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

Rate Studies on the Interaction of BF_3 with Bovine Plasma Albumin[1a,b]

BY R. SRINIVASAN AND SIDNEY W. BENSON

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A kinetic study of the interaction of gaseous BF_3 with solid bovine plasma albumin has been carried out in order to establish the conditions under which sorption equilibrium between the gas and the solid is attained. Measurements were made in a modified McBain sorption balance, over a range of sorption pressures and at three temperatures. The rate data did not fit any simple kinetic equation. The observed activation energy, which had a maximum value of 3.5 kcal./mole, and the pressure dependence of the interaction, suggested that the process is diffusion controlled. This is confirmed by a plot of amount sorbed *vs.* square root of time, which is linear. Runs made with mixtures of helium and BF_3, and methane and BF_3, to detect the existence of temperature gradients in the system, showed that such gradients did not influence the rate significantly. In the light of the above data, the interaction of BF_3 with solid proteins is explained.

Introduction

Gaseous BF_3 interacts with solid, dry proteins, the process being very exothermic in its initial stages. Benson and Seehof,[2a,b] who investigated this process in some detail, showed the existence of a large hysteresis between the sorption and desorption paths. They also pointed out that the amount of permanently bound BF_3, which is a measure of the hysteresis, is dependent on the pressure at which BF_3 is sorbed by the protein.

The present work was undertaken as a detailed study of the irreversible binding of BF_3 by proteins, to see if a correlation between the amount of BF_3 permanently bound and the number of functional groups in the proteins could be found. Such a correlation has been reported with HCl and the free basic groups in several proteins.[2a,b] The early work in this direction with BF_3 was promising.[2b]

It seemed unlikely, in view of the results reported with HCl, that the amount of BF_3 bound can increase continuously with increasing sorption pressure. In an attempt to determine the pressure limit at which a maximum value for this quantity will be reached, rate studies on the sorption of BF_3 by bovine plasma albumin (hereafter abbreviated to BPA) were undertaken. The influence of temperature, pressure and foreign gases on the sorption process were investigated to find out if sorption equilibrium was reached under practical conditions. These results are reported here.

Experimental

Materials.—The various gases used in this research were (1) BF_3 (Matheson Chemical Co.) from commercial cylinders was purified by bulb-to-bulb distillations, and stored in glass vessels. (2) Methane (Matheson Chemical Co.), and (3) helium (Air Reduction Co.) were used directly as obtained from cylinders. The BPA used (Armour and Co., Ill.) was spray frozen[3] from a 2% aqueous solution. The product was sieved, and only that portion passing through a No. 30 mesh and retained by a No. 100 mesh, which corresponds to a particle diameter 0.6 to 0.15 mm. was used in the experiment.

Apparatus.—The apparatus that was used was a modified McBain sorption balance. A 50-mg. sample of the protein was taken in a glass bulb of weight 150 mg. hanging from a quartz helix of high sensitivity. The helix was enclosed by a water-jacketed cylindrical glass vessel with suitable leads to the vacuum line, BF_3 reservoir and a mercury manometer. The reaction between BF_3 and the protein was followed by the extension of the helix caused by the increase in weight of the protein. The Gaertner cathetometer used to measure this extension could be read to 0.005 cm. The helix used had a sensitivity of 26.83 mg./cm., so that the accuracy of the balance was 0.2 mg., which for the sample size taken corresponded to ±0.05 mmole of BF_3/g. of protein (hereafter mmole/g.). The water in the jacket was thermostated to ±0.25°.

In runs in which a gaseous mixture was used, it was sometimes necessary to stir the gas. This was achieved by connecting the vessel to a Toepler pump, which alternately filled and emptied, at the rate of 3 cycles/minute. The volume of the Toepler bulb was $^1/_{26}$ of the volume of the vessel. The 4% change in pressure caused by the pulsing of the Toepler pump was shown not to be significant in the experiment.

Apiezon N grease was used in all the ground joints and stopcocks. The grease turned black slowly in the presence of BF_3, but control experiments showed that it absorbed very little or no BF_3.

