parallelism between the enthalpy, or entropy, of binding and the type of spectrum given by the complex.

It is of interest, nevertheless, to note that compounds IX and VI, with the flexible side chains, show the smallest entropies of binding. Since the spectroscopic behavior of IX and VI indicates that the flexible side chains are twisted backward in order to accommodate themselves to the specific protein sites involved in the binding, this restriction on free rotation may reduce the configurational entropy and thus compensate in large part for the increase in entropy due to release of water molecules on binding. In line with this view are the substantially larger entropies of binding of these compounds, IX and VI, by bovine albumin. With the bovine protein, the spectra are "normal" and show no isomeric specificities, so that there is not as restricted a configuration for the flexible side chains of these bound molecules.

Conclusions.—The thermodynamic quantities for the binding process thus reflect the contributions of all portions of the small molecule in the formation of the complex with the protein. If the substituents on a particular molecule are not situated in positions sterically favorable for interaction with particular side chains of the protein, binding may still be strong because of interaction with other portions of the macromolecule.

Spectra, on the other hand, reflect the interactions of particular sites on the protein with specific substituents of the bound molecule, even though these bonds may not be the major contribution to the energetics of binding. Since these are specific interactions, the spectra are very sensitive to steric effects. With the present series of molecules two types of interaction are observed. Molecules (I, 3) and (VII) form one type of spectroscopic complex, whereas (I, 1) and (I, 2) comprise a second type. The distances between substituents of each molecule of the latter pair are essentially the same, 12–13 Å.; the separations of substituents in the former pair are markedly different, 9 and 15 Å., respectively, and yet they behave alike. Spectra of the complexes thus reflect very clearly differences in distances between substituents of the small molecule and hence between specific sites on the protein.

Acknowledgments.—These investigations were assisted by grants from the Office of Naval Research, Department of the Navy (NR 124-054), and from the Rockefeller Foundation.

EVANSTON, ILLINOIS

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

A Study of Compound Formation between BF_3 and Proteins[1]

BY R. SRINIVASAN AND SIDNEY W. BENSON

RECEIVED APRIL 9, 1956

A detailed study of the nature of the complexes formed by solid proteins on sorbing BF_3 has been carried out. The first method that was used was to find the amounts of BF_3 bound irreversibly by several proteins and see if any correlation existed between these values and the amino acid compositions of the proteins. The method proved inconclusive since the BF_3 irreversibly bound was a function of the temperature of desorption in all the cases studied. The more rigorous method of examining the desorption isotherm for isobaric regions was also undertaken. Owing to the practical difficulties in this method only the egg albumin–BF_3 system was studied. No stoichiometric compounds were observed. From the continuity in the desorption rate data, and the similarity between such data for all the proteins, it was concluded that stoichiometric compounds did not exist in any of these systems. A hypothesis as to the nature of protein–BF_3 complexes is proposed. Supporting evidence is obtained from the fit of the data to the Freundlich equation, and the interaction of poly-γ-benzyl-L-glutamate and Nylon with BF_3.

Among the polar gases whose interaction with proteins is of interest from the point of view of compound formation, BF_3 offers much scope for investigation. It is a strong Lewis acid, which can form coördinate links with the oxygen, nitrogen, and possibly the sulfur atoms in proteins.[2] Sufficient information is available in the chemical literature to indicate that in simple organic compounds the same functional groups which are found in the amino acid residues constituting the protein chain, do form stoichiometric compounds with BF_3. Thus the ω-amino group in lysine can be expected to react with 1 mole of BF_3 in the manner of ethylamine.[3] Though the guanidino group in arginine, and the imidazole group in histidine contain 2 nitrogen atoms apiece, it is probable from steric considerations that they would interact with only one mole of BF_3 each. The work of Landolph[4] and Bowlus and Nieuwland[5] indicates that amides and BF_3 form 1:1 compounds. Hence the amide groups in proteins can be expected to bind BF_3.[6]

Both oxygen and sulfur atoms have been known to form coördination compounds with BF_3. Hence the oxygen atoms in the alcohol (serine, threonine), phenol (tyrosine), and carboxyl (aspartic and glutamic acids; also the ester group in poly-γ-benzyl-L-glutamate) groupings of a protein are all poten-

(1) (a) This work has been supported by a research grant (G-3541-C2) from the U. S. Public Health Service, National Institutes of Health, Bethesda, Maryland. (b) The material presented in this paper is taken from the dissertation submitted by R. Srinivasan to the Graduate School of the University of Southern California in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(2) Carbon acts as a donor only in olefinic compounds. These are not encountered in proteins.

