STUDIES ON THE LIPOLYTIC ENZYME ACTION

V. A NOTE ON THE ACTIVATION ENERGY

by

F. SCHØNHEYDER AND K. VOLQVARTZ
Biochemical Institute, Aarhus University (Denmark)

As a supplement to previously published studies on the kinetics of the action of pancreatic lipase[1] and liver esterase[2], the results of the determination of the energy of activation of the enzymic hydrolysis for rac.l-caprylyl glycerol are given in what follows. These studies were extended to include an examination of the influence of temperature on the rate of the enzymic hydrolysis of emulsified *n*-propyl caprylate.

In spite of the fact that the influence of temperature on the reaction rate of the enzymic hydrolysis of esters has been studied several times for more than fifty years there are hardly any data available which allow a quite exact determination of the activation energy for processes catalyzed by pancreatic lipase and liver esterase.

On the basis of experiments of KASTLE AND LOEVENHART[3], LINEWEAVER[4] and MOELWYN-HUGHES[5] have computed $E = 4{,}200$ cal for the hydrolysis of ethyl butyrate by pancreatic lipase. Owing to the low value found the latter author doubts that the calculated value is actually an expression of the true E of the reaction, as it corresponds to the variation of the diffusion coefficient with temperature. MOELWYN-HUGHES warns against attaching too much importance to the results which are obtained at varying temperature in heterogeneous systems. In this connection we wish to point out that KASTLE AND LOEVENHART's experiments were carried out with emulsions of the volatile ethyl butyrate in unbuffered mixture and with a non-homogeneous enzyme preparation. The method employed for the determination of the rate of reaction does not yield values corresponding to the initial reaction velocity, the acid liberated during the hydrolysis not being measured until 30 mins after mixing enzyme with substrate. EULER[6] has calculated E from TAYLOR's[7] experiments with triacetin and found 16,700 cal. SIZER AND JOSEPHSON[8] have computed E of about 7,600 cal on the basis of experiments by TERROINE[9], who worked with pancreatic lipase and triacetin, ethyl butyrate and olive oil, and on experiments by NICLOUX[10], who worked with ricinus lipase and cottonseed oil. For similar reasons which have already been mentioned in connection with the experiments by KASTLE AND LOEVENHART we are of the opinion that the experiments of TAYLOR, TERROINE and NICLOUX are not sufficiently reliable to allow calculation of E. SIZER AND JOSEPHSON[8] have performed some frequently quoted experiments with tributyrin and pancreatic lipase. Above 0° C they obtained $E = 7{,}600$ cal whereas below zero $E = 37{,}000$ was determined. They worked with heterogeneous reaction mixtures which were not shaken and contained bile salts, $CaCl_2$ and NH_4Cl-NH_4OH buffer, and determined the time required to bring about one per cent. hydrolysis. SCHWARTZ[11] has found E to be about 8,800 cal in heterogeneous systems containing pancreatic lipase and triglycerides (tributyrin to tricaprylin). His system contained gum arabic as emulsifier and a buffer consisting of K_2HPO_4 and KOH. As a measure of the initial velocity he used the time required to attain a certain change in the pH of the enzyme-substrate system. SCHWARTZ thinks that he has the same degree of dispersity for each substrate at different temperature. SINGER AND HOFSTEE[12] have recently determined the energies of activation of the enzymic hydrolysis to be 7,850 cal/mole for monobutyrin and 5,710 cal/mole for triacetin using wheat germ lipase. They used a manometric method. Apparently no calculation of E for ester hydrolysis catalyzed by liver esterase has been reported.

PROCEDURES

A homogeneous solution containing 0.33 mmol rac. l-caprylyl glycerol in 1% NaCl was used as substrate. The enzyme material was obtained by extracting chopped acetone- and ether-dried

References p. 309.

pig pancreatic and rabbit liver tissue with 1% NaCl. The continuous titration technique used was identical with that described previously[1]. In some experiments the very slightly water-soluble *n*-propyl caprylate was used as substrate. In these experiments the reaction mixture was shaken vigourously by hand.

