STUDIES OF THE POLYGALACTURONASE-(PG)-ENZYMES OF MOULD ORIGIN

VII. KINETICS OF THE PECTIN DECOMPOSITION OF TYPE I. PG*

By

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(Received April 9, 1964)

Introduction

It was established that the PG which was once considered a single enzyme, consists of several components. Thus, simultaneously, the glycosidic linkages of pectin are hydrolised parallel with several enzymes. DEMAIN and PHAFF [1] were the first to classify PG-enzymes and later followed by DEUEL and STUTZ [2]. Accordingly, distinction is to be made between PGs of types I., II. and III. — Type I. is a PG-component of liquefying property. The precondition of splitting the glycosidic linkage of pectin is that, besides the linkage, there should be free carboxylic groups, too.

Type II. PG hydrolises the highly esterified, so-called native pectin far more rapidly than the polymer of low-degree esterification. Therefore, around the glycosidic bond to be split, there must be present methylated carboxylic groups. Pectinesterase (PE) inhibits the activity of Type II. PG. Type III. PG is a component of saccharifying property that splits galacturonic acid units from the end of the pectin chain [2].

Theoretically, on the enzymic hydrolysis of pectin, kinetic conclusions can be drawn from the results obtained by the direct determination of reducing groups that become freed, and from the decrease in viscosity of the pectin solution, respectively. Many authors have already used both the increase of the reducing content of the pectin solution (3—5) and the decrease in viscosity (6—8) for their kinetic calculations. To study the kinetics of the enzymic hydrolysis of pectin is however considered to be a hard task. I.e., the results are influenced not only by the degree of esterification and polymerization of the substrate and by the way of activity-measuring (determination of the reducing material content, viscosity measuring); the problem is rendered complex also by the fact that the three PG-components as well as PE simultaneously affect the pectin. Therefore, since research workers have simultaneously measured the resultant of the joint effect of several pectin decomposing

* VIth publication: Die Nahrung **7**, 326 (1963).

enzyme components, and from this they have concluded the kinetics of pectolysis — the results obtained so far in the field of the kinetics of enzymic decomposition of pectin are to be accepted with some reserve.

We have tried to ensure the optimum conditions for a single PG-component: Type I. PG, thus eliminating the effect of all the other pectin decomposing enzyme components. In the present paper we shall discuss the kinetical regularities as experienced under these conditions.

Experimental procedure

Materials and methods

Substrate

For our kinetic studies a Na-pectate that was purified by the KERTÉSZ method [9] and having an esterification degree of 6 per cent, was used. (Obipektin A. G. Bischofszell, Schweiz.) First the proper quantity of Na-pectate was wetted with a small quantity of methanol and was then agitated on a shaker with a solution of 0.125 M NaCl solution until it became completely dissolved, finally it was diluted with a 0.125 M NaCl solution to a pectin concentration of 1 per cent. This was the stock solution of which with a NaCl solution of 0.125 M, the test dilutions of an optional pectin concentration was produced. Since, according to experiences, it is only with fresh pectin solutions that reproduceable results can be obtained, a freshly produced pectin solution was always used for the tests. The Na-pectate concentration (c_s) is given in per cents.

Viscosity measuring

The viscosity measurings was performed in a buffer-free medium (pH = 4) in a water bath that had been regulated to 20 °C by way of the Höppler ultrathermostate, with the modified Oswald capillar viscosimeter of 63.62 sec. water value [10]. The relative viscosity referred to the NaCl solution of 0.125 M, the effluent time of which, measured on the viscosimeter, was 64.6 sec.

Enzyme solution

In our laboratory, right before the test, with the KOCH method [11] we produced a 5 per cent enzyme extract from a dry mould bran preparation that was prepared with the aid of the high pectolytic Asp. niger strain No. 21 [12]. According to the original prescription, the mould bran is to be wetted with ethanol, then it must be extracted for one hour on a shaker. As proved by our tests [12], ethanol wetting did not seem to be suitable as it resulted in considerable inactivating. This is the reason why, when making the enzyme

extract, the ethyl alcoholic wetting was omitted. The enzyme concentration (c) is given in the dimension of dry mould bran g/litre.

