[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

A Study of the Kinetics of the Hydrolysis of Waxy Maize Starch by Crystalline Pancreatic Amylase from Swine[1]

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Reproducible and comparable values for the affinity constant of swine pancreatic amylase and waxy maize starch have been determined as a function of temperature. The affinity constant exhibited the temperature dependence of an equilibrium constant between 15 and 40°. The rate constant for the formation of products deviates from Arrhenius behavior in this same temperature range. The physical significance of the affinity constant is discussed taking the above temperature dependence into consideration. The apparent heat and entropy of enzyme–substrate complex formation have been calculated from the temperature dependence of the affinity constant. The values have been compared with the few possibly comparable values reported for other enzyme systems. Because of the limited number of enzyme systems so far investigated on the basis given here, an extensive comparison cannot be made. However, it is interesting to note that the values for the apparent heat of enzyme–substrate complex formation for the three carbohydrases, invertase, emulsin and pancreatic amylase, are similar in sign and magnitude. Enzyme concentration has been found to have little effect on the value of the affinity constant of swine pancreatic amylase provided the velocities of hydrolysis are measured under conditions in which the enzyme is stable. With minor modifications, the procedure and method developed here should be applicable for use with other α-amylases and should make possible significant and comparable new studies of the kinetics of the early action of these important enzymes.

Introduction

Although swine pancreatic amylase has been studied by numerous workers for many years, a thorough investigation of its kinetics has not been reported. Only in recent years has it been possible to prepare crystalline homogeneous pancreatic amylase[4,5] in sufficiently high yields[6] and to obtain the homogeneous starch fractions[7-10] necessary to make such a study worthwhile.

It was the purpose of this investigation to study the applicability of Michaelis–Menten kinetics[11-14] to the action of swine pancreatic amylase on a well-characterized substrate, waxy maize starch. A procedure[15] has been established by means of which reproducible and comparable affinity constants of swine pancreatic amylase can be determined conveniently. Furthermore, the temperature dependence of this constant was studied in order the better to understand its physical significance. The temperature dependence study also permitted the calculation of the apparent heat and entropy of enzyme–substrate complex formation.

Investigation of the kinetics of the action of pancreatic amylase is complicated by the fact that the molecular state of the substrate changes during the course of hydrolysis.[16-22] Moreover, the velocity of hydrolysis depends upon the molecular state of the substrate.[16-22] This dependence is illustrated by the data[20] for the hydrolysis of waxy maize starch by swine pancreatic amylase given in Table I. Because of this dependence of the reaction on the molecular state of the substrate, the initial velocity was defined in this investigation in terms of the reaction time necessary for the hydrolysis of a given per cent., 2.3%, of the glucosidic bonds of the substrate.[15] The data listed in Table I indicate that there is little if any change in the velocity of the reaction during the hydrolysis of the first 4% of the glucosidic bonds of waxy maize starch by swine pancreatic amylase.[20] Therefore, the "initial" velocity as defined above and used here does not differ significantly from the velocity at zero time.[15]

With minor modifications, the procedure and method developed in this work should be applicable for use with other α-amylases and should make possible significant and comparable new studies of the kinetics of the early action of these important enzymes.

Experimental

Amylase.—Thrice recrystallized swine pancreatic amylase[6,23] that had been found by a number of criteria to be

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TABLE I

COMPARISON OF THE RELATIVE RATES OF HYDROLYSIS AND DEGREE OF POLYMERIZATION OF RESULTING DEXTRINS AT DIFFERENT EXTENTS OF HYDROLYSIS OF WAXY MAIZE STARCH BY SWINE PANCREATIC AMYLASE[a]

Extent of hydrolysis,[b] %	Relative rates of hydrolysis,[c] V/V_0	Degree of polymerization,[d] DP	Dextrin by wt.,[e] %
1.1	1	200	99.5
2.1	1	90	98
4.2	1	50	92
20.8	0.2	7–8	87
31.7	.005	5–6	70
35.7	.0005	5–6	63