For the determination of the initial velocities (v_0) for hydrolysis of rac. l-caprylyl glycerol by pancreatic lipase was used the equation $1/v_0 = (A/a) - B$, which is obtained by differentiation of the equation $t = A\ (\ln a - \ln (a-x)) - Bx$, which holds for the first 40% of the hydrolysis, *cf.* paper I[1], p. 155. v_0 in the experiments with liver esterase can easily be determined graphically from the slope of the first part of the reaction curve, x/t being almost constant in the first 10% of the hydrolysis catalyzed by this enzyme, *cf.* paper II[2]. v_0 in the experiments with *n*-propyl caprylate and pancreatic lipase was determined graphically from the first part of the reaction curve.

RESULTS AND DISCUSSION

When the log of the reaction rate is plotted against the reciprocal of the absolute temperature, as in Fig. 1, a straight line is obtained from zero up to about 40° C, showing that the ARRHENIUS equation is strictly obeyed both in the case of pancreatic lipase and liver esterase. From the slope of the lines in Fig. 1, the corresponding E values are calculated. For pancreatic lipase and rac.l-caprylyl glycerol the slope is -0.2014 ± 0.0050, and hence $E = 9{,}200$ cal/mole. For liver esterase and rac.l-caprylyl glycerol, with a slope of -0.3300 ± 0.0033, $E = 15{,}100$. The difference between the E values of the two enzymes towards the same substrate has been verified several times using different enzyme preparations. It may be of interest to mention that KASTLE AND LOEVENHART have pointed out on the basis of experiments with ethyl butyrate that the activity of hepatic lipase varies much more with temperature between 0° and 40°C than that of pancreatic enzyme. This is in agreement with our findings with another substrate.

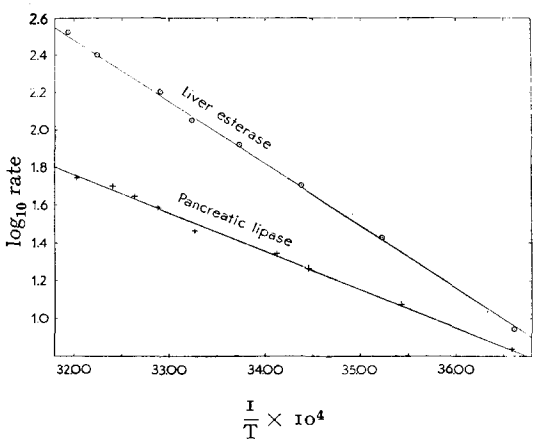

Fig. 1. The effect of temperature on the hydrolysis of an ester catalyzed by liver esterase and pancreatic lipase. The ordinate represents the $\log_{10}$ of the initial reaction rate for the hydrolysis multiplied by 10^4. E for rac. *l*-caprylyl glycerol and liver esterase = 15,100 cal/mole; for the same ester and pancreatic lipase, E = 9,200 cal/mole.

It appears from previously published investigations on the kinetics of pancreatic lipase and liver esterase activity towards rac. *l*-caprylyl glycerol[1,2] and tripropionin[13] that the different time course relationship for the two enzyme reactions may be explained by assuming different reaction schemes. As we have not been able to use purified enzyme preparations and thus do not know the true concentration of enzyme in our systems, it is not possible to put the E values found in a closer relation to the postulated reaction schemes.

In connection with the experiments mentioned it might be of interest to report the results from a series of experiments concerned with the influence of temperature on the hydrolysis of *n*-propyl caprylate by pancreatic lipase. This system is heterogeneous, the ester being only very slightly soluble in water. The reaction mixture was shaken vigorously by hand. It is seen from Table I that under the given experimental conditions the

References p. 309.

influence of temperature on the rate of hydrolysis is negligible. This may be explained as a consequence of the reaction occurring on a saturated surface where the rate is independent of concentration and temperature within fairly wide limits, TAYLOR[14]. Owing to the vigorous shaking the change of velocity with temperature due to a varying diffusion coefficient will be eliminated.