Theoretical considerations

The kinetics of pectin decomposition can be studied in an exact manner only if the kinetic conclusions are drawn from the changes ensuing on the effect of a single PG-component only. In the present experiments we have chosen such test conditions and have worked with such measuring methods that the obtained changes could be attributed to the effect of a single PG-component. The experiments were performed with Na-pectate substrate and the progress of pectin hydrolysis was established by viscosity measuring. The disturbing effect of PE cannot be felt on Na-pectate. Type II. PG hydrolyses Na-pectate very slowly or not at all. Type III. PG splits off monogalacturonic acid units from the end of the pectin chain; during the short period of reaction this, of course, will not perceptibly influence the average mol.wt. — and viscosity.

From the data of viscosity-measurements kinetic calculations can be made on the basis of the following conceptions: with the progress of hydrolysis the viscosity of the Na-pectate solution decreases. From the specific viscosity (η_{sp}), with the aid of the MARTIN function [13], the value of the intrinsic viscosity ($[\eta]$) can be determined,

$$\frac{\eta_{sp}}{c_s} = [\eta] \cdot e^{k_2 \cdot [\eta] \cdot c_s} \quad \text{and} \quad \log \frac{\eta_{sp}}{c_s} = \log [\eta] + 0.4343 \cdot k_2 \cdot [\eta] \cdot c_s \qquad (1)$$

where k_2 is a constant depending on the form and size of the molecule. (According to OWENS [14], the value of k_2 is, in the case of pectin 0.42.)

Between the intrinsic viscosity and the degree of polymerization (V) of pectin, OWENS [14] has established the following empiric relation:

$$[\eta] = 1.528 \cdot 10^{-3} \cdot V^{1.34}. \qquad (2)$$

With the progress of the reaction, from the change of the degree of polymerization we might conclude on the number of split bonds which makes it possible for us to make kinetic calculations. (In seeking for the basic principle discussed in this paper, we have mainly relied on the works of SLAVIČKOVA [6] and DURFEE [15].)

With pectin hydrolysis, on following DURFEE [15], any bond could split up on the basis of statistical probability and then the average mol. wt. according to the weight, and the degree of polymerization respectively, would be:

$$\overline{M}_w = \frac{2n - p + 1}{p + 1} \cdot m \tag{3}$$

and

$$\frac{\overline{M}_w}{m} = V_x = \frac{2n - p + 1}{p + 1} \tag{4}$$

where $\overline{M}_w$ = the average mol. wt. according to weight,
V_x = the degree of polymerization,
n = the number of monomers in the molecule,
m = mol. wt. of the monomers
p = the number of particles obtained after decomposition.

With the above derivation DURFEE presumed that

a) pectin molecules are built up of identical number of units: i.e. the starting substrate is a homogeneous polymer,

b) the decomposition occurs at random and its probability is the same, e.g. in case of a double-member unit and a long chain. Naturally, the first stipulation is not met with in the case of the substrate, but this is not essential for on the basis of the second assumption the enzyme will of necessity produce within a short time the most different polymerization mixture.

If we introduce the following markings:
the number of the bonds of the original molecule, $a = n - 1$,
the number of scission-bonds, $x = p - 1$,
and if we replace these in formula 4., then

$$V_x = \frac{2a + 2 - x}{x + 2}$$

and since $a = V_0 - 1$ (where V_0 is the initial degree of polymerization), the number of the scission-bonds will be:

$$x = \frac{2(V_0 - V_x)}{V_x + 1} \tag{5}$$

Assuming a monomolecular reaction, the reaction rate constant will be:

$$k = \frac{1}{t} \ln \frac{a}{a - x} \tag{6}$$

substituting a and x, we obtain:

