THE EFFECT OF TEMPERATURE ON THE RATE OF HYDROLYSIS OF TRIGLYCERIDES BY PANCREATIC LIPASE

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Few studies have been made concerning the effect of temperature on the rate of hydrolysis of triglycerides by lipase. Balls, Matlack, and Tucker (1937) reported that the triglycerides of fatty acids containing less than 8 carbon atoms were not split by pancreatic lipase any faster at 40° than at 20°C. and that the hydrolysis was remarkably rapid even at 0°C.

Shortly after the completion of the present investigation Sizer and Josephson (1942) published data on the effect of temperature on pancreatic lipase hydrolyzing excess tributyrin from −70° to 50°C. In accordance with the practice of Crozier (1926), they applied the Arrhenius equation:

$$\ln \frac{K_2}{K_1} = \frac{\mu}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)$$

where μ represents the slope of the line drawn through the experimental points when log rate of the reaction is plotted against the reciprocal of the absolute temperature. The temperature characteristic (μ) is considered to be the energy of activation of the reaction expressed in calories per mol. These investigators found a μ of 7,600 for the enzyme acting from 0° to 50°C.

With the exception of this report the energy of activation of lipase has not been experimentally determined although a few attempts have been made to calculate the value from scattered data in the literature. For example, a μ = 16,700 was computed (Waksman and Davison, 1926; Euler, 1920) from the work of Taylor (1906), in which the rate of hydrolysis of triacetin by castor bean lipase was compared at 18° and 28°C. Similarly, Lineweaver (1939) secured a μ = 4,200 for the hydrolysis of ethyl butyrate by pancreatic lipase calculated from data of Kastle and Loevenhart (1900). A temperature characteristic of approximately 8,000 can be calculated for the same system, however, from the data in a report by Kastle, Johnston, and Elvove (1904). This value of 8,000 agrees well also with values computed from results of Terroine (1910) and Nicloux (1904). Since these temperature characteristics were computed from very meager data little significance can be given to them.

Method

The method suggested by Schwartz (1942) for the study of the hydrolysis of triglycerides was used with certain modifications. This procedure depended upon the

measurement of change in pH by the glass electrode due to the liberation of fatty acid. In the present work the reciprocal of the time required to hydrolyze a given fat to the same extent at the different temperatures was taken as a measure of the rate of reaction. A buffer of pH 9 consisting of K_2HPO_4 and KOH, an emulsifier of gum arabic, and a glycerol extract of lipase was used in all experiments. With dilute fat emulsions it was found that hydrolysis to a pH of approximately 8.1, for example, would take place at the higher temperatures in about 10 minutes. With greater concentrations of fat, however, this pH was reached in too short a time to enable convenient measurement, and consequently the hydrolysis was allowed to proceed to a lower more suitable pH. Preliminary investigations showed that this improved the accuracy of the results.

Inasmuch as the pH of a buffer is a function of the temperature the hydrolysis of a fat emulsion to the same pH at different temperatures would not represent the same degree of hydrolysis. Accordingly, the effect of temperature on the pH was determined for several buffers ranging from 8.1 to 6.5. These were prepared by adding

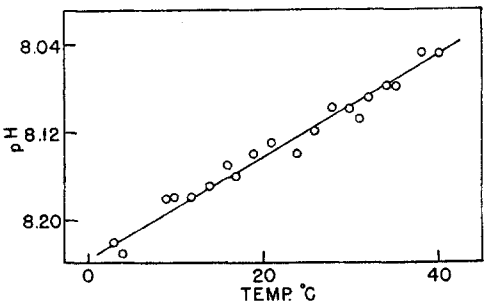

FIG. 1. The effect of temperature on the pH of a phosphate buffer (see text).

graded quantities of butyric acid to the original phosphate buffer. For each buffer the pH was directly proportional to the temperature over the short range studied, 2° to 40°C. (Fig. 1), although the slopes of the lines were different. Thus the enzymatic hydrolysis of a fat emulsion was allowed to proceed at a given temperature to that pH read from the curve plotting pH against temperature. The reciprocal of the time required for this to take place was recorded.