Results and Discussion

Reproducibility.—Figure 1 shows the results of two runs on the rate of sorption of BF_3 by BPA, at 0.25°. The pressure of BF_3 was 64.0 cm. of mercury and was controlled manually to within 1% of this value. These runs were made at an interval of two weeks, using protein samples from the same batch. The reproducibility is seen to be within the experimental error.

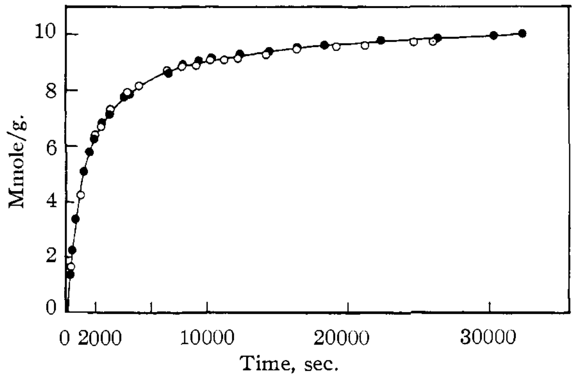

Fig. 1.—Reproducibility of rate of sorption of BF_3 by BPA; $T = 0.25°$; BF_3 pressure 64.0 cm. ○ and ● belong to two different runs.

Kinetic Studies.—From Fig. 1, it can be seen that there is a rapid initial reaction, which slows down considerably, in a short time. In this instance, the initial rate, which was 0.006 mm./g./sec.,

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[2] (a) S. W. Benson and J. M. Seehof, THIS JOURNAL, **73**, 5053 (1951); **75**, 3925 (1953); (b) J. M. Seehof and S. W. Benson, *ibid.*, **77**, 2579 (1955).

[3] S. W. Benson and D. A. Ellis, *ibid.*, **70**, 3563 (1948).

diminished to 0.002 mm./g./sec. after 17 minutes, but even after 82,000 seconds[4] the rate was 0.00009 mm./g./sec. The rate was barely measurable with this apparatus after 5 × 10^5 seconds. The amount of BF_3 that had been sorbed at that point was 11.15 mmole/g. The experimental data did not fit a first, second or fractional-order rate equation. The special type of second-order plot, *i.e.*, 1 mmole per g. *vs.* 1/time that Benson and Richardson[5] found applicable to the interaction of H_2O, C_2H_5OH and CH_3OH with egg albumin and BPA was not applicable in this case. The observation that 50% of the sorption was over in 0.03% of the total time suggested that the process was very complex and possibly the sum of two or more steps.

The effect of varying the temperature on the rate, at constant BF_3 pressure, is shown in Fig. 2. The rate is seen to change very little in the temperature range studied. The energy of activation was calculated from the slopes at equal amounts of BF_3 sorbed. In view of the difficulty in reading the small slopes involved, it was only possible to estimate a maximum value, which was 3.5 kcal./mole. This small value suggests that the process may be diffusion controlled.

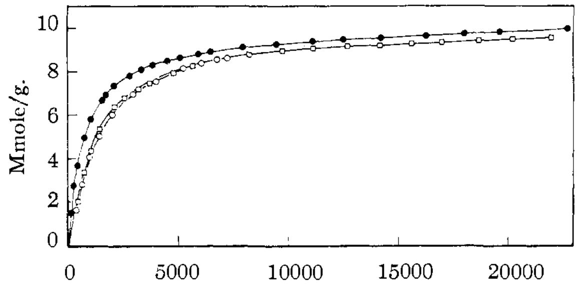

Fig. 2.—Influence of temperature on rate of sorption of BF_3 by BPA; BF_3 pressure 63.1 cm.: ○, T = 0.25°; □, T = 14.00°; ●, T = 28.25°.

The effect of varying the pressure of BF_3 on the rate is shown in Fig. 3. While a 3-fold change in pressure had a large effect on the rate, no simple correlation could be found between the two. In general, an increase in pressure can be expected to change the diffusion coefficient.

