comparatively much less sorbed, as it is in the case of heat denatured EA. The observed effects seem to indicate that heat denaturation produces a more compact structure, at least to a degree which would cause greater deformation for EtOH as compared to H_2O. (It is interesting to note that Neurath and Bull[20] report that the density of ovalbumin increases from 1.2655 to 1.2940 upon heat denaturation.)

E. Effect of Temperature.—The hysteresis cycles for EtOH on EA at 25.0 and 40.3° are shown in Fig. 8. Planimeter measurements show that there is a rather small decrease in area of the hysteresis loop (amounting to about 8%) at the higher temperature. This is consistent with the view that the area of the loop is related to the total amount of sorption, this latter also being smaller at the higher temperatures.

If the Clausius–Clapeyron equation is used for the calculation of the net partial molar heats of sorption and desorption, from these data, it is found that $-\Delta\bar{H}_s$(net) is about 2.0 kcal. at 0.3 saturation and decreases slowly to 0.8 kcal. at 0.8 saturation. For the desorption, $-\Delta\bar{H}_d$(net) is about 2.0 kcal. near 0.9 saturation and appears to go through a small maximum of 2.7 kcal. at 0.5 saturation from which it decreases to 1.6 kcal. at 0.2 saturation. These data are in sharp contrast to the data obtained for the sorption of H_2O on BSA by Benson and Keilin.[8] There the heats of sorption appeared to be less than the heat of condensation of pure water and showed a steady decrease with increasing sorption to values near 0.7 saturation which were almost $^1/_2$ the heat of condensation of pure water! This seemed quite anomalous at the time and the present results obtained with EtOH which are quite normal would indicate that the water sorption should probably be reexamined.

F. Empirical and Thermodynamic Treatment.—The foregoing sets of data indicated qualitative relations between (1) the amount of sorption, (2) the size of the adsorbate molecule and (3) the area

(19) H. Neurath and K. Bailey, "The Proteins: Chemistry, Biological Activity and Methods," Vol. 1, Part B, Academic Press, Inc., New York, N. Y., 1953, p. 807.

(20) H. Neurath and H. B. Bull, *J. Biol. Chem.*, **115**, 519 (1936).

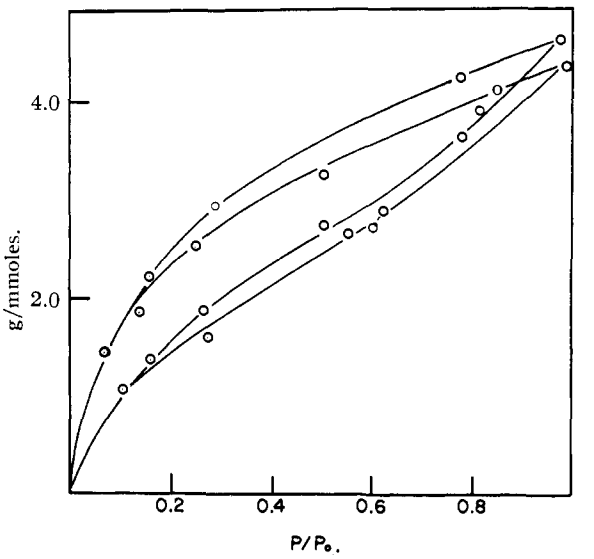

Fig. 8.—Effect of temperature on the hysteresis loop of C_2H_5OH on egg albumin at 25 and 40.3°: open circles, 40.3°; closed circles, 25°. Lower branches are sorption; upper branches desorptions.

of the hysteresis loop. A planimeter was used to measure the area of the hysteresis loop, L, and the area under the sorption path, D. The latter value was weighted by V^0, the liquid molar volume of the adsorbate, expecting that this product might give some measure of the stress imposed upon the protein network and hence relate in some way to the magnitude of the hysteresis effect, represented by L. A plot of log DV^0 *vs.* log L indicated an approximate adherence to the equation

$$L = \left(\frac{DV^0}{7.4}\right)^{1/2}$$

It subsequently was found that the ratio of observed hysteresis loop areas to the calculated areas were 1.2, 1.3, 1.4, 0.9, 1.1 and 5.1 for EtOH, *i*-C_4H_9OH, H_2O, Et_2O, EtCl and MeOH on EA at 25.0°. The erratic deviation of MeOH discouraged further calculations along these lines.

Another approach was made by evaluating the cyclic value of ΔF_h, the free energy dissipated by a mole of protein upon completing a hysteresis loop.