(3) C. A. Kraus and E. H. Brown, THIS JOURNAL, **51**, 2690 (1929).

(4) F. Landolph, *Ber.*, **12**, 1578 (1879).

(5) H. Bowlus and J. A. Nieuwland, THIS JOURNAL, **53**, 3835 (1931).

(6) Tryptophan, which contains an indole group, has not been considered, since, among the proteins that were studied, it amounts to only 0.16 mmole/g. in fibrin and less than 0.1 mmole/g. in all the other cases.

tially capable of binding BF_3.[7] However, from steric considerations, only one BF_3 molecule can be expected to add on to the carboxyl group. This is borne out by the work on esters and acids.[8] The results on the coördination compounds of organic sulfur derivatives with BF_3 is limited to the work of Axe.[9] From this work, the methyl thio group (in methionine and cysteine) and the dimethyl disulfide group (in cystine) in proteins seem likely to interact with BF_3.

The peptide group contains both a nitrogen and an oxygen atom. Since this group constitutes more than $^3/_5$ of all of the functional groups in a protein capable of binding BF_3,[10] it is necessary to consider in detail the steric considerations involved in the coördination of BF_3 to either the nitrogen or oxygen atom or both. This has been presented in the discussion below.

The work of Benson and Seehof[11] showed that solid proteins sorbed large quantities of BF_3, a large part of which was irreversibly bound. They stated that the pressure dependence of this irreversible binding should be fully studied before any correlation between the structure of the protein and the BF_3 content can be found.

In this paper, the results on the interaction of BF_3 with several proteins and two synthetic polypeptides are reported. Sufficient data are now available so that it is possible to draw some conclusions concerning the nature of protein–BF_3 complexes.

Experimental

The preparation of protein samples in a finely divided form suitable for interaction with gases has been described already.[12] The proteins that were studied were egg albumin, natural and denatured, β-lactoglobulin, zinc insulin, lactalbumin, edestin, bovine plasma albumin (hereafter BPA) and casein. The synthetic polypeptides, Nylon and poly-γ-benzyl-L-glutamate (hereafter PBG) that were also used in this study were obtained in a finely divided form by special methods. Nylon[13] was dissolved in pure, molten phenol (about 5% solution). This solution was sprayed on the surface of an 0.1*N* aqueous solution of NaOH kept in a shallow trough and heated to 80°. When the droplets of the phenol solution hit the aqueous phase, Nylon was precipitated as a thin film, which was skimmed off mechanically. The film was washed with water followed by ethanol, which removed the last traces of phenol. Photo-micrograph and electron microscopic studies showed that the film had an open sieve-like structure with filaments of 0.2 to 2 μ in thickness. The BET surface area of the sample,[14] which was calculated from N_2 isotherm measurements, was found to be 11 sq. meters/g.

PBG[15] was prepared for study by taking a 2% solution of the sample in chloroform and adding ethanol, whereupon the polypeptide was precipitated in a fibrous form. This was washed with alcohol and dried in a vacuum. X-Ray diffraction photographs of this sample[16] indicated that it had the same structure as the form studied by Bamford, *et al.*,[17] and interpreted by Pauling and Corey[18] to be an α-helical structure.

Results

Pressure Dependence.—The amount of BF_3 permanently bound to a protein was found by Seehof and Benson[11] to be function of the sorption pressure, up to a partial pressure of 10 cm. The explanation for this pressure dependence is the influence of pressure on the rate of sorption of BF_3 which has been discussed already.[19] It was found from the present study that when the sorption pressure was over 20 cm. the amount of BF_3 in the protein at the sorption maximum reached a value greater than the amount of BF_3 permanently bound at room temperature,[20] in a few days. However, in order to increase the rate of sorption, the partial pressure of BF_3 during sorption was held at 76 cm. in all the results reported here.