It is essential to compare the rates of hydrolysis of fat emulsions at the different temperatures when they are in the same degree of emulsification. Therefore, a stock emulsion was prepared, stored at a low temperature, and 20 ml. portions of it removed when needed. Esters which are hydrolyzed by water alone, such as tripropionin, were not studied because the amount of hydrolysis during the storage period and during the actual determination itself would be erroneously attributed to the enzyme. Triglycerides from butyrin to caprylin inclusive were studied since they are not hydrolyzed by water to any measurable extent under the experimental conditions. The temperature of the emulsion was controlled to ± 0.05°C. by means of a water bath.

RESULTS

The effect of temperature on the rate of hydrolysis of triglycerides by pancreatic lipase was analyzed so that temperature characteristics could be meas-

ured for the reactions. Fig. 2 illustrates the data obtained for the hydrolysis of 0.05 M tributyrin by the enzyme for 36 temperatures between 2° and 37.2°C. Since the relationship between the reciprocal of the absolute temperature and log rate of hydrolysis is linear from 2° to 28°C., the data can be expressed by the Arrhenius equation. The value of μ was calculated to be 8,900 ± 100. Above 28°C. the relationship is no longer linear, probably because of thermal inactivation of the lipase. When only a few temperatures (10 to 12) were studied the μ for the same concentration of tributyrin was approximately 8,900 ± 1,000. It was believed, nevertheless, that the evaluation of μ from 10 to 12 determina-

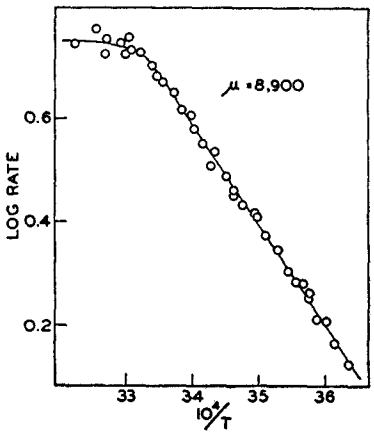

FIG. 2

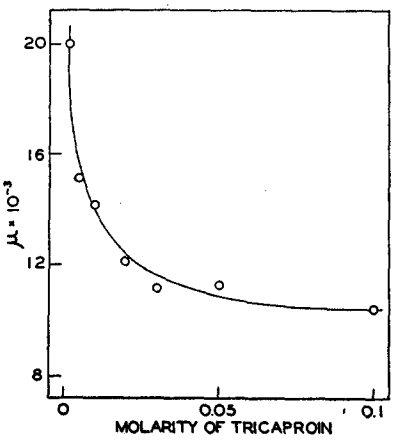

FIG. 3

FIG. 2. Log (rate of hydrolysis × 100) of 0.05 M tributyrin by lipase plotted against $10^4/T$.

FIG. 3. Relationship between the molarity of tricaproin and the temperature characteristic.

tions would be sufficiently accurate to establish the effect of variation of substrate concentration on the temperature characteristic.

The values determined in this way for various concentrations of several triglycerides are recorded in Table I. In the case of tributyrin, the mean of the μ values for all concentrations, excepting the value obtained with the most dilute concentration, was 8,500. This value compares favorably with that of 8,900 determined more accurately for one concentration. The temperature characteristic obtained for the most dilute emulsion of tributyrin, 10,200, is outside the experimental error of the mean and this deviation may be significant. The values found for the highest and lowest concentrations of trivalerin were 9,100 and 12,400 respectively, revealing a trend to higher values as the concentration decreases. The temperature characteristics for concentrations of tricaproin (0.1, 0.05, and 0.03) were constant (10,400 to 11,200). Further dilution resulted

in progressively larger values, 20,000 being obtained with 0.002 M concentration. Fig. 3 illustrates the curvilinear increase in μ upon dilution of the emulsion of caproin. This trend is emphasized further with heptylin. The highest concentration gave a low μ = 9,600, while the value for the lowest concentration,

