THE KINETICS OF ENZYME REACTIONS: SCHÜTZ'S LAW

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Emil Schütz,[1] who followed polarimetrically the course of the peptic digestion of globulin-free albumin, discovered that the quantity x of albumin digested in a given time t was proportional to the square root of the amount ϵ of pepsin employed:

$$x = \text{const. } \sqrt{\epsilon} \quad \ldots\ldots(1)$$

Borissov[2] and Samojloff,[3] who independently arrived at the same result for tryptic and peptic digestion respectively, showed in addition that when the quantity of enzyme employed was maintained constant the extent (x) of digestion was proportional to the square root of the time:

$$x = \text{const. } \sqrt{t} \quad \ldots\ldots(2)$$

These joint observations may be written in the form:

$$x = k_s \sqrt{\epsilon t}$$

or

$$k_s = \frac{x}{\sqrt{\epsilon t}} \quad \ldots\ldots(3)$$

which is known as Schütz's Law. A repetition of the work of Emil Schütz by Julius Schütz,[4] who estimated the quantities of peptone produced by means of nitrogen determinations after precipitation of unchanged protein, substantially confirmed the conclusions arrived at by the earlier investigator, but indicated that the rule is approximate

[1] Euler, *Chemie der Enzyme*, Munich, 3rd edition, 1925, **2**, 511.
[2] Euler, General Chemistry of the Enzymes, New York, 1st edition, 1912, 175.
[3] Euler, General Chemistry of the Enzymes, New York, 1st edition, 1912, 175.
[4] Schütz, J., *Z. physiol. Chem.*, 1900, **30**, 1.

only, since with high concentrations of enzyme the quantity of protein digested is lower than that demanded by equation (3). Arrhenius,[5] analysing the electrical conductivity data of Sjöqvist[6] on the peptic digestion of egg albumin, concludes that Schütz's Law in this case holds for the first half of the reaction. Schütz's Law has been found to hold by Vernon[7] for the tryptic digestion of fibrin, by Engel[8] for the action of pancreatin on egg yolk emulsion, by Rubner[9] for the fermentation of 20 per cent cane sugar solutions, determined calorimetrically, and by Arrhenius[10] for the action of steapsin on fats. The data of Armstrong[11] on the hydrolysis of lactose by emulsin conform better with the Schütz equation (3) than with the ordinary unimolecular velocity constant equation, particularly when the concentration of emulsin is low. In more recent years W. van Dam[12] has found that equation (1) holds for the peptic hydrolysis of casein, while Northrop[13] finds that when the quantity of substrate is high and the amount of enzyme employed is low, Schütz's Law holds for the hydrolysis of peptone by pepsin. A similar behaviour is encountered in the case of the tryptic hydrolysis of casein.[14] Willstätter, Waldschmidt-Leitz, Duñaiturria and Künstner,[15] show that, when the quantity of enzyme is small in comparison with that of the substrate, Schütz's Law holds for the hydrolysis of casein by trypsin-kinase, provided the early stage of the reaction is neglected.

In general it may be concluded that Schütz's Law is an empirical relation which has been found to have a wide applicability to data on enzyme reactions, particularly when the concentration of enzyme is

[5] Arrhenius, Immunochemie, Leipsic, 1st edition, 1907, 53.

[6] Euler, *Chemie der Enzyme*, Munich, 3rd edition, 1925, **2**, 512.

[7] Vernon, *J. Physiol.*, 1901, **26**, 421.

[8] Euler, *Chemie der Enzyme*, Munich, 3rd edition, 1925, **2**, 21.

[9] Rubner, *Chem. Centralblatt.*, 1905, **76**, 39.

[10] Arrhenius, Quantitative Laws in Biological Chemistry, London, 1st edition, 1915, 46.

[11] Armstrong, *Proc. Roy. Soc. London, Series B*, 1904, **73**, 507.

[12] van Dam, W., *Z. physiol. Chem.*, 1912, **79**, 247.

[13] Northrop, *J. Gen. Physiol.*, 1920, **2**, 471.