[a] Data by Mindell.[20] [b] Per cent. theoretical glucose. [c] V = rate at given per cent. hydrolysis; V_0 = initial rate. [d] DP = number average degree of polymerization of the dextrins remaining after the removal of maltose and glucose. [e] Per cent. dextrin by weight:per cent. by weight of the polysaccharide remaining after removal of maltose and glucose.

homogeneous as a protein and also as an enzyme[6] was used in this investigation. The crystalline protein has been reported to have a molecular weight of 45,000, based on diffusion data[24] and of 50,000, based upon analytical data.[25] The molar concentration of the amylase was calculated on the basis of a saccharogenic activity[6] of 16,000 per mg.[6] and a molecular weight of 45,000[24] for the crystalline protein.

Substrate.—The substrate was waxy maize starch[26] that had been used in previous studies with this amylase.[20,21] The starch had been defatted exhaustively[8] and gave no evidence of contamination by linear components.[9,10,27] It had been washed thoroughly with cold distilled water to remove low molecular weight polysaccharides and other soluble impurities. It offered a branched substrate that had undergone a minimum of manipulation. This branched substrate had the advantage of being much more soluble in water than the linear fractions of starches and of having little, if any, tendency to complicate the results by retrogradation. A stock 0.1% starch solution was prepared daily. This was diluted to the desired concentration. In order to protect the amylase from inactivation and permit optimal enzymic activity, all starch reaction mixtures and enzyme solutions were adjusted to *p*H 7.2; 0.01 *M* phosphate; 0.02 *M* or 0.05 *M* chloride[28]; 2.2×10^{-4} *M* calcium ion.[29] The concentration of waxy maize starch is expressed here as moles of glucosidic bonds per liter.[30]

Methods.—In their attack on the 1,4-α-D-glucosidic linkages of very large molecules, α-amylases cause a large reduction in the average molecular weight for each glucosidic linkage hydrolyzed.[16–22] The free aldehyde groups thus formed on very high molecular weight fragments are difficult to detect or to determine quantitatively. Therefore, methods based upon measurements of the increase in such groups are not ideal for investigations of the initial action of α-amylases. For this reason, the initial stages of the action of pancreatic amylase upon waxy maize starch reported here were measured by a spectrophotometric method developed for this purpose.[31] This method is based upon spectrophotometric measurements of complexes of iodine with waxy maize starch and with certain of its hydrolysis products. It is similar to spectrophotometric procedures developed to study iodine complexes of linear components of starches and of their early hydrolysis products.[16,22,32–34] The absorption ratios or A.R. values[35] obtained by the spectrophotometric method were referred to the percentage of glucosidic linkages hydrolyzed by use of a calibration curve that correlated the percentage of bonds hydrolyzed with the A.R. values[35] of hydrolyzates obtained under comparable conditions.[31] The bonds hydrolyzed were measured by an iodometric procedure[36] that had been modified[37] to increase the sensitivity of the determination. Use of the calibration curve or of the equation calculated from it[31,38] made it possible to express the results obtained by the spectrophotometric method in terms of glucosidic linkages broken.

The precision of the spectrophotometric method is high even with very dilute starch solutions such as those used in this investigation. The standard deviation for a single determination was found to be equivalent to a standard deviation of ±0.09% theoretical glucose for a 0.00625% waxy maize starch solution.[31]

