lower than those reported in Table II for triethylamine and methyl iodide and similar data obtained for triethylamine and ethyl iodide. Moreover, the reaction of quinuclidine with methyl iodide is so rapid (it is essentially complete in four minutes at room temperature with reagents present in as low concentrations as 0.0132 mole per liter), that in this reaction especially the results are subject to considerable uncertainty. It is estimated that the errors in the energies of activation may be as large as 500 cal.

The rate constants obtained are listed in Table III.

TABLE III

RATE CONSTANTS FOR THE REACTION OF TRIETHYLAMINE AND QUINUCLIDINE WITH ALKYL IODIDES

Amine	Alkyl iodide	T, °C.	k (liters mole^{-1} sec.$^{-1}$)
Triethylamine	Methyl	25.0	3.29×10^{-2}
Triethylamine	Ethyl	25.0	1.92×10^{-4}
		35.0	3.81×10^{-4}
		45.0	7.24×10^{-4}
Quinuclidine	Methyl	5.5	0.610
		15.0	1.06
		25.0	1.88
Quinuclidine	Ethyl	5.5	1.27×10^{-2}
		15.0	2.55×10^{-2}
		25.0	4.87×10^{-2}
		35.0	8.34×10^{-2}
Quinuclidine	Isopropyl	25.0	7.97×10^{-4}
		35.0	17.2×10^{-4}
		45.0	31.6×10^{-4}

Values for the energies of activation and log PZ calculated from these values for the specific rate constants are listed in Table I.

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Summary

1. Quinuclidine has been prepared in good yield starting with the commercially available 4-pyridineëthanol. Quinuclidine hydrochloride has been prepared and characterized.

2. Kinetic studies have been made of the reaction of triethylamine with ethyl iodide and of quinuclidine with methyl, ethyl and isopropyl iodide in nitrobenzene solution at several temperatures.

3. A number of generalizations are proposed for the effects of changing steric requirements on displacement reactions. These generalizations, based largely on analogy with the effect of steric requirements on the stability of addition compounds, permit a simple interpretation of the experimental data. The results do not support previous interpretations based primarily on postulated polar effects of structural changes in alkyl groups.

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[CONTRIBUTION FROM CHEMISTRY DEPARTMENT, NORTHWESTERN UNIVERSITY MEDICAL SCHOOL]

Monolayers of Pepsin and of Insulin

BY HECTOR A. DIEU[1] AND HENRY B. BULL

Monolayers of egg albumin and of β-lactoglobulin have been investigated at surface pressures below one dyne per centimeter.[2] The equation represents the relation between the area A and the film pressure F for monolayers of these proteins in the low pressure region. When FA is plotted against F, the slope of the line (α) yields the gaseous area of the film and at 25° the intercept β is equal to 24.6×10^2/mol. wt. where mol. wt. is the number average molecular weight of the film molecules.

$$FA = \alpha F + \beta \qquad (1)$$

It has been found that spread films of pepsin and of insulin obey eqn. 1, and it has thus been possible to calculate the film molecular weights as well as the gaseous area of these proteins.

Experimental

The pepsin was prepared according to Northrop,[3] and its activity was found to be 0.19 Hb. U. per mg. of nitrogen. The pepsin solutions were dialyzed against 0.2 M acetate buffer at pH 5.2 before use. The pepsin concentration was determined by micro-Kjeldahl using the factor indicated by Northrop.[3]

We are grateful to Armour and Company for a supply of crystallin insulin. Before use the insulin was dialyzed against a buffer at pH 3.5 for seventy-two hours at 0°. The concentration of insulin was determined by micro-Kjeldahl.

A Wilhelmy balance has been used to register the film pressures. The wettability of the slides has been improved by the addition of glycerol to the 5% ammonium sulfate solution to the extent of 2% by volume. It is necessary to exercise care in the preparation of the substrate solutions, and the glycerol was exhaustively extracted with petroleum ether and the ether removed completely before addition to the ammonium sulfate solution. The ammonium sulfate solutions were treated with activated charcoal and the charcoal removed by filtering before the addition of the glycerol. The protein solutions were added to the surface with a Blodgett pipet which delivered 0.085 cc. of solution.

Results

The molecular weights and the gaseous areas of spread films of pepsin and of insulin have been calculated with the aid of eqn. 1. Tables I and II

(1) Research Fellow of the Belgian American Foundation.