$$k = \frac{1}{t} \ln \frac{(V_0 - 1)(V_x + 1)}{(V_x - 1)(V_0 + 1)} \tag{7}$$

A great many circumstances argue in favour of the DURFEE hydrolysis (performed at random). However, neither is the possibility precluded that with hydrolysis the pectin chain becomes "halved" i.e., the enzyme always attacks the middle of the longest molecule. Accepting the hypothesis of the halving decomposition of EKENSTAM [16], then, similarly to the above train of thought, and if it is the x-bond that becomes scissioned, the degree of polymerization (V_x) will be:

$$V_x = \frac{V_0}{x + 1}$$

and from this

$$x = \frac{V_0 - V_x}{V_x} \tag{8}$$

and

$$k = \frac{1}{t} \ln \frac{V_x (V_0 - 1)}{V_0 (V_x - 1)} \tag{9}$$

(Further the x and k values computed from the equations that had been deduced on the basis of the DURFEE decomposition, will be marked with index 1, while the values calculated on basis of the "halving hypothesis" are marked by index 2.)

Results

Determination of the intrinsic viscosity of pectin

It is the MARTIN-equation (formula 1.) that establishes correlation between reduced viscosity $\left(\frac{\eta_{sp}}{c_s}\right)$ and intrinsic viscosity ($[\eta]$) of macromolecules. With polymers that do not contain ionizable groups, if the reduced viscosity is graphically described in the macromolecule concentration function, we obtain a straight line and from this the values of the intrinsic viscosity can be established at $c_s = 0$. With polyelectrolites and also with pectin, the $[\eta]$ cannot be determined with this method, i.e. neither is the MARTIN-equation effective. (Table 1, Fig. 1.) It is apparent that with a small pectin concentration instead of decreasing, the value of the reduced viscosity will abruptly increase.

The intrinsic viscosity values shown in Table I, were determined with the aid of the MARTIN-equation. The MARTIN-equation can be solved by series development and then, for instance, by the NEWTON approximation solution and the nomograph method, respectively. We have chosen the latter. (Making up the different c_s values by whole-number values $[\eta]$, we have calculated

Table I

The values of relative viscosity $\left(\frac{\eta_{sp}}{c_s}\right)$ and of intrinsic viscosity $[\eta]$ of solutions with different Na-pectate concentrations

Na-pectate concentration %	Dissolved in water		In NaCl solution of 0.125 M	
	$\frac{\eta_{sp}}{c_s}$	$[\eta]$	$\frac{\eta_{sp}}{c_s}$	$[\eta]$
0.01	9.00	8.90	1.30	1.30
0.025	6.40	6.00	1.50	1.48
0.05	5.20	4.96	1.50	1.44
0.10	4.30	3.74	1.60	1.46
0.20	3.60	3.04	1.60	1.40
0.30	3.46	2.54	1.80	1.47
0.40	3.35	2.29	1.82	1.43
0.60	3.45	2.06	2.10	1.47

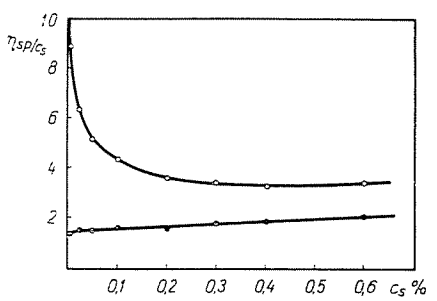

Fig. 1. Determination of the intrinsic viscosity of Na-pectate solution by way of graphic method

—○—○— reduced viscosity values in aqueous solution,

—●—●— reduced viscosity values $\frac{\eta_{sp}}{c_s}$ measured in 0.125 M NaCl solution.

C_s = Na-pectate concentration in per cent

the log $[\eta]$ backwards, the $0.4343 \cdot 0.42 \cdot c_s \cdot [\eta]$, the log $\frac{\eta_{sp}}{c_s}$ and from this the value of $\frac{\eta_{sp}}{c_s}$. With the different pectin concentrations (c_s) we summarized the values of $\frac{\eta_{sp}}{c_s}$ belonging to the intrinsic viscosity in a table, and then constructed a nomogram from the data of the table, on which the intrinsic viscosities belonging to the optionally reduced viscosity values, can easily be read.