BF_3 Permanently Bound by Proteins.—It was realized that the permanent binding of BF_3 by proteins is dependent, among other factors, on the desorption temperature. The criteria involved in the definition of "permanently" bound gas have been discussed in detail elsewhere.[21] It is sufficient to state here that, since these criteria describe a rate phenomenon, and since this rate is itself temperature dependent, the criteria should include an arbitrary temperature. In Table I the amounts of BF_3 permanently bound by six proteins at various temperatures are presented. In all these cases the sorption was carried out at 20° at a BF_3 pressure of 76 cm. Desorption at each temperature was continued until the loss of weight of the sample in a 24-hour period fell below 0.05 mmole/g. These values show that the amount of BF_3 permanently bound decreases continuously with increasing temperature in all the cases, the value falling to zero in three cases. Similar results were obtained with lactalbumin, fibrin and denatured egg albumin. In Figs. 1 and 2 the rate of desorption of BF_3 from egg albumin and β-lactoglobulin at different temperatures has been presented graphically. The limiting value for the BF_3 content at each temperature is the BF_3 permanently bound for that protein at that temperature. These are the values given before in Table I. The rate data did not fit any simple kinetic equation. This is not surprising if, as we shall see, the rates represent a complex process, involving diffusion as well as desorption from a number of energetically different sorption sites. The rate data further show that in spite of increasing the temperature, the rate of desorption diminishes continuously with decreasing

(7) H. S. Booth and D. R. Martin, "Boron Trifluoride and its Derivatives," John Wiley and Sons, Inc., New York, N. Y., 1949, p. 42.

(8) H. S. Booth and D. R. Martin, ref. 7, pp. 65, 74.

(9) W. N. Axe, U. S. Patent 2,378,968 to Phillips Petroleum Co., (June 26, 1945).

(10) The sum of the amounts of functional groups capable of binding BF_3, in various proteins has been included in Table I.

(11) S. W. Benson and J. M. Seehof, THIS JOURNAL, **73**, 5053 (1951); **75**, 3925 (1953); also J. M. Seehof and S. W. Benson, *ibid.*, **77**, 2579 (1955).

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

(13) The sample of Nylon used was very kindly supplied by E. I. du Pont de Nemours and Company, Wilmington, Delaware. It has a number average mol. wt. of 14,500.

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

(15) The sample of PBG was kindly supplied by Dr. A. Elliott of Courtaulds, Ltd., England.

(16) We are indebted to Dr. Jerry Donohue of this Laboratory for his help in carrying out the X-ray studies.

(17) C. H. Bamford, W. E. Hanby and F. Happey, *Proc. Roy. Soc. (London)*, **A205**, 30 (1951).

(18) L. Pauling and R. B. Corey, *Proc. Natl. Acad. Sci.*, **37**, 241 (1951).

(19) R. Srinivasan and S. W. Benson, THIS JOURNAL, **78**, 2405 (1956).

(20) BF_3 permanently bound at room temperature was less than 10 mmoles/g. in all the cases studied. See Table I.

(21) S. W. Benson and R. Srinivasan, THIS JOURNAL, **77**, 6371 (1955).

TABLE I

BF_3 PERMANENTLY BOUND BY PROTEINS AND POLYPEPTIDES AT VARIOUS TEMPERATURES (VALUES IN MILLIMOLES BF_3/G. PROTEIN)

		Egg albumin	Zinc insulin	β-Lacto-globulin	Edestin	Bovine plasma albumin	Casein	Nylon	PBG
Total amount of functional groups[a]		13.35	13.16[b]	13.27	13.70	13.64	12.82	8.98[b]	9.12
Temp., °C.	0	9.2	....	...	...	9.1	8.6	9.2	..
	24	7.0	5.0	7.2	9.6	7.1	7.9	9.0	3.0
	39	6.0	3.9	6.2	7.6	5.8	6.3	9.0	0
	60	3.9	3.5	4.4	5.9	...	...	...	..
	70	2.7	2.2	2.8	4.6	...	...	...	..
	80	0	0	0	1.8	...	...	...	..

[a] Data on protein composition from G. R. Tristram, "The Amino Acid Composition of Proteins," Chapter 3 of "The Proteins," H. Neurath and K. Bailey, Academic Press, Inc., New York, N. Y., 1953. [b] Includes end groups.