[14] Northrop, *J. Gen. Physiol.*, 1923–24, **6**, 723.

[15] Willstätter, Waldschmidt-Leitz, Duñaiturria, and Künstner, *Z. physiol. Chem.*, 1926, **161**, 191.

relatively small compared with that of the substrate, and when the temperature of investigation is maintained low, so as to minimise any complications introduced by the spontaneous inactivation of the enzyme. Differentiating equation (3) with respect to t, it is found that the relation may be written:

$$\frac{dx}{dt} = \frac{k_s^2 \cdot \epsilon}{2x} \qquad (4)$$

i.e. Schütz's Law implies that the rate of reaction is directly proportional to the concentration ϵ of enzyme, inversely proportional to the concentration x of products, and independent of the concentration of substrate.

A Theoretical Deduction of Schütz's Law on the Basis of the Law of Mass Action

The deduction of Schütz's Law given here is due originally to Arrhenius;[16] it is quoted by Euler[17] and is restated by Northrop[14] as follows:

When the concentration of enzyme varies as a result of some cause other than the spontaneous inactivation of the enzyme we have:

$$\frac{dx}{dt} = k\,(A - x)\,Q \qquad (5)$$

where A is initial concentration of substrate, x is the quantity which has undergone hydrolysis in time t; Q the quantity of free (active) enzyme is a function of time. Considering the following equilibrium between enzyme and products of hydrolysis, ϵ being the *total* enzyme content:

$$\text{Enzyme} + \text{Inhibitor} \rightleftharpoons \text{Enzyme-Inhibitor (complex)}$$

$$[Q] \qquad [x - (\epsilon - Q)] \qquad\qquad [\epsilon - Q]$$

$$K = \frac{[Q]\,[x - (\epsilon - Q)]}{[\epsilon - Q]}$$

[16] Arrhenius, *Medd. Nobel. Inst.*, 1908, **1**, 1.

[17] Euler, *Chemie der Enzyme*, Munich, 3rd edition, 1925, **1**, 154.

Arrhenius[16] shows that when $x \gg \epsilon$, this equation can be written in the form:

$$K = \frac{Qx}{\epsilon} \quad \text{or} \quad Q = \frac{K\epsilon}{x} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (6)$$

substituting this value of Q in equation (5) we arrive at the relation:

$$\frac{dx}{dt} = k \; K\epsilon \; \frac{A - x}{x} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (7)$$

on integration:

$$k\,K\epsilon = \frac{1}{t}\left\{A \; ln \; \frac{A}{A - x} - x\right\} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (8)$$

From equation (7) it is seen that the rate is inversely proportional to the concentration of products; hence this equation cannot be expected to hold during initial stages of the reaction. Since the concentration of substrate is high, it can be assumed that $(A - x)$ is roughly constant over a certain range, hence equation (7) reduces to the following approximation:

$$\frac{dx}{dt} = k \,.\, K\epsilon \,.\, \frac{A}{x} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (9)$$

Integration of this expression shows that:

$$\sqrt{2\,k \,.\, K\,A} = \frac{x}{\sqrt{\epsilon\,t}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (10)$$

which is Schütz's Law. Arrhenius has thus shown that Schütz's Law is but a modified form of the unimolecular law and can be derived on the assumptions that (a) the concentration of substrate is much greater than the concentration of enzyme, (b) heat inactivation of the enzyme does not play an appreciable part in altering the active enzyme content, (c) products of hydrolysis form a complex with the enzyme, and this inhibits further hydrolysis, and (d) the concentration of substrate remains sensibly constant throughout the range of reaction considered.

It should, perhaps, be pointed out that since Schütz's Law (equation 10) is but an approximate form of the more general equation

(8), it must follow that any reaction which obeys Schütz's Law, must also obey the Arrhenius equation (8). This has been found to be true in certain cases where both equations (8) and (10) have been applied to the experimental data. Arrhenius found this to be so for the saponification of ethyl acetate by ammonia.[10] Bayliss[18] also found equation (8) to hold for the tryptic digestion of casein and gelatin. Northrop[14] compares the applicability of the Schütz Law and of the Arrhenius equation to data obtained by him for the hydrolysis of casein by trypsin.