Results

Influence of Temperature upon the Affinity Constant and Rate Constant of Swine Pancreatic Amylase. Determination of Affinity Constants.—The affinity constants,[39] K_a, of swine pancreatic amylase and waxy maize starch were determined at 15, 30 and 40°. Four concentrations of substrate were used at each temperature: 3.08×10^{-3} *M*, 0.770×10^{-3} *M*, 0.513×10^{-3} *M* and 0.385×10^{-3} *M*. Each substrate solution and enzyme solution was adjusted to 0.01 *M* phosphate; 0.05 *M* chloride; 2.2×10^{-4} *M* calcium ion; *p*H 7.0–7.2; conditions that had been found to prevent any measurable inactivation of pancreatic amylase even in very dilute enzyme and substrate solutions such as those used here and at the temperatures being investigated.[28,29] The approximate enzyme concentrations in the more dilute substrate solutions were 1.1×10^{-10} *M* for hydrolyses at 15°; 3.6×10^{-11} *M* for those at 30°; 1.8×10^{-11} *M* for those at 40°. For the higher substrate concentration of 3.08×10^{-3} *M*, the enzyme concentrations were twice those used for the hydrolyses of the lower substrate concentrations. Therefore, by multiplying the actual times of hydrolysis of the more concentrated substrate by two, it was possible to compare the data for all of the substrate

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(39)

$$\text{E} + \text{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{ES} \xrightarrow{k_2} \text{E} + \text{Products}$$

$$K_M = \frac{(\text{E})(\text{S})}{(\text{ES})}; \quad K_a = \frac{1}{K_M} = \frac{(\text{ES})}{(\text{E})(\text{S})}; \quad V = \frac{k_2(\text{E})_0 S(K_a)}{1 + SK_a};$$

(E) = enzyme concentration; $(\text{E})_0$ = enzyme concentration at zero time; S = initial substrate concentration; V = velocity; K_M = Michaelis–Menten constant; K_a = affinity constant.

TABLE II

INFLUENCE OF SUBSTRATE CONCENTRATION UPON THE HYDROLYSIS OF WAXY MAIZE STARCH BY SWINE PANCREATIC AMYLASE

Substrate concentration: Glucosidic bonds/l.,[a] moles × 10^3	g./100 ml.	Time of hydrolysis, min.	Absorption ratio, A.R. 1	2	Glucosidic bonds broken, %	Glucosidic bonds broken/l moles × 10^5
			A, at 15°			
3.08[b]	0.0500	36	0.875	0.877	1.3	4.0
		48	.827	.829	1.9	5.0
		60	.790	.782	2.5	7.7
		84	.702	.700	3.6	11.1
0.770[c]	.0125	10	.892	.892	1.1	0.84
		13	.844	.850	1.7	1.3
		16	.803	.795	2.3	1.8
		22	.723	.716	3.4	2.6
.513[c]	.0083	8	.884	.883	1.3	0.67
		10	.846		1.7	0.87
		12	.800	.793	2.3	1.2
		14		.766	2.8	1.4
		16	.722		3.3	1.7
		18		.690	3.8	2.0
.385[c]	.00625	5	.909	.911	0.9	0.35
		7	.853	.861	1.6	.62
		9	.802	.807	2.2	.85
		13	.701	.709	3.6	1.4
			B, at 30°			
3.08[b]	0.0500	36	0.854	0.852	1.6	4.9
		48	.819	.804	2.2	6.8
		60	.766	.769	2.7	8.3
		84	.681	.684	3.9	12.0
0.770[d]	.0125	13	.831	.835	1.9	1.5
		16	.795	.784	2.4	1.8
		19	.752	.744	3.0	2.3
		25	.670	.670	4.0	3.1
.513[d]	.0083	10	.819	.838	1.9	0.97
		12	.767	.801	2.5	1.3
		14	.755	.761	2.9	1.5
		18	.677	.681	3.9	2.0
.385[d]	.00625	7	.881	.873	1.3	0.50
		9	.833	.827	1.9	.72
		11	.787	.780	2.5	.96
		15	.704	.685	3.7	1.4
			C, at 40°			
3.08[b]	0.0500	36	0.870	0.868	1.4	4.3
		48	.827	.833	1.9	5.0
		60	.792	.792	2.4	7.4
		84	.711	.711	3.5	10.8
0.770[e]	.0125	15	.833	.846	1.8	1.4
		18	.811	.811	2.2	1.7
		21	.774	.767	2.7	2.1
		27	.707	.696	3.6	2.8
.513[e]	.0083	12	.847	.850	1.7	0.87
		14	.823	.813	2.1	1.1
		16	.787	.794	2.4	1.2
		20	.717	.732	3.3	1.7
.385[c]	.0062	9	.885	.887	1.2	0.46
		11	.835	.850	1.8	.69
		13	.793	.811	2.3	.88
		17	.709	.737	3.3	1.3