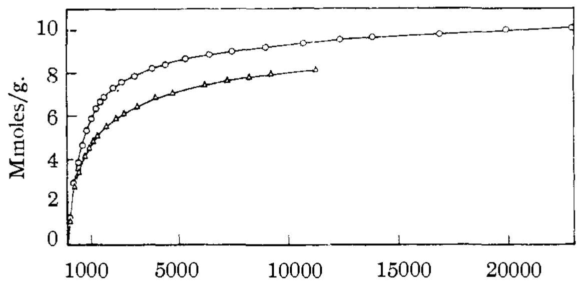

Fig. 3.—Influence of pressure on rate of sorption of BF_3 by BPA; T = 28.25°: ○, P = 68.2 cm.; △, P = 20.6 cm.

If the rate controlling step in the early stages of the sorption process can be considered equivalent to the diffusion of the gas through an infinite solid, then the amount sorbed, which is the amount diffusing through the solid, should be a linear function of $t^{1/2}$, where t is the time.[6] In Fig. 4, the amount of BF_3 sorbed has been plotted as a function of $t^{1/2}$, t in seconds, at two different temperatures. The agreement with a linear relationship is seen to be satisfactory, considering the difficulty in measuring the time and pressure accurately in the early stages of the reaction. Other examples of a reaction between a gas and a solid being diffusion controlled, and where the data fit a similar plot, are found in the literature, *e.g.*, the oxidation of iron at 200° studied by Vernon, *et al.*[7]

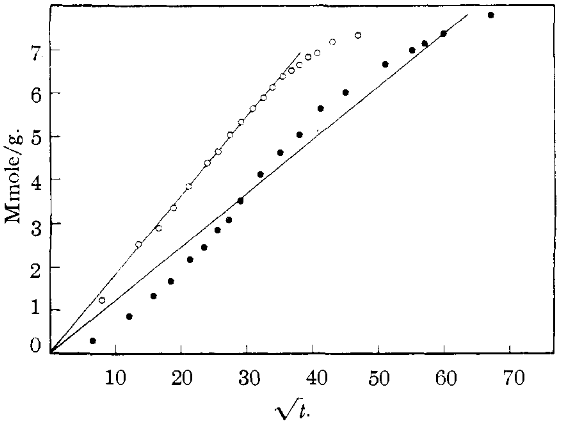

Fig. 4.—Plot of amount sorbed *vs.* $\sqrt{t}$ to show diffusion controlled process; time in seconds: ○, T = 28.25°; P = 64.2 cm.; ●, T = 0.50°; P = 61.6 cm.

In this system, it is necessary to examine if the temperature of the protein is the same as that of the water in the jacket especially in the early stages of the reaction. Seehof[8] observed that the interaction of BF_3 with egg albumin was exothermic to the extent of about 20 kcal./mole of BF_3 sorbed. Benson[9] has pointed out that in exothermic reactions, the temperature gradients in the reaction zone may be of considerable magnitude.

Several runs were carried out to determine whether temperature gradients did play a part in the interaction of BF_3 with BPA. The results of these runs are summarized in Fig. 5. Curve (a) indicates the rate using BF_3 alone at a pressure of 4.4 cm. of mercury at 28.50°. Curves (b) and (c) indicate the rate when using a mixture of 4.4 cm. of BF_3 and 63.8 cm. of helium, at the same temperature. In (b) the gaseous mixture was stirred, in (c) it was not. The purpose in using helium as a diluent was that if temperature gradients were large enough to influence the rate, then helium would reduce or eliminate them by virtue of its large heat conductivity. Since the rate curves (a) and (b) differ very little, it is to be concluded that temperature gradi-

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[4] This part of the curve is not included in the Fig. 1.

[5] S. W. Benson and R. L. Richardson, This Journal, **77**, 2585 (1955).

[6] W. Jost, "Diffusion in Solids, Liquids and Gases," Academic Press, Inc., New York, N. Y., 1952, p. 41.

[7] W. H. J. Vernon, *et al.*, *Proc. Roy. Soc.* (*London*), **216A**, 375 (1953).

[8] J. M. Seehof, private communication.