In the range of low pectin concentration the increase of the reduced viscosity value of pectin and, consequently, that of the intrinsic viscosity

value is due to the fact that the pectic acid is in a highly ionized state as a consequence of severe dissociation the pectin molecule becomes elongated and this change of form results in the increase of viscosity. According to OWENS [14, 17] one reason for the increase in viscosity is that the solvent inhibits the moving of the ionized molecule.

SWANSON [13] observed that in a solution containing electrolite, the cellulose xanthate behaves like a polymer not containing ionizable groups. Like SWANSON we, too, have tried to add electrolite (NaCl) to the pectin solution. On basis of the preliminary tests [12] we have decided on the 0.125 M NaCl concentration because from 0.155 M on pectin was precipitated by NaCl. With different pectin concentrations, the reduced viscosity of the solutions containing 0.125 M NaCl and the intrinsic viscosity values corresponding to the $\frac{\eta_{sp}}{c_s}$ as determined by a nomograph, are shown in Table I. We have demonstrated the relation of $\frac{\eta_{sp}}{c_s}$ and the pectin concentration also in Fig. 1.

It can be seen that by adding the electrolite, we obtain a "normal" straight line the axial section of which will supply $[\eta] \cdot ([\eta] = 1.46)$.

The instrinsic viscosity values determined with the MARTIN-equation (nomogram) display a striking likeness to the above mentioned, graphically determined $[\eta]$. The max. deviation from the mean $[\eta]$ of the values $[\eta]$ calculated with the different pectin concentrations, is ± 3.6 per cent. The molecule weights calculated from these intrinsic viscosity values and the degree of polymerization are also in good agreement (see later).

When interpreting the straight line seen in Fig. 1, it is to be presumed that in the absence of electrolites, the electrically charged groups will repulse each other, this resulting in the alignment of the pectin chain. By adding electrolite, the repulsing effect of the adjacent groups will become less — due to the shadowing effect of electrolite. It is to be assumed that the electrolite exerts an influence on the solvation of molecules. As a result of all this the MARTIN-equation will become effective in the NaCl medium.

The effect of pectin concentration on the reaction rate

On hydrolysing Na-pectate the split bond-values x_1 and x_2 that can be calculated from the change in the degree of polymerization by the formulae 5 and 8., refer to one molecule. With the different pectin concentrations the reaction rate can only be determined if the milliequivalent quantity (x_1', x_2) of split bonds is determined. To compute x_1' and x_2' we have used the formulae $x_1' = = 50.5 \cdot \frac{x_1}{V_0} \cdot C_s$ and $x_2' = 50.5 \cdot \frac{x_2}{V_0} C_s$ where 50.5 is a constant value obtained from the mol. wt. of the monomeric part (198) of the Na-pectate,

by g/litre conversion. If we are illustrating the milliequivalent quantity of the split bonds in the function of time, such a curve or straight line will be obtained, the direction tangent of which is equivalent to the momentary rate of reaction.

With different pectin concentrations we performed hydrolysis experiments with the aid of an enzyme concentration of 0.125 g/l. From the measuring data of viscosity determined at different times, we have computed the values of the degree of polymerization and the x, x' and k-values belonging to these, respectively. In order to demonstrate the method, in Table II we have summar-

Table II

The changes of polymerization degree values (V), the changes of the number of split bonds (x_1 and x_2), of bond milliequivalents ($x_1' \cdot 10^{-2}$ and $x_2' \cdot 10^{-2}$) as well as of reaction rate constants ($k_1 \cdot 10^{-4}$ and $k_2 \cdot 10^{-4}$) as experienced at the enzymic hydrolysis of 0.5 per cent. Na-pectate solution ($C_S = 0.5$). Enzyme concentration (C) = 0.125 g/liter