[a] Moles of glucosidic bonds per liter.[30] [b] Enzyme concentration used for 3.08 × 10^{-3} *M* substrate was twice that used for the other substrate concentrations. Data have been referred to one enzyme concentration by multiplying actual times of hydrolysis for 3.08 × 10^{-3} *M* substrate by two. [c] Enzyme concentration = 1.1 × 10^{-10} *M*. [d] Enzyme concentration = 3.6 × 10^{-11} *M*. [e] Enzyme concentration = 1.8 × 10^{-11} *M*.

concentrations on the basis of the same enzyme concentration.[40,41]

In measurements of the course of hydrolysis, the time intervals, which varied with substrate concentration, were chosen so that in a plot of extent of reaction *versus* time, the experimental points would occur at about the same percentage hydrolysis.

Two sets of duplicate measurements were made at each temperature. The data for one set are given in Table II. It should be noted that all measurements at each temperature were made on the same day in order to make certain that the stock starch and enzyme solutions were identical. By use of the equation[31,38] relating absorption ratio (A.R.)[35] to the fraction of glucosidic linkages hydrolyzed, the absorption ratios have been translated into the fraction of glucosidic linkages broken (columns 6 and 7, Table II). Because of the dependence of the velocity[15-22] and of the affinity constant[42] on the extent of hydrolysis, it is important to define the initial velocities in a reproducible way. In this investigation, all velocities were referred to a given extent of hydrolysis of 2.3% of the glucosidic bonds. The time required to reach this extent of hydrolysis at each temperature and enzyme concentration was interpolated from curves such as those given in Fig. 1. Initial velocity then was calculated from the equation

$$V = \frac{0.023S}{t}$$

where V = velocity; S = initial substrate concentration in moles of glucosidic bonds per liter[30]; t = time required to reach 2.3% hydrolysis.

The velocities of hydrolysis at the different temperatures and substrate concentrations studied are summarized in Table III. Affinity constants were calculated from the slope and intercept of plots of the reciprocal of the velocity ($1/V$) *versus* the reciprocal of the substrate concentration ($1/S$)[13] by the method of least squares. The affinity constants and their standard deviations, calculated from the standard deviations of the slope and intercepts,[43] are listed in the last column of Table III. As would be expected, the affinity constants are lower at the higher temperatures. Their temperature dependence is discussed later.

Bernfeld and Studer-Pécha[44] have published a value for the Michaelis–Menten constant of crystalline swine pancreatic amylase acting on corn amylopectin at 20°. In terms of percentage solutions they reported 0.018% for the Michaelis–Menten constant. As waxy maize starch and corn amylo-

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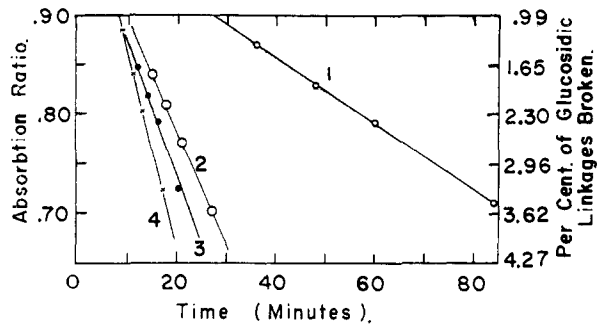

Fig. 1.—Influence of substrate concentration upon hydrolysis of waxy maize starch by swine pancreatic amylase at 40°. Substrate concentrations in moles of glucosidic bonds per liter[30]: curve 1, 3.08×10^{-3} *M*; curve 2, 0.770×10^{-3} *M*; curve 3, 0.513×10^{-3} *M*; curve 4, 0.385×10^{-3} *M*; A.R. = $(O.D.)_t/(O.D.)_0$[35].