(2) Bull, THIS JOURNAL, **67**, 4 (1945); **68**, 745 (1946).

(3) Northrop, *J. Gen. Physiol.*, **30**. 177 (1946).

show the gaseous areas and molecular weights of pepsin as a function of the elapsed time between spreading and compression. The results in Table I were obtained using a spreading concentration of 0.38 mg. of pepsin per square meter, while Table II shows results obtained using 0.74 milligram per square meter as the spreading concentration of pepsin.

TABLE I

FILM MOLECULAR WEIGHTS AND GASEOUS AREAS OF PEPSIN WHOSE SPREADING CONCENTRATION WAS 0.38 MG./SQ. M.

Time, minutes	Gaseous area, sq. m./mg.	Molecular weights
0.5	0.60	32,000
1	.74	32,500
2	.86	35,000
5	.92	35,400
10	1.08	36,200
60	1.24	33,000
360	1.35	37,000

TABLE II

FILM MOLECULAR WEIGHTS AND GASEOUS AREAS OF PEPSIN WHOSE SPREADING CONCENTRATION WAS 0.74 MG./SQ. M. ON 5% AMMONIUM SULFATE

Time, minutes	Gaseous area, sq. m./mg.	Molecular weights
0.5	0.4–0.50	35,200
2	.61	34,600
6	.73	34,000

Table III shows the influence of elapsed time between spreading and compression on the properties of pepsin films spread on hydrochloric acid at *p*H 1.2 instead of on ammonium sulfate.

TABLE III

FILM MOLECULAR WEIGHTS AND GASEOUS AREAS OF PEPSIN WHOSE SPREADING CONCENTRATION WAS 0.38 MG./SQ. M. ON HYDROCHLORIC ACID AT *p*H 1.2

Time, minutes	Gaseous areas, sq. m./mg.	Molecular weights
1	1.25	33,400
15	1.40	34,500
16	1.48	37,000
100	1.48	37,000

Table IV shows the influence of cupric ions on the pepsin films.

TABLE IV

FILM MOLECULAR WEIGHT AND GASEOUS AREA OF PEPSIN WHOSE SPREADING CONCENTRATION WAS 0.38 MG./SQ. M. ON 5% AMMONIUM SULFATE CONTAINING 0.08 MOLAR COPPER SULFATE

Time, minutes	Gaseous area sq. m./mg.	Molecular weights
1	1.40	34,000
20	1.44	32,500
45	1.49	35,000

Table V shows the influence of time on an insulin film whose spreading concentration was 0.417 mg./sq. m. on 5% ammonium sulfate.

TABLE V

FILM MOLECULAR WEIGHTS AND GASEOUS AREAS OF INSULIN WHOSE SPREADING CONCENTRATION WAS 0.417 MG./SQ. M. ON 5% AMMONIUM SULFATE

Time, minutes	Gaseous areas, sq. m./mg.	Molecular weights
0.5	0.70	19,700
2	1.07	18,000
30	1.13	19,000
270	1.26	20,100
1200	1.74	18,700

Table VI shows the influence of cupric ions on the film molecular weight and on the gaseous area of insulin films whose spreading concentration was 0.417 mg./sq. m. The films were compressed two minutes after spreading.

TABLE VI

FILM MOLECULAR WEIGHTS AND GASEOUS AREAS OF INSULIN FILMS SPREAD ON 5% AMMONIUM SULFATE CONTAINING VARYING AMOUNTS OF COPPER SULFATE

$CuSO_4$, moles/liter	Gaseous areas, sq. m./mg.	Molecular weights
0	1.07	18,000
2×10^{-4}	0.98	35,100
4×10^{-4}	.97	38,200
100×10^{-4}	.89	37,900

Table VII shows the influence of time on the properties of an insulin film spread on a 5% ammonium sulfate solution containing 1×10^{-2} molar copper sulfate.

TABLE VII

MOLECULAR WEIGHTS AND GASEOUS AREAS OF INSULIN WHOSE SPREADING CONCENTRATION WAS 0.417 MG./SQ. M. ON A 5% AMMONIUM SULFATE CONTAINING 1×10^{-2} MOLAR COPPER SULFATE

Time, minutes	Gaseous area, sq. m./mg.	Molecular weights
2	0.89	37,900
30	1.05	35,800
120	1.13	37,200

Discussion

Apparently, the film molecular weight of pepsin is influenced neither by the spreading process nor by the composition of the substrate solution. The average value of the film molecular weight of pepsin is 34,400 which compares favorably with that reported for the molecular weight of pepsin in solution.[4] We did find a film molecular weight of about 18,000 for a pepsin solution which had been incompletely dialyzed and no doubt contained split products of pepsin. Curiously, the addition of copper sulfate to the substrate solution in this case restored the molecular weight to 35,000.