A Theoretical Deduction of Schütz's Law on the Basis of the Adsorption Theory

The deduction of Schütz's Law on the basis of the theory of adsorption is due originally to Langmuir,[19] whose treatment is followed here with certain modifications and extensions to cover stages of enzymic hydrolysis other than those represented by Schütz's Law.

Enzymes are regarded as colloids on the surface of which chemical reaction takes place. Consider the catalytic change $A \rightarrow B$. Let Θ_A and Θ_B be the areas of the enzyme surface covered with molecules of A and B respectively. Both Θ_A and Θ_B are, in general, functions of time and depend on the extent of the reaction. As used by Langmuir the limiting value of each such term is unity. The rate of reaction will be, in general, proportional to Θ_A, hence

$$\frac{dx}{dt} = k_L \theta_A \qquad (11)$$

It is to be noted that k_L is a proportionality factor measuring the average "reactivity" per adsorbed molecule, and is not necessarily the same as Langmuir's term ν_A, which is the rate of desorption of molecules of A from the enzyme surface. Considering a special case in which even at low bulk concentration of A, its adsorbability is so great that it covers practically the whole of the enzyme surface, Θ_A would be nearly unity, and would remain constant over a wide range of change in bulk concentration, *i.e.* the observed rate, $\frac{dx}{dt}$, of reaction would remain constant through the range of bulk concentration

[18] Euler, *Chemie der Enzyme*, Munich, 3rd edition, 1925, **2**, 477.
[19] Langmuir, *J. Am. Chem. Soc.*, 1916, **38**, 2221.

considered, thus giving a zero order reaction. It has not been possible to find any data on enzyme action to substantiate this point, probably because the concentrations of substrate necessary for the conditions which would give a zero order reaction are too low to allow of accurate measurements. Waldschmidt-Leitz,[20] referring to the data of Michaelis and Davidsohn[21] on the hydrolysis of sucrose by saccharase, states that a "rule which might be expected in a purely catalytic reaction does not appear to hold here, namely, the non-dependence of reaction velocity upon the concentration of substrate."

Clearly, however, this condition is not that under which the Schütz behaviour is observed. Some disturbing circumstance is occurring in the latter case and this is taken by Langmuir to be due to a displacement of A molecules by B molecules. As a consequence of this, Θ_A, instead of remaining constant, diminishes during the reaction, but its variable area is still independent of the bulk concentration of A, because the variation in Θ_A is due to the operation of B molecules, an independent factor.

Obviously the displacement effect produced by the B molecules cannot sensibly operate right at the beginning; we have therefore to consider a suitable stage in the process. The assumption that B molecules can displace A molecules means that the adsorbability of B must be still greater than the adsorbability of A in spite of the latter being taken (necessarily) as highly adsorbable. At the end of the process the whole surface must be closely packed with B molecules.

When we are in the stage corresponding to the Schütz conditions we have the Langmuir equation holding, *i.e.*

$$\alpha_B \theta_A \mu_B = \nu_B \theta_B \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (12)$$

where μ_B is rate of adsorption of B molecules.

ν_B is rate of desorption of B molecules.

α_B is, in general, nearly unity.

This equilibrium must be attained very rapidly compared with the rate of chemical change $A \rightarrow B$. This is implied in the assumption

[20] Waldschmidt-Leitz, Enzyme Actions and Properties, London, 1st edition, 1929, 27.