TABLE III

VELOCITY OF HYDROLYSIS OF WAXY MAIZE STARCH BY SWINE PANCREATIC AMYLASE AS A FUNCTION OF SUBSTRATE CONCENTRATION AND TEMPERATURE

Temp., °C.	Substrate concn.,[a] $a \times 10^3$	Velocity,[a] moles l.$^{-1}$ min.$^{-1}$ 1 $V \times 10^6$	2 $V \times 10^6$	Affinity constant, $K_a \times 10^{-3}$, l. moles^{-1}[a]
15	3.08	1.26	1.32	6.14 ± 0.43
	0.77	1.11	1.09	
	.513	1.00	1.02	
	.385	0.96	0.95	
30	3.08	1.40	1.34	3.32 ± 0.25
	0.77	1.16	1.12	
	.513	1.02	0.96	
	.385	0.87	0.82	
40	3.08	1.26	1.25	2.29 ± 0.13
	0.77	0.96	0.99	
	.513	.79	.79	
	.385	.68	.69	

[a] Moles of glucosidic bonds per liter.[30]

pectin are very similar branched-chain substrates, it would be expected that the value for the amylopectin might be comparable with those obtained in this investigation. Translated into the units used here, the affinity constant found by Bernfeld and Studer-Pécha[44] for swine pancreatic amylase and corn amylopectin is 1×10^3 liter mole^{-1} at 20°, a value even lower than the 2.29×10^3 liter mole^{-1} found in this work at 40°, as shown in Table III. The lower value for the affinity constant reported by Bernfeld and Studer-Pécha[44] can be attributed to the fact that they did not consider the extent of hydrolysis in measuring their velocities.[15] Their data show that in their experiments the extent of hydrolysis varied with substrate concentration and covered a range from 0.2 to 40%.

Influence of Enzyme Concentration upon the Affinity Constant of Swine Pancreatic Amylase at 40°.—If the affinity constant were a function of amylase concentration, the results reported above would be of little significance. However, the data reported here show that the concentration of pancreatic amylase has little effect on the value of the affinity constant obtained with waxy maize starch provided the hydrolyses are carried out under conditions in which the enzyme is stable.

Three sets of measurements of the hydrolysis at 40° of waxy maize starch solutions with concentrations of 3.08×10^{-3} *M* to 0.385×10^{-3} *M* were made. Each hydrolysis mixture was adjusted to 0.02 *M* chloride ion, 2.2×10^{-4} *M* calcium ion and 0.01 *M* phosphate buffer, *p*H 7.0–7.2.[28,29] The experimental procedure and method were similar to those used for the temperature dependence studies, with the exception that the relative enzyme concentrations in the hydrolyses of any given set of measurements were exactly proportional to the substrate concentrations. Thus, within each determination, the enzyme concentration was varied by a factor of eight. Furthermore, the enzyme concentration in one of the sets was about twice that used in the other two. The data for the three sets of determinations are summarized in Table IV.

The times of hydrolysis, *t*, required to reach an absorption ratio[31,35] of 0.8, equivalent to 2.3% hydrolysis, were determined in the usual way, and the velocities, *V*, for the different initial substrate concentrations, *S*, were calculated from the equation[15,31,40,41,45]

$$V = \frac{0.023S}{E_{rel}t}$$

Velocities were referred to a relative enzyme concentration (E_{rel}) of 1.0 by multiplying the times of hydrolysis (*t*) by the relative enzyme concentration.[45]

Values for the affinity constant, calculated by least squares analysis as previously described, also are summarized in Table IV along with their standard deviation. There is no significant difference among the three values obtained, an indication that a twofold increase in enzyme concentration has little effect on the value of the affinity constant of swine pancreatic amylase for waxy maize starch. The average of the three values obtained for the affinity constant is 2.01×10^3 liter mole^{-1}. This value agrees reasonably well with the value of 2.29×10^3, liter mole^{-1}, obtained above.