BF_3 content.[22] But at 80° there is a sudden increase in the rate of desorption, and the rate curve is seen to dip below zero BF_3 content. Since the BF_3 content of a protein cannot have negative values, it was inferred that part of the protein molecule was also volatilizing with the BF_3. To test this, microanalyses were conducted on the original sample of β-lactoglobulin and on the end product after desorbing all the volatile matter that came off at 80°. The compositions in the two cases corresponded to $C_1N_{0.26}H_{1.93}$ and $C_1N_{0.24}H_{1.84}$.[23] This showed that the protein had lost nitrogen presumably as ammonia, which would have formed NH_3-BF_3. From a blank run in which pure dry β-lactoglobulin was heated by itself to 80° for 15 days in a vacuum, it was established that this was not sufficient to give rise to volatile products (*i.e.*, no loss in weight).

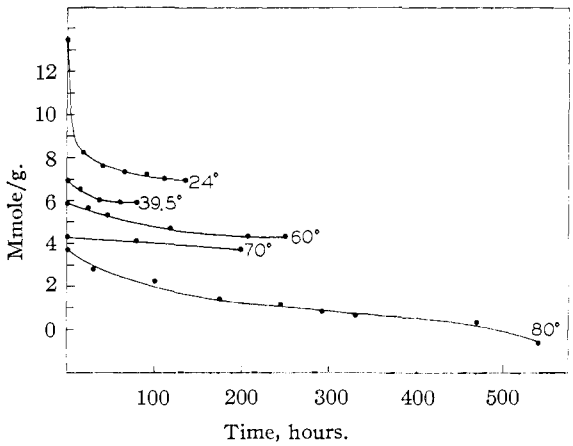

Fig. 1.—Rate of desorption of BF_3 from egg albumin at various temperatures. Sorption at 1 atm. BF_3, 20°. Desorption carried out on same sample at successively higher temperatures.

Desorption Isotherm.—The above results showed that the only way of establishing the existence of stoichiometric compounds in the protein–BF_3 system is to measure the equilibrium vapor pressure of BF_3 above the protein–BF_3 complex as a function of composition, at constant temperature. The presence of isobaric regions would indicate the presence of stoichiometric compounds, since in a region of composition where the equilibrium is

$$\text{Protein}:BF_3(s) \rightleftharpoons \text{Protein}(s) + BF_3(gas)$$ [24]

operative, fixing the temperature should fix the vapor pressure too.

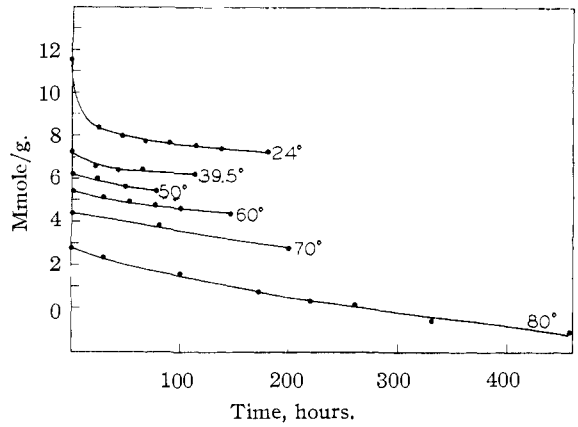

Fig. 2.—Rate of desorption of BF_3 from β-lactoglobulin at various temperatures. Sorption at 1 atm. BF_3, 20°. Desorption carried out on same sample at successively higher temperatures.

The isotherm for the system egg albumin–BF_3 was determined at 31.5°. By using a mercury manometer in conjunction with a specially designed McLeod gage, it was possible to read gas pressures continuously from 76 cm. down to 10^{-5} cm. It was found that equilibrium between the gas and solid was reached in about 24 hours in the range down to 10^{-3} cm. Below this pressure, equilibrium was reached only after about 48 hours and the outgassing of the walls in that time introduced an error for which the measured pressures had to be corrected. For this reason, the isotherm was not extended to pressures lower than 10^{-4} cm.