[21] Michaelis and Davidsohn, *Biochem. Zeit.*, 1913, **49**, 333.

regarding the excessively high adsorbability of B molecules. The equilibrium represented by equation (12) is therefore constantly maintained although Θ_A is diminishing, and Θ_B is increasing with time. (This is possible with rapid adjustment since μ_B is increasing with time.) Combining equations (11) and (12) we get:

$$\text{chemical rate} = \frac{k_L \nu_B \theta_B}{\alpha_B \mu_B} = k_L \left(\frac{\nu_B}{\alpha_B}\right) \frac{\theta_B}{\mu_B} \quad \ldots\ldots\ldots\ldots\ldots\ldots (13)$$

i.e. the rate is directly proportional to Θ_B, inversely proportional to μ_B (*i.e.* concentration of B), and independent of concentration of A. If the chemical reaction has progressed sufficiently Θ_B will approach unity and when this is approximately the case Schütz's expression holds.

It is thus possible to deduce Schütz's Law theoretically by applying Langmuir's theory of heterogeneous catalysis to the conversion of A molecules to B molecules under the influence of an enzyme, provided (a) both A and B molecules are highly adsorbable, (b) the adsorbability of B molecules is greater than that of A molecules, and (c) the initial stages of the reaction are not considered.

The True Critical Increment for Enzymic Hydrolysis as Calculated from the Schütz Empirical Constant k_s

The following section is intended to direct attention to a possible error which may be introduced into the calculation of the critical increment, E, for enzymic reactions if the experimental values of the Schütz constant (k_s of equation (3)) are used in the well known equation

$$\frac{d \ln k}{dT} = \frac{E}{RT^2}.$$

A comparison of equations (3) and (10) or of the differential equations (4) and (9) shows that:

$$k_s^2 = 2 k K A \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (14)$$

where k_s = the Schütz empirical constant, determined experimentally by means of equation (3),

k = the true (hypothetical) unimolecular velocity constant for the enzymic reaction,

K = equilibrium constant governing complex formation,
and A = initial concentration of substrate.

It follows that:

$$2\frac{d\ ln\ k_s}{dT} = \frac{d\ ln\ k}{dT} + \frac{d\ ln\ K}{dT}$$

since A is independent of temperature. If each of these terms is multiplied by RT^2 we have the critical increments and heat effect of the processes to which k_s, k and K refer, *i.e.*

$$2\ E_s = E_{uni} + Q_v$$

or

$$E_{uni} = 2\ E_s - Q_v \quad \text{.....(15)}$$

i.e. the true critical increment for the hydrolytic process is equal to twice the critical increment value calculated from the Schütz constant k_s, minus the heat of decomposition of the enzyme-products complex. Generally we may assume that Q_v will be negligibly small compared with the two other terms involved, so that

$$E_{uni} = 2\ E_s \quad \text{.....(16)}$$

It can readily be shown that the critical increment calculated from k_L (equation (11)) is a true value, identical with that calculated for the theoretical unimolecular constant k (equation (9)).

Application of the Foregoing Considerations to the Case of the Hydrolysis of Casein by Trypsin-Kinase

Experiments were carried out on the hydrolysis of casein by trypsin completely activated with enterokinase, under conditions for which Schütz's Law was found to be valid.

Preparations

(a). *Enzyme Extract.*—Pigs' pancreas, free from fat, was prepared by drying with acetone and ether according to the procedure of Willstätter and Waldschmidt-Leitz.[22] 5 gm. of the dried gland powder were added to 100 cc. of a glycerol-water mixture containing 4 volumes of glycerol (B.D.H., A.R.*) to 1 vol. of dis-

[22] Willstätter and Waldschmidt-Leitz, *Z. physiol. Chem.*, 1923, **125**, 132.

*B. D. H. stands for British Drug Houses, Limited; A. R. stands for analytical reagent.

tilled water. The powder was dispersed through the liquid by shaking and the dispersion kept for 3 hours at 30°C. The finely divided suspension was centrifuged for about 90 min., and the supernatant liquid finally filtered. The clear extract thus obtained remains for some months without deterioration if kept in an ice chest.

(b). *Kinase Solution.*—Pig's intestinal mucosa was treated with acetone, acetone-ether mixture and with ether successively.[23] 2 gm. of the dried mucosa were dispersed in 100 cc. of 0.05 N ammonia and kept at 30°C. for 2 hours. The suspension was filtered, and the clear filtate evaporated to half its volume by means of a rapid current of air, warmed to 30°—35°C. The resulting solution was kept in the ice chest.