Determination of Rate Constants

Although it would be possible to determine the rate constant and the affinity constant from the same set of determinations, it was not practicable to do so. For this reason, separate measurements of the velocity of hydrolysis of 3.08×10^{-3} *M* waxy maize starch were made. The velocity (*V*) obtained was extrapolated to the maximum velocity (V_m) by the following form of the Michaelis—Menten equation

$$V_m = V\left(\frac{1 + K_aS}{K_aS}\right)$$

where K_a = affinity constant and *S* = initial substrate concentration.

The rate constant for the breakdown of the enzyme substrate complex to products was determined from the maximum velocity of the reaction by the equation: $V_m = k_2E_0$; where V_m = maximum velocity; k_2 = rate constant for the break-

(45) E_{rel} = relative enzyme concentration = $\frac{\text{enzyme concentration (moles/l.)}}{1.3 \times 10^{-11}\ M}$

t = time; *S* = initial substrate concentration in moles of glucosidic bonds per liter[30]; 1.3×10^{-11} *M* = unit enzyme concentration.

TABLE IV

THE INFLUENCE OF SUBSTRATE CONCENTRATION UPON THE HYDROLYSIS OF WAXY MAIZE STARCH

	Substrate concn., moles/l.[a]								
	3.08		0.770		0.512		0.125		
	I. Relative enzyme concn. (E_{rel}[b])								
	15.00		3.75		2.50		1.88		
Time of hydrolysis, min.	A.R.[c]	Glucosidic bonds hydrolyzed %	A.R.[c]	Glucosidic bonds hydrolyzed %	A.R.[c]	Glucosidic bonds hydrolyzed %	A.R.[c]	Glucosidic bonds hydrolyzed %	Affinity constant, $K_s \times 10^{-3}$, l. mole^{-1}
4	0.857	1.6	0.890	1.1	0.945	0.4	0.950	0.3	2.09 ± 0.13
8	.698	3.6	.782	2.5	.814	2.1	.862	1.5	
12	.593	5.0	.652	4.3	.719	3.4	.758	2.9	
16	.515	6.1	.582	5.2	.630	4.5	.660	4.2	
		II. Relative enzyme concn. (E_{rel}[b])							
		7.00		1.75		1.17		0.88	
8	0.864	1.5	0.897	1.0	0.932	0.6	0.950	0.3	1.97 ± 0.18
12	.783	2.6	.854	1.6	.876	1.3	.904	0.9	
16	.726	3.3	.795	2.4	.845	1.7	.862	1.5	
20	.677	3.9	.737	3.1	.795	2.4	.825	2.0	
24	.618	4.9	.695	3.7	.743	3.1	.780	2.6	
8	.852	1.6	.906	0.9	.932	0.6	.958	0.2	1.96 ± 0.10
12	.791	2.4	.854	1.6	.895	1.1	.910	0.9	
16	.721	3.3	.800	2.3	.848	1.7	.868	1.4	
20	.674	4.0	.743	3.1	.798	2.3	.816	2.1	
24	.624	4.6	.703	3.6	.760	2.8	.777	2.5	

[a] Moles of glucosidic bonds per liter.[30] [b] E_{rel} = relative enzyme concentration = (moles of pancreatic amylase/l.)/(1.3 × 10^{-11} mole/l.)[46]; unit enzyme concentration = 1.3 × 10^{-11} mole/l. See ref. 35.

down of the enzyme substrate complex to products; E_0 = initial enzyme concentration which, at maximum velocity, is equal to the concentration of the enzyme substrate complex, because at maximum velocity the enzyme is saturated with substrate.

The experimental conditions and procedure were similar to those employed for the determination of the affinity constants. For the measurements at 15 and at 23°, the enzyme concentration was twice that used for the measurements at 30° and at 40°. This adjustment was made to keep the times of hydrolysis in these experiments comparable with those studied previously in the investigation of the stability of the amylase.[37,40] The data for two series of measurements, each carried out in duplicate, are summarized in Table V. The calculated velocities, maximum velocities and rate constants are summarized in Table VI.