Employing the Gibbs–Duhem equation and the well-known relation between partial molar free energy and activity, the following equation was derived for ΔF_h

$$\Delta F_h = \frac{-RT}{n_2} \oint \frac{n_1}{a_1} \, da_1$$

wherein the symbols have their usual meaning and the adsorbate component is indicated by both n_1 and a_1. Further substitution led to the working equation

$$\Delta F_h = \frac{-2.3\,RTM_2}{10^3} \oint x \, d\left[\ln\left(\frac{P}{P_0}\right)\right]$$

wherein

M_2 = mol. wt. of the protein adsorbent
x = mmoles of adsorbate/g. of adsorbent
P/P_0 = relative pressure of the adsorbate

The evaluation of this integral by the graphical in-

tegration of mm./g. *vs.* log P/P_0 was rather unsatisfactory at the lower values of P/P_0. However, upon noting that θ, the exponent in the Freundlich sorption isotherm, was approximately 0.7 it developed that quite satisfactory evaluations in this region could be made with a plot of $[\text{mm./g.}/(P/P_0)^{0.7}]$ *vs.* $[(P/P_0)^{0.7}]$. This follows from the fact that an integral of the form $\int x\, d(\ln p)$ is equal to $1/\theta \int x/p^\theta \cdot d(p^\theta)$. Moreover, if $x = kp^\theta$, *i.e.*, if the sorption can be represented by the Freundlich isotherm in the region of low relative pressures, then the plot of (x/p^θ) *vs.* p^θ will give straight line extrapolations to zero relative pressure.

Using data for various polar gases on EA at 25.0° and setting M_2 = 42,000 the following ΔF_h values were obtained in kcal./mole of protein: EtCl = 38, H_2O = 66, EtOH = 79 and MeOH = 118. It is noteworthy that these values follow a definite trend with respect to the stress factor, DV^0, employed in the empirical treatment. The values for DV^0 in (cc.) (unit area)/(mole) were: EtCl = 1680, H_2O = 6060, EtOH = 7440 and MeOH = 7520. However, again, the departure of MeOH from the linear trend of the other adsorbates was disturbing. It tends to emphasize certain omissions in the present treatment which do not account for the specific geometry of adsorbate–R group arrangements.

LOS ANGELES, CAL.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

The Binding of Organic Ions by Proteins. Volume Changes

BY ROBERT M. ROSENBERG[1,2] AND IRVING M. KLOTZ

RECEIVED NOVEMBER 3, 1954

The volume change in the reaction between bovine serum albumin and sodium dodecyl sulfate has been determined from pycnometric measurements of the apparent molal volumes of bovine serum albumin, sodium dodecyl sulfate and the complex between them. For solutions in which approximately 13 dodecyl sulfate anions are bound per mole of albumin, the volume change for bound anion is 6.7 ± 1.4 ml. The results have been compared with predictions based on a simple electrostatic theory, and possible explanations for the deviations have been given.

Introduction

There has been much discussion in the literature[3–6] of the factors which determine the strength of binding of anions to serum albumin. Since previous measurements of the thermodynamic quantities, ΔF and ΔS of binding,[7] have suggested the importance of electrostatic effects in the binding of anions, it was considered worthwhile to determine yet another thermodynamic property, the volume change of the reaction, which should also reflect the contribution of electrostatic factors.

The reaction studied was the binding of sodium dodecyl sulfate by bovine serum albumin. The free energy and entropy changes in this reaction had been determined previously by Karush and Sonenberg.[8] The number of dodecyl sulfate ions bound is sufficient to produce a measurable volume change.

The formation of the complex may be represented by the equation

$$P + rA \rightleftharpoons PA_r \qquad (1)$$

where P represents free protein, A free anion and PA_r the complex containing r bound anions. The volume change for this reaction was determined by measurement of the densities of solutions of protein, small ion, and mixtures of the two, and calculation of the apparent molal volumes of the respective species by standard methods.[9] The volume change may be computed from the expression

$$\Delta V = \phi(V)_{PA_r} - \phi(V)_P - r\phi(V)_A \qquad (2)$$

where $\phi(V)$ represents the apparent molal volume.

Experimental

Materials.—Crystallized bovine serum albumin was purchased from Armour and Company. The moisture content, determined by heating to constant weight at 100° over phosphorus pentoxide in an Abderhalden drying pistol, was 3.0%. The dry protein was found to contain 0.74% sodium chloride.[10]

The sodium dodecyl sulfate was a specially purified sample generously supplied by the Fine Chemicals Division of E. I. du Pont de Nemours and Company.

Toluene used in the thermoregulator was of C.P. grade and was further purified by shaking it over mercury overnight and by allowing it to stand over mercury one week.

Sodium hydroxide was J. T. Baker Analyzed grade.

Distilled water from the laboratory tap was further redistilled from alkaline permanganate in an all-Pyrex apparatus before use in solutions for the density measurements.

Methods.—The densities of solutions of bovine serum albumin, sodium dodecyl sulfate and mixtures of the two were measured by a differential pycnometric method. The pycnometers (of about 60-ml. volume) were constructed by Dr. C. E. Moser for an investigation of the apparent molal volumes of amino acids and related compounds. The measurements, with minor modifications, were carried out by the method of Gucker and Moser.[11,12]

The differential method provides a precise value for the difference in density $d - d_0$ between solution (d) and solvent (d_0), as required for calculation of the apparent molal

(1) Atomic Energy Commission, Predoctoral Fellow in the Physical Sciences, 1949–1950; H. E. Russell Fellow of Trinity College, 1947–1949.

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