In Fig. 3 the isotherm at 31.5° for the egg albumin–BF_3 system has been plotted as log composition *vs.* log P. The figure shows the absence of isobars in the range of composition studied, thus indicating that stoichiometric compounds do not exist. For a system which obeys the Freundlich equation

$$x/m = KP^a$$

where x is the amount of gas sorbed by m units of

(22) It is the initial rates of desorption at different temperatures that are compared.

(23) The oxygen content was not specified; the end product did not contain any fluorine.

(24) This is a general equation; more specifically a similar equation can be written for each type of functional group that binds BF_3.

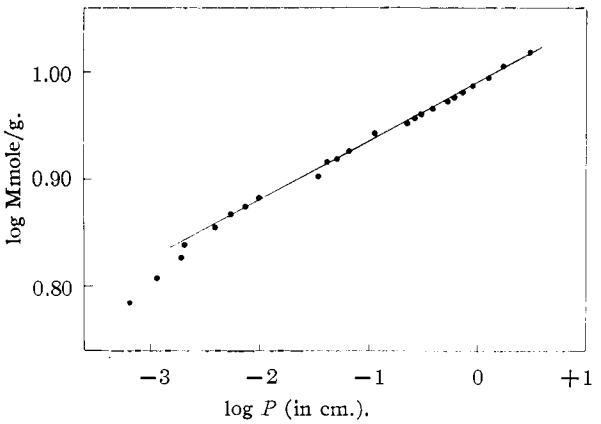

Fig. 3.—Freundlich plot of data on egg albumin–BF_3 system; temp. 31.5°.

sorbent and P the partial pressure of the gas, the plot of log composition *vs.* log P should be linear. This is found to be so in Fig. 3 over the range of pressures from 3.1 to 0.005 cm.

The results for the synthetic polypeptides PBG and Nylon are also included in Table I. The behavior of PBG was very similar to that of the proteins in that (1) the BF_3 bound permanently, even at its maximum, was considerably less than the total number of functional groups present in the molecule, (2) this value tended to decrease on increasing the temperature, becoming zero ultimately.[25] A study of the X-ray diffraction pattern of PBG:BF_3 complex also was made. The pattern was not significantly different from that of the starting material itself, indicating that the coiled structure of the PBG molecule had not been altered or destroyed on sorbing BF_3.

In contrast to PBG, Nylon appeared to form a stable complex made up of 9.0 mmole./g. of BF_3. This was found to be stable up to 39°. The agreement between this composition and the number of functional groups in Nylon, which is 8.98 mmole/g. is striking.

Discussion

From the absence of isobars in the isotherm at 31.5° for the system egg albumin–BF_3, it can be definitely said that stoichiometric compounds of BF_3 with the protein do not exist in this range of composition. From the continuity in the rate data on the desorption of BF_3 from this protein (Fig. 1) it seemed reasonable to extend this conclusion to the whole range of composition. From the similarity in the desorption rate data for egg albumin and the other proteins studied,[26] it appears likely that stoichiometric compounds do not exist in any of these systems. However, it is relevant to discuss the nature of the complexes formed by BF_3 with proteins.

The fit of the desorption data to the Freundlich equation is noteworthy since Zeldowitch[27] showed that this would be true if the sorption sites possessed an exponential distribution of binding energies.[28] Such a spread in the binding energies could be due to the dependence of the free energy of binding not only on the nature of the atom to which BF_3 coördinates, but on the position of the atom in the amino acid residue. The latter factor would determine the steric hindrance that has to be overcome to form a bond. The sequence of the amino acid residues may also be a contributing factor. Such steric hindrance has been recognized even in the interaction of BF_3 with simple substances.[29] It is possible to illustrate it in the case of the peptide groups in the protein chain, which constitute more than 60% of the BF_3 binding capacity of the proteins studied. In Fig. 4 two views of the peptide