Temperature Dependence of the Affinity Constant and the Rate Constant.—The temperature dependence of the rate constant exhibits a significant deviation from the Arrhenius equation,[46] ($\ln k = \ln A - \Delta E/RT$) within the temperature interval between 15 and 40°. This deviation is shown by the plot of the natural logarithm of the average values for the rate constant *versus* the reciprocal of the absolute temperature as given in Fig. 2. The calculated energies of activation (ΔE) are 12.1 kcal. mole^{-1} between 30 and 40°, 13.8 kcal. mole^{-1} between 23 and 30° and 15.8 kcal. mole^{-1} between 15 and 23°.

It should be noted, however, that between 23 and 40°, the data can be fitted to the Arrhenius equation as shown in Fig. 2. Within this temperature interval, deviation of the experimental values from the least squares line is within an experimental error in the rate constants of about ±2%. The average energy of activation for this temperature interval is 12.8 ± 0.4 kcal. mole^{-1}. This pseudo Arrhenius behavior agrees well with an average

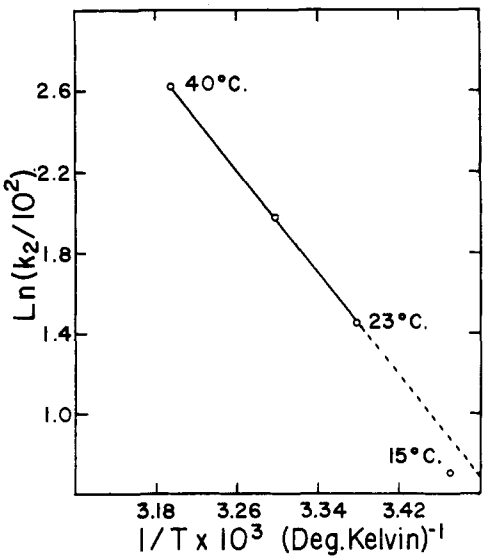

Fig. 2.—Plot of the natural logarithm of the rate constant *versus* the reciprocal of the absolute temperature. Straight line fitted by the method of least squares to the points between 23 and 40°.

energy of activation for pancreatic amylase of 12.5 kcal. mole^{-1} between the temperatures of 21 and 40° which is obtained by calculations based on results reported by Kendall and Sherman.[47]

Fischer and de Montmollin[48] have listed a value of 13.5 kcal. mole^{-1} as the energy of activation of crystalline swine pancreatic amylase in a paper dealing with the action of alpha amylase from *Aspergillus oryzae*. Unfortunately, no information about experimental conditions was given, making it difficult to evaluate the significance of the differ-

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TABLE V

INFLUENCE OF TEMPERATURE UPON THE HYDROLYSIS OF 3.08 × 10^{-3} *M* WAXY MAIZE STARCH[a] BY SWINE PANCREATIC AMYLASE

Enzyme concn., mole/l., (E) × 10^{10}	Temp., °C.	Time of hydrolysis, min.	Absorption ratio, A.R. 1	2	Glucosidic bonds hydrolyzed, %
		Series I			
6.34	15	9	0.834	0.835	1.8
		12	.785	.750	2.7
		15	.718	.690	3.6
		21	.638	.613	4.6
6.34	15	9	.815	.813	2.1
		12	.776	.757	2.7
		15	.725	.716	3.3
		21	.633	.637	4.5
3.17	30	6	.797	.803	2.3
		8	.740	.732	3.2
		10	.678	.665	4.0
		14	.581	.587	5.1
3.17	40	3	.810	.820	2.1
		4	.750	.757	2.9
		5	.697	.686	3.7
		7	.592	.598	5.0
3.17	40	3	.812		2.1
		4	.759		2.8
		5	.704		3.6
		7	.603		4.9
		Series II			
5.08	15	9	0.833	0.832	1.9
		12	.785	.800	2.5
		15	.733	.748	3.1
		21	.659	.650	4.2
5.88	15	9	.822		2.0
		12	.787		2.5
		15	.733		3.2
		21	.659		4.2
5.88	23	6	.783	.779	2.6
		8	.718	.713	3.4
		10	.634	.669	4.3
		14	.545	.551	5.6
5.88	23	5	.805	.814	2.2
		7	.743	.730	3.1
		9	.680	.653	4.1
		13	.582	.605	5.0
2.94	30	6	.805	.810	2.2
		8	.747	.756	2.9
		10	.689	.699	3.7
		14	.602	.615	4.8
2.94	30	6	.813		2.1
		8	.766		2.6
		10	.711		3.5
		14	.616		4.7