The gaseous area of pepsin films is greatly dependent both on the spreading concentration and on the elapsed time between spreading and compression. It appears from Table I and particularly from Table II that at short time intervals and

(4) Northrop, Kunitz and Herriott, "Crystalline Enzymes," Columbia University Press, New York, N. Y., 1948.

at higher spreading concentrations that the gaseous area is considerably smaller than 0.80 sq. meters per milligram which is the area occupied by a fully compressed monolayer of peptide chains.[5] The fact that the correct value for the molecular weight of pepsin was obtained indicates that all the pepsin applied to the surface remained on the surface. The expansion of the film with time arises, therefore, from the expansion of individual pepsin molecules and not from the appearance of additional pepsin molecules on the surface. The initial step in the spreading of pepsin appears to be the formation of a duplex film. A monomolecular film is quickly formed from the duplex film and this transformation is followed by a slow expansion of the individual gaseous molecules. Twenty hours after spreading, the gaseous area of pepsin is about 1.60 sq. meters per milligram. It can be estimated that the area of a β-keratin chain lying flat on the surface would be about 1.70 sq. meters per milligram.[5] Evidently, after a time the pepsin approaches the condition of a β-keratin film on the surface. This slow expansion of the gaseous area may be regarded as a type of surface denaturation.

Insulin molecules tend to dissociate on the surface as shown in Table V. It will be recalled that insulin in solution likewise tends to dissociate.[6] The gaseous area of the insulin film expands with time and after twenty-four hours reaches the area of a β-keratin chain lying flat on the surface. The results given in Table VI demonstrate that a small amount of copper sulfate in the substrate solution prevents the dissociation of the insulin molecules. This parallels the behavior of films of β-lactoglobulin.[2]

We have investigated the coefficient of compressibility of pepsin and of insulin films at higher film pressures spread on 5% ammonium sulfate solutions. The minimum coefficient of compressibility of a pepsin film compressed fifteen minutes after spreading was 0.0275 centimeter per dyne which corresponded to a film pressure of 5 dynes per centimeter and to an area of 0.82 sq. meter per milligram. A pepsin film compressed six hours after spreading yielded a minimum compressibility coefficient of 0.025 centimeter per dyne corresponding to a film pressure of 10 dynes per centimeter and to an area of 1.08 sq. meters per milligram. Evidently, the area of a film at higher pressures depends significantly on the time between spreading and compression. The results shown in Tables I and II indicate that the amount of protein on the surface is independent of time and, accordingly, the variation of area with time is simply a reflection of the expansion of the individual protein molecules. This consideration means that some of the variable protein film areas reported in the literature may not be due to loss of protein in the substrate solution but unequal expansion of the surface protein molecules.

The insulin film was compressed within two minutes of spreading and the coefficient of minimum compressibility was 0.025 centimeter per dyne which corresponded to an area of 0.90 sq. meter per milligram and to a surface pressure of 7.6 dynes per centimeter. In accord with the discussion elsewhere,[5] it is believed that the point of minimum compressibility represents the smallest area to which a film can be compressed without partial collapse of the film occurring.

(5) Bull, "Advances in Protein Chemistry," Vol. III, 1947.
(6) Gutfreund, *Biochem. J.*, **42**, 156 (1948).

Summary

1. The film molecular weights and the gaseous areas of films of pepsin and of insulin have been determined as a function of several variables.

2. The film molecular weight of pepsin is about 34,400 and pepsin molecules do not dissociate on the surface.

3. The gaseous area of the pepsin film increases with time and is smaller the greater the spreading concentration. It appears that the initial step in the spreading of pepsin is the formation of a duplex film which quickly changes into a monolayer. Following the transformation, the gaseous pepsin molecules slowly expand to an area which would be occupied by a β-keratin chain lying flat on the surface.

4. Insulin molecules dissociate on the surface but a small amount of copper ions in the substrate solution prevents this dissociation.

5. The pepsin and insulin films have also been examined in the high pressure region and the coefficients of compressibility of these films calculated and reported.

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