[9] S. W. Benson, *J. Chem. Phys.*, **22**, 46 (1954).

ents influence the rate only very slightly. Even the small difference between (a) and (b) may not be due to this effect alone, but may be caused by the slight fluctuation in pressure set up by the pulsing of the Toepler pump. If the reaction were carried out without stirring, as in (c) the rate decreased greatly. This must be attributed to the diminution in the concentration of BF_3 near the surface of the solid, as the reaction proceeded. It is realized that the fluctuation in pressure in (b) alters the conditions in this run as compared to (a) and (c). But since these results were qualitative no attempt was made to carry out these runs under more rigorous conditions. In the case of (d), the helium was replaced by an equal pressure of methane, and the mixture was stirred. In this instance, the diminution in rate can only be due to a depletion in the concentration of BF_3 in the pores of the solid, in spite of stirring. The relative sizes of the helium atom (diam. 2.66 Å.) and the methane molecule (diam. 3.2 Å.) makes this likely.

From the foregoing results it is possible to give a definite picture of the reaction between BF_3 and a protein, based on the model proposed by Benson and Seehof.[2a] On exposing the protein to BF_3 there is a rapid exothermic reaction[10] between BF_3 and the most accessible groups in the protein. The activation energy for the formation of a coördinate bond between the nitrogen atom and BF_3 in the gas phase, has been found to range from 2 kcal./mole for NH_3 to zero in the heavier amines.[11] If the activation energies for the other groups are of this order, it would explain the rapid reaction at room temperature.

The reaction slows down when BF_3 has to diffuse farther into the solid to find unreacted groups. The local heating effect, even though it cannot be measured accurately,[8] must be appreciable, since the heat of formation of a coördinate bond between the nitrogen in NH_3 and BF_3 is -42 kcal./mole[12]; between the oxygen atom in dimethyl ether and BF_3, about -14 kcal./mole.[13] The "solvation" process which is presumed to occur in the interaction of proteins with water, or HCl, or ammonia does not appear to take place with BF_3, since the amount of BF_3 in the protein, when the reaction reaches an end, is not more than the sum of the number of functional groups in the protein, which are capable of binding BF_3. This absence of solvation may be due to the large size of the BF_3 molecule compared to NH_3, HCl and H_2O. The fact that the temperature at which these studies were made is farther from the critical temperature of BF_3 (on a relative basis) than for the other three gases is also a factor. With the sorption of a large amount of BF_3 and especially on standing, the breakdown of the protein occurs. Studies on this and on desorption will be reported elsewhere.

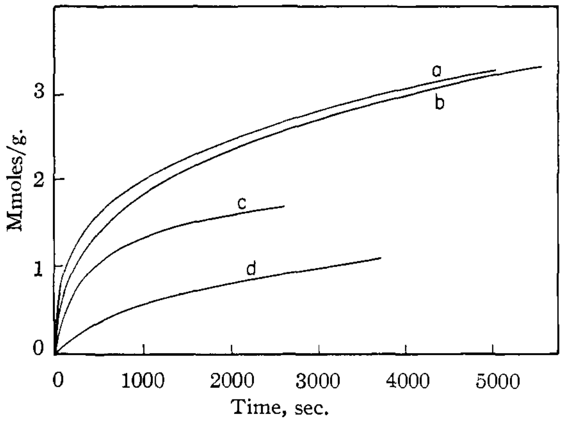

Fig. 5.—Rate of sorption by BPA using gas mixtures; $T = 28.5°$, in all cases: a, BF_3 (P = 4.4 cm.), no stirring; b, BF_3 (P = 4.4 cm.) + helium (P = 63.8 cm.), with stirring; c, BF_3 (P = 4.4 cm.) + helium (P = 63.4 cm.), no stirring; c, BF_3 (P = 4.4 cm.) + methane (P = 63.8 cm.), with stirring.

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[10] At the temperatures and pressures involved in this study, capillary condensation can be ruled out.

[11] D. Garvin and G. B. Kistiakowsky, *J. Chem. Phys.*, **23**, 338 (1955).

[12] S. H. Bauer, *et al.*, THIS JOURNAL, **65**, 889 (1943).

[13] H. S. Booth and D. R. Martin, "Boron Trifluoride and its Derivatives," John Wiley and Sons, New York, N. Y., 1949, p. 42.