(c). *Casein Solution.*—A solution of casein was prepared by addition of 100 cc. 0.025 N ammonia to 6 gm. casein (Kahlbaum-Hammarsten), stirring and keeping for 1 hour at 30°C. At the end of this time the slight amount of insoluble matter was filtered off. The clear filtrate is referred to as a 6 per cent casein solution.

(d). *Buffer Solution.*—A mixture of equal volumes of N ammonia and N ammonium chloride was used as buffer mixture. The pH of this buffer is stated by Willstätter, Waldschmidt-Leitz, Duñaiturria and Künstner[15] to be 8.6 at 20° C., 8.9 at 30° C. The pH was measured at 20°C. with the glass electrode and found to be 8.6.

Experimental Procedure

The course of the tryptic hydrolysis of casein was followed according to the method described by Willstätter, Waldschmidt-Leitz, Duñaiturria and Künstner.[15]

An equal volume, always less than 1 cc., of enzyme extract was measured into each of a number of 50 cc. Jena glass flasks. This volume of enzyme extract was completely activated in each case by addition of 0.3 cc. kinase solution and distilled water to make the total volume 3 cc., and by leaving the contents for 30 min. at 30°C. At the end of this period of activation 2 cc. buffer solution and 5 cc. of 6 per cent casein solution were added, making the total volume of reaction mixture in each flask equal to 10 cc. At intervals reckoned from the instant at which the casein was added, the increase in acidity of the solutions was estimated by the Willstätter method of stepwise-titration, in 50 per cent and 90 per cent alcohol, using 0.2 N NaOH (prepared in 90 per cent alcohol). In each case a blank experiment was performed with casein and buffer alone for the particular time-interval, the enzyme and kinase being added only at the end of this period, immediately before titration. In the results tabulated below, the alkali titres of the blank experiments have been subtracted from the total titres so that x cc. of 0.2 N NaOH) in the tables is a measure of the actual increase in acidity of the casein solution due to the tryptic action.

The enzyme quantity, ϵ, is expressed in terms of the trypsin unit defined by Willstätter, Waldschmidt-Leitz, Duñaiturria, and Künstner,[15] preliminary work

[23] Waldschmidt-Leitz, *Z. physiol. Chem.*, 1923–24, **132**, 204.

having led to a confirmation of the definition given by these authors. The data obtained for the tryptic hydrolysis of casein, using the method described above, are summarized in Tables 1 and 2.

TABLE 1

Temp. 30°C.

Total Volume of Reaction Mixture 10 Cc., Containing 2 Cc. of Buffer Having pH 8.9 at 30° C.

ϵ = 0.5 trypsin unit			ϵ = 0.75 trypsin unit		
t	x (cc. of 0.2 N NaOH in 90 per cent alcohol)	$k_s = \frac{x}{\sqrt{\epsilon t}}$		x (cc. of 0.2 N NaOH in 90 per cent alcohol)	$k_s = \frac{x}{\sqrt{\epsilon t}}$
min.			*min.*		
20	0.56	(0.177)	20	0.80	(0.201)
30	0.86	(0.221)	30	1.14	(0.240)
40	1.14	0.254	40	1.34	0.253
60	1.35	0.247	50	1.57	0.256
80	1.62	0.256	60	1.67	0.248
100	1.74	0.246	80	1.97	0.254
120	1.92	0.248	100	2.14	0.247

TABLE 2

Temp. 40°C.