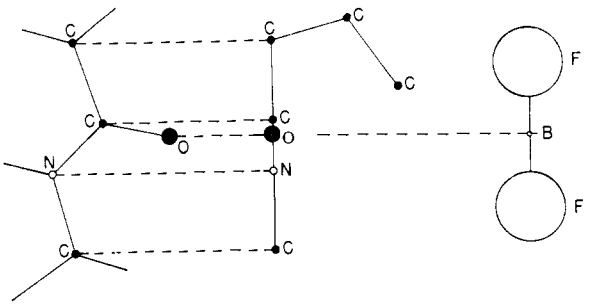

Fig. 4.—BF_3 molecule and two views of peptide group. Only fluorine atoms drawn on same scale as bond lengths. Dimensions of N, C, O and B atoms arbitrary B–F bond distance after Braune and Pinnow.[31] Radius of F atom after Pauling.[32] Peptide bond distances after Corey and Donohue.[30]

group have been drawn to scale, based on the dimensions proposed by Corey and Donohue,[30] from their results on X-ray studies on simple peptides. In the same figure, a picture of the BF_3 molecule is also drawn. The B–F bond distance was taken from the electron diffraction studies of Braune and Pinnow,[31] while the covalent bond radius of the fluorine atom is the value of 0.64 Å. given by Pauling.[32] It is seen that where the R-group of the amino acid residue is composed of two or more carbon atoms it will offer considerable hindrance to the close approach of the BF_3 molecule to either the nitrogen or the oxygen atom. Hence, depending on the nature of the R-group, BF_3 may not or at best only weakly coördinate with either of these atoms to form a complex. In the light of this hypothesis, the behavior of PBG and nylon can be easily explained. PBG, in which the R-group is composed of a three carbon chain with a terminal ester group, did not sorb, even at the sorption maximum, the stoichiometric amount of BF_3, which would correspond to its total content of functional groups. Even this amount of BF_3 was lost easily on desorp-

(25) On sorbing BF_3, PBG turned a deep orange color. When all the BF_3 was removed, the material became colorless again. Some experiments with simple organic compounds indicated that this color is characteristic of complexes of BF_3 with esters containing a benzyl radical. This is the most direct evidence showing that BF_3 that is sorbed by PBG is actually coördinated to the polypeptide molecule.

(26) The desorption rate curves for all the proteins studied were qualitatively similar to those in Figs. 1 and 2. These have not been reproduced here.

(27) J. Zeldowitch, *Acta Phys. Chem., U.R.S.S.*, 1, 961 (1934).

(28) Similarly, the desorption of water, methanol and ethanol from egg albumin also fit the Freundlich equation. *Vide* R. L. Richardson, Ph.D. Thesis, University of Southern California, 1954, p. 51.

(29) H. S. Booth and D. R. Martin, ref. 7, p. 83.

(30) R. B. Corey and J. Donohue, THIS JOURNAL, **72**, 2899 (1950).

(31) H. Braune and P. Pinnow, *Z. physik. Chem.*, **B35**, 239 (1937).

(32) L. Pauling, "Nature of the Chemical Bond," Cornell University Press, Ithaca, New York, 1948, p. 164, Table 21.2.

tion, at moderate temperatures. On the other hand, Nylon, in which the R-group is only a hydrogen atom, sorbed the stoichiometric amount of BF_3 to form a complex which did not break down on desorbing at temperatures up to 39°.

The evidence presented thus indicates that the strong, "permanent" binding of BF_3 by proteins is not stoichiometric in nature. On the other hand the fact that the total BF_3 sorption can be experimentally separated into chemically (*i.e.*, permanent) and physically sorbed parts does point to a specific, chemical interaction for this permanently bound portion despite the absence of stoichiometry. The reason for the non-stoichiometric addition of BF_3 may be at least threefold. One is the dispersity of particle size of the solid protein samples used; the second is the interaction between sorbed groups and the third is lack of true equilibrium in the system.

The first explanation cannot be completely ruled out by the results of the present investigation since no systematic studies were made of the effects of particle size on BF_3 sorption. However, the fact that different samples of protein did give quite reproducible results is an indication that this may not be too important an effect.[33] In addition the results of Benson and Zwanzig[34] on H_2O sorption indicates that in a comparable system, particle dispersity is an unimportant factor.