[a] Moles of glucosidic bonds per liter.[30]

ence between the two values. It is of interest to note, however, that Fischer and de Montmollin[48] also found changes in their values for the energy of activation in the neighborhood of 15–20° for the crystalline alpha amylase from *Bacillus subtilis* and for the beta amylase from wheat and from barley malt.[48]

The temperature dependence of the affinity constant within the temperature interval studied here is that characteristic of an equilibrium constant ($\ln K = -\Delta H/RT + \Delta S/R$). Evidence for this statement is given by the linear relation obtained in a plot of the natural logarithm of the affinity constant *versus* the absolute temperature. The deviation of the experimental values from the linear relation corresponds to a standard deviation in the values for the affinity constant of ±2%. This deviation is well within the limits of experimental error.

TABLE VI

INFLUENCE OF TEMPERATURE ON THE RATE CONSTANT AND ON THE VELOCITY OF HYDROLYSIS OF 3.08 × 10^{-3} *M* WAXY MAIZE STARCH[a] BY SWINE PANCREATIC AMYLASE

Series no.	Temp., °C.	Velocity of hydrolysis,[b] $V \times 10^7$	$(1 + K_aS)/K_aS$	Max. velocity[b,c] $V_m \times 10^7$	Enzyme concn., moles/l. $(E)_0 \times 10^{10}$	Rate constant,[d] sec.$^{-1}$ k_2
1	40	3.65	1.14	4.15	3.17	1310
	30	1.97	1.10	2.16	3.17	680
	15	1.16	1.05	1.22	6.34	193
2	30	1.88	1.10	2.06	2.94	702
	23	2.24	1.07	2.40	5.88	409
	15	1.11	1.05	1.16	5.88	189

[a] Moles of glucosidic bonds per liter.[30] [b] Moles of glucosidic bonds per liter per second. [c] $V_m = [V(1 + K_aS)/K_aS]$. [d] $k_2 = V_m/(E)_0$.

The values for the apparent heat and entropy of enzyme–substrate complex formation (−7.1 ± 0.2 kcal. mole^{-1} and −7.1 ± 0.8 cal. deg.$^{-1}$ mole^{-1}) calculated from the temperature dependence of the affinity constant are compared in Table VII with values reported for other enzyme systems. Because of the limited number of enzymes studied, an extensive comparison cannot be made. However, it is interesting to note that the values for the apparent heat of enzyme–substrate complex formation for the three carbohydrases are similar in sign and magnitude.

TABLE VII

VALUES FOR APPARENT HEAT AND ENTROPY OF ENZYME–SUBSTRATE COMPLEX FORMATION OF SWINE PANCREATIC AMYLASE COMPARED WITH VALUES REPORTED FOR OTHER ENZYMES

Enzyme system	ΔH, kcal./mole	ΔS, cal. deg.$^{-1}$ mole^{-1}
Invertase[49]	− 2.0	− 1.0
Emulsin[50,51]		
Methyl β-D-glucoside	− 7.5	
Ethyl β-D-glucoside	− 4.5	
Swine pancreatic amylase	− 7.1	− 7.1
Citric acid dehydrogenase[52]	−13.4	−40.0
Carbonic anhydrase[53]	−20.0	−59.8
Urease[54]	3.6	17.5

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