TABLE I

Variation of μ with Molarity of Triglyceride

Molarity	Butyrin	Valerin	Caproin	Heptylin	Caprylin
0.1	9,500	9,100	10,400	—	—
0.075	7,800	—	—	—	8,800
0.05	8,900	10,700	11,200	9,600	8,800
0.03	7,800	11,600	11,100	11,200	10,300
0.02	8,500	11,400	12,100	13,500	12,000
0.01	7,900	12,400	14,100	14,600	15,300
0.005	9,200	—	15,100	18,800	17,900
0.003	10,200	—	—	—	—
0.002	—	—	20,000	22,300	23,700

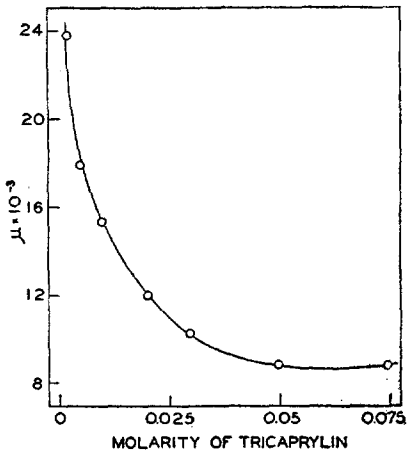

FIG. 4. Relationship between the molarity of tricaprylin and the temperature characteristic.

0.002 M, was 22,400. The μ for tricaprylin increased in a similar curvilinear manner from 8,800 with concentrated emulsions to a maximum value of 23,700 with 0.002 M (Fig. 4).

A given concentration of triglyceride gave a constant μ value regardless of the quantity of enzyme used or to what per cent hydrolysis the reaction was continued. In the hydrolysis of 0.02 M heptylin, for example, the same μ was obtained even when the degree of splitting was varied by 650 per cent.

DISCUSSION

Sizer and Josephson (1942) reported a temperature characteristic of 7,600 for the lipolytic hydrolysis of a concentrated tributyrin emulsion. This value compares well with the mean value of 8,500 for the hydrolysis of tributyrin by lipase obtained in this study. Although these determinations were made by two widely different methods, the values differ by only 900.

From data in the literature, temperature characteristics of 4,200 and 8,000 can be calculated for hydrolysis catalyzed by pancreatic lipase. The values obtained in this study varied from 7,800 to 23,700 and are characteristic of lipase acting on different substrates in a variety of concentrations in this particular heterogeneous medium.

Haldane (1930) has pointed out that if the dissociation constant of the compound formed between enzyme and substrate does not have a low temperature coefficient, the true energy of activation will not be determined in low substrate concentrations. He states, "Sub-maximal substrate concentrations should give a spuriously low or high temperature coefficient, the increase in the velocity of transformation of the enzyme-substrate compound being partly counteracted by the decreased formation of the compound or augmented by its increased formation." Thus it may be that the compound formation between glyceride and enzyme is endothermic and this results in increased μ values at low substrate concentrations.

It is possible too that the temperature characteristic of the reaction between enzyme and substrate differs for mono-, di-, and triglycerides. At the lower concentrations, particularly with the longer chained fats, a greater proportion of the insoluble di- and monoglycerides may be hydrolyzed. In a previous report Schwartz (1942) presented data indicating that more and more of the di- and monoglycerides are split by the enzyme as the length of the carbon chain increases.

SUMMARY

1. The temperature characteristics for the hydrolysis of various concentrations of tributyrin, trivalerin, tricaproin, triheptylin, and tricaprylin have been determined.

2. The μ values for the hydrolysis of all concentrations of tributyrin by pancreatic lipase, except the most dilute, were found to be constant within the experimental error, 8,500 $\pm$ 1,000.

3. The temperature characteristics for the hydrolysis of trivalerin, tricaproin, triheptylin, and tricaprylin varied from approximately 8,500 $\pm$ 1,000 for the high concentrations to 12,400, 20,000, 22,400, and 23,700 respectively for the most dilute concentration of each.

4. An interpretation of these results was presented.

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