The second and third explanations cannot be completely disentangled. The high concentrations of permanently bound BF_3 (*i.e.*, about 10 *M* per volume of dry protein) leads to an average distance of separation of about 10 Å. between binding sites. In view of the diverse nature of the functional groups present in the protein and the relatively large size of the BF_3 molecule it is not at all unreasonable to expect a "smearing" out of the differences in binding energies and in chemical potential of the different sites leading to a behavior resembling that of solid solution. Such behavior would still be compatible with the observations of Seehof and Benson,[11] that in back-titration with NH_3, stoichiometric relations could be found between very strongly binding groups and the amounts of BF_3 not back-titrated by NH_3. If these back-titrations are not all fortuitous, they could be interpreted as arising from "energy bands" in the solid protein which could appear as non-stoichiometric binding from the rigorous test of a phase diagram though still stoichiometric from the point of view of NH_3:BF_3 formation if the "gaps" between the different sites were sufficiently large. For a more rigorous test of this model, it would be necessary to study the effects of temperature on the back titrations.

LOS ANGELES, CALIF.

(33) Even though such samples usually came from the same "batch," it is unlikely that the particle dispersity was the same since the larger particles usually accumulate at the bottom of the bottle.

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

[CONTRIBUTION FROM THE INSTITUTE FOR ENZYME RESEARCH, UNIVERSITY OF WISCONSIN]

On the Mechanism of Dehydrogenation of Fatty Acyl Derivatives of Coenzyme A. IV. Kinetic Studies

BY JENS G. HAUGE[1]

RECEIVED JANUARY 25, 1956

The kinetics and the equilibrium of the enzymatic α,β-oxidation of fatty acyl derivatives of CoA were investigated. With indophenol as acceptor the following three step mechanism of reaction appears likely: electrons are passed from substrate to a primary dehydrogenase (flavoprotein), thence to a specific electron transferring enzyme (flavoprotein) and finally to the dye. The interaction of the two enzymes concerned with their respective substrates, *i.e.*, fatty acyl CoA and reduced dehydrogenase, follows Michaelis–Menten behavior. The three primary dehydrogenases differ markedly in their ability to react with phenazine–cytochrome *c* as acceptor system as compared to flavoprotein–indophenol. With pyocyanine as acceptor a measurable equilibrium was established, and the oxidation–reduction potential E_0' of the system butyryl CoA–crotonyl CoA was determined to be −0.015 v. It is emphasized that oxidative enzyme systems, the components of which have tightly bound prosthetic groups, have unique kinetic properties. Traditional Michaelis constants and turnover numbers of such enzymes cannot be obtained when over-all reactions are measured. The observed constants give the kinetic characteristics of the total substrate–enzyme–acceptor system.

During the last two years four flavoproteins have been shown to participate in the first oxidative step of the fatty acid cycle as it occurs in pig liver mitochondria.[2-5] The evidence indicates that FAD is the prosthetic group in at least three of these enzymes.[6] Three of these are primary dehydrogenases, differing in their substrate specificity and other characteristics. They have been named butyryl dehydrogenase,[5,7] general fatty acyl dedrogenase[2] and palmityl dehydrogenase,[4] abbreviated G, Y and Y′, the letters indicating their green and yellow colors, respectively.

The fourth of these flavoproteins functions in the

(1) Postdoctoral trainee of the National Heart Institute, National Institutes of Health. Present address, Department of Biochemistry and Microbiology, Central Institute for Industrial Research, Oslo, Norway.

(2) F. L. Crane, S. Mii, J. G. Hauge, D. E. Green and H. Beinert, *J. Biol. Chem.*, **218**, 701 (1956).

(3) F. L. Crane and H. Beinert, *ibid.*, **218**, 717 (1956).

(4) J. G. Hauge, F. L. Crane and H. Beinert, *ibid.*, **219**, 727 (1956).

(5) F. L. Crane, J. G. Hauge and H. Beinert, *Biochim. Biophys. Acta*, **17**, 293 (1955).

(6) The following abbreviations will be used: CoA, coenzyme A; C_n, acyl CoA with *n* carbon atoms in the acyl moiety; FAD, flavin-adenine dinucleotide; indophenol, 2,6-dichlorophenolindophenol; OD, optical density; Δ_{OD}, change of OD; phenazine, phenazine methosulfate; Tris, tris-(hydroxymethyl)-aminomethane; G, Y, Y′ and ETF are defined in the text; E_p, primary dehydrogenase (G, Y, Y′); GH_2, YH_2, etc., are the reduced forms of G, Y, etc.; $C_4 - E_p$, C_4 used with E_p.

(7) D. E. Green, S. Mii and H. R. Mahler, *J. Biol. Chem.*, **206**, 1 (1954).