*Total Volume of Reaction Mixture 10 Cc., Containing 2 Cc. Buffer Having pH 8.9 at 30°C.**

ϵ = 0.60 trypsin unit			ϵ = 1.20 trypsin units		
t	x (cc. of 0.2 N NaOH in 90 per cent alcohol)	$k_s = \frac{x}{\sqrt{\epsilon t}}$		x (cc. of 0.2 N NaOH in 90 per cent alcohol)	$k_s = \frac{x}{\sqrt{\epsilon t}}$
min.			*min.*		
30	1.61	0.379	20	1.79	0.365
40	1.78	0.363	30	2.23	0.372
50	2.03	0.371	40	2.60	0.375
60	2.15	0.359	50	2.87	0.370
80	2.58	0.372	60	3.03	0.358

* The change in pH of the system due to an increase in temperature of 10° will be about 0.2 pH. This magnitude of change—*at the optimum pH region for the tryptic action on casein*—may be neglected, since it may be seen from the curves obtained by Northrop[24] that a change of 0.2 pH, round the optimum pH region, will not introduce any appreciable error into the rate of hydrolysis.

[24] Northrop, *J. Gen. Physiol.*, 1922–23, **5**, 263.

From the figures in Table 1 the average of 10 values for k_s at 30°C. is equal to *0.251*. The values given in brackets are somewhat lower than this mean, and refer to stages of the reaction for which Schütz's Law does not hold.

From the figures in Table 2 the average of 10 values for k_s at 40°C. is equal to *0.368*. Substituting these values of k_s (0.251 at 30°C.; 0.368 at 40°C.) into the equation $\frac{d\ ln\ k_s}{dT} = \frac{E_s}{RT^2}$, it is found that the observed critical increment E_s is 7,200 calories. This, however, is an empirical value related to the true critical increment of hydrolysis according to equation (15). Employing the approximate relation of equation (16), it is found that E_{uni} becomes 14,400 calories.

It is to be noted that this value should be identical with that calculated from observed *unimolecular* constants for the tryptic hydrolysis of casein obtained at two temperatures. Unfortunately, however, values of unimolecular coefficients k_{uni} for this reaction have not been determined at two temperatures. Northrop[25] has succeeded in obtaining a unimolecular constant for the reaction at 0°C., but no temperature coefficient is recorded. The difficulties in the way of determining the value of E_{uni} have been (1) the necessity of accurate temperature control at low temperatures, and (2) the correct evaluation of A, *i.e.* the total extent of reaction. An attempt is being made at present to investigate this point more fully.

Waldschmidt-Leitz[20] has pointed out that the data available on the effect of temperature on enzymic reactions are largely taken from the older work where the significance of H^+ ion concentration, for example, was not realised, and consequently it is difficult to obtain data with which the results cited in this paper may with certainty be compared.

Vernon [26] in a study of the hydrolysis of Witte's peptone by trypsin, found that the time required to digest a given percentage of the peptone varied inversely as the quantity of enzyme employed, *i.e.*

$$et = \text{const. for a constant value of } x.$$

[25] Northrop, *J. Gen. Physiol.*, 1923–24, **6**, 417.

[26] Vernon, *J. Physiol.*, 1904, **30**, 330.

Thus it is not clear whether the Schütz Law or the unimolecular relation is the significant expression for the reaction as carried out by Vernon.

From Vernon's data, however, it is possible to calculate a value of E_s which is 7,150 calories, a value which is in agreement with that obtained in the present work. The value calculated by Euler is 14,300 calories, on account of the fact that he substituted the values of t, at the two temperatures, in the equation for the critical increment, instead of the value of $\sqrt{t}$ at the two temperatures.

It is interesting to observe that Auld[27] finds a value for E_{uni} of 14,670 calories for the hydrolysis of salicin by emulsin.

SUMMARY

1. A review of the applicability of Schütz's Law to enzymic reactions is given.

2. The theoretical deductions of the Law, (a) on the basis of the law of mass action, (b) on the basis of the adsorption theory, are given and the significance of the assumptions made in these deductions pointed out.

3. It is shown that the true critical increment for an enzymic reaction is equal to twice the critical increment calculated from the Schütz constant k_s, if the heat of decomposition of the enzyme-products complex be neglected.

4. Experiments are described on the tryptic hydrolysis of casein at 30°C. and 40°C. The foregoing considerations are applied to the experimental results obtained.

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[27] Auld, *Trans. Chem. Soc.*, 1908, **93**, 1275.