TABLE I

EFFECT OF TEMPERATURE ON THE RATE OF HYDROLYSIS (v_o) OF *n*-PROPYL CAPRYLATE BY PANCREATIC LIPASE

The ester (0.265 mmol) was *suspended* in 50 ml reaction mixture containing 1 ml veronal buffer

Temp *C*	v_o *m.equiv. acid liberated per 10 min*
10.6	0.0107
14.3	0.0114
17.0	0.0114
20.0	0.0119
23.4	0.0114
26.3	0.0121
31.0	0.0114
32.5	0.0107
36.0	0.0115
39.4	0.0115

ACKNOWLEDGEMENT

This work has been aided by a grant from "Carlsberg Foundation".

SUMMARY

1. A review is given of previous publications on the rates of hydrolysis of esters by lipolytic enzymes at varying temperature.

2. The energies of activation for the hydrolysis of rac. l-caprylyl glycerol in aqueous homogeneous solution by pancreatic lipase and liver esterase are found to be 9,200 cal/mole and 15,100 cal/mole respectively.

3. In a heterogeneous system which contains *n*-propyl caprylate and pancreatic lipase and is vigorously shaken, no change in the initial reaction velocity with temperature between 10.6° and 39.4° C could be demonstrated.

RÉSUMÉ

1. Nous donnons un exposé des publications précédentes sur la vitesse d'hydrolyse d'esters par des enzymes lipolytiques à diverses températures.

2. Pour les énergies d'activation de l'hydrolyse du l-caprylyl glycérol racémique en solution par la lipase pancréatique et l'estérase hépatique nous calculons respectivement 9,200 cal/mol et 15,100 cal/mol.

3. Dans un système hétérogène contenant du caprylate de *n*-propyl et de la lipase pancréatique et qui est agité vigoureusement, aucun changement de la vitesse de réaction initiale avec la température entre 10.6° et 39.4° C n'a pu démontré.

ZUSAMMENFASSUNG

1. Eine Übersicht über frühere Arbeiten betreffs der Geschwindigkeiten der Esterhydrolyse durch lipolytische Enzyme bei verschiedenen Temperaturen wird gegeben.

2. Für die Hydrolyse des rac. l-Caprylylglycerols in wässriger homogener Lösung durch

References p. 309.

Pancreaslipase und durch Leberesterase werden die Aktivierungsenergien 9,200 cal/Mol, beziehungsweise 15,100 cal/Mol berechnet.

3. In einem heterogenen System, welches *n*-Propylcaprylat und Pancreaslipase enthält und kräftig geschüttelt wird, konnte keine Änderung der Anfangs-Reaktionsgeschwindigkeit mit der Temperatur im Temperaturbereich 10.6°–39.4° C nachgewiesen werden.

REFERENCES

1 F. SCHØNHEYDER AND K. VOLQVARTZ, *Biochim. Biophys. Acta*, 6 (1950) 147.
2 F. SCHØNHEYDER, *Biochim. Biophys. Acta*, 6 (1951) 461.
3 J. H. KASTLE AND A. S. LOEVENHART, *Am. Chem. J.*, 24 (1900) 491.
4 H. LINEWEAVER, *J. Am. Chem. Soc.*, 61 (1939) 403.
5 E. A. MOELWYN-HUGHES, *Ergeb. Enzymforsch.*, Bd. II (1933) 1.
6 H. EULER, *Chemie der Enzyme*, Bd. 1, München and Wiesbaden, 1920.
7 A. E. TAYLOR, *J. Biol. Chem.*, 2 (1906) 87.
8 I. W. SIZER AND E. S. JOSEPHSON, *Food Research*, 7 (1942) 201.
9 E. J. TERROINE, *Biochem. Z.*, 23 (1910) 404.
10 M. NICLOUX, *Compt. rend. soc. biol.*, 56 (1904) 839.
11 B. SCHWARTZ, *J. Gen. Physiol.*, 27 (1943) 113.
12 T. P. SINGER AND B. H. J. HOFSTEE, *Arch. Biochem.*, 18 (1948) 245.
13 F. SCHØNHEYDER AND K. VOLQVARTZ, *Biochim. Biophys. Acta*, 8 (1952) 407.
14 H. S. TAYLOR, *Chem. Rev.*, 9 (1931) 1.

Received December 17th, 1951