Incubation time (min)	V	x		$x_1' \cdot 10^{-2}$	$x_2' \cdot 10^{-2}$	$k_1 \cdot 10^{-4}$	$k_2 \cdot 10^{-4}$
		x_1	x_2				
0	203	—	—	—	—	—	—
37	194	0.092	0.046	1.14	0.57	0.11	0.06
65	188	0.159	0.080	1.97	0.99	0.12	0.06
95	181	0.242	0.122	3.00	1.51	0.12	0.06
130	177	0.292	0.147	3.63	1.82	0.11	0.06
165	166	0.443	0.223	5.50	2.77	0.13	0.06
260	144	0.815	0.410	10.40	5.09	0.13	0.06
320	140	0.895	0.450	11.11	5.59	0.13	0.07
380	135	1.000	0.503	12.42	6.25	0.13	0.06
440	129	1.140	0.574	14.32	7.20	0.12	0.06
500	121	1.340	0.679	16.65	8.44	0.13	0.07

ized the polymerization degrees obtained with the 0.5 per cent pectin hydrolysis and the values deduced from these. The values of the reaction rate constants as experienced with the enzymic hydrolysis of solutions having different pectin concentrations, are summarized in Table III. (We have omitted to make known the values of k_2 since k_2 — as it is to be seen from Table II. — is always the half of the existing k_1.) The number of split bonds measured or calculated with the individual pectin concentrations at different times, were illustrated in the function of time (Fig. 2.), and with the aid of the direction tangents of the thus obtained straight lines, we have determined the rates of the enzymic pectin decomposition belonging to the various pectin concentrations. The values of reaction rates as experienced with the different Na-pectate concentrations, are summarized in Table IV. From the reaction rate experienced with the various pectin concentrations the Michaelis—Menten constant (K_m) can easily be determined. The reaction rate as illustrated in the function of

Table III

Reaction rate constants ($k_1 \cdot 50^{-4}$) a measured at different times in the enzymic hydrolysis of solutions with different pectine concentrations (C). Enzyme concentration (C) = 0.125 g/l)

$c_s = 0.05\%$		$c_s = 0.075\%$		$c_s = 0.1\%$		$C_s = 0.2\%$		$C_s = 0.4\%$		$C_s = 0.5\%$	
cubation me (min.)	$k_1 \cdot 10^{-4}$	Time (min.)	$k_1 \cdot 10^{-4}$	Time (min.)	$k_1 \cdot 10^{-4}$	Time (min.)	$k_1 \cdot 10^{-4}$	Time (min.)	$k_1 \cdot 10^{-4}$	Time (min.)	$k_1 \cdot 10^{-4}$
0	—	0	—	0	—	0	—	0	—	0	—
30	1.11	40	0.65	34	0.70	40	0.32	30	0.24	37	0.11
65	0.62	70	0.67	62	0.78	70	0.37	60	0.25	65	0.12
90	0.91	100	0.66	92	0.61	130	0.35	90	0.19	95	0.12
120	0.96	160	0.68	123	0.63	160	0.34	155	0.19	130	0.11
183	0.79	220	0.61	155	0.65	195	0.38	195	0.19	165	0.13
248	0.71	280	0.52	217	0.59	250	0.36	228	0.19	260	0.13
308	0.67	400	0.63	277	0.60	310	0.34	350	0.20	320	0.13
368	0.69			337	0.59	370	0.38	415	0.19	380	0.13
428	0.77									440	0.12

Table IV

The values of reaction rates ($v \cdot 10^{-4}$) as experienced with the different Na-pectate concentrations (C_s). Reaction rate values were determined from Fig. 2, by graphic method

C_s	$\frac{1}{C_s}$	$v \cdot 10^{-4}$	$\frac{1}{v} \cdot 10^3$	$\frac{C_s}{v} \cdot 10^3$
0.05	20.0	1.77	5.65	2.82
0.075	13.3	2.35	4.24	3.18
0.10	10.0	2.92	3.42	3.42
0.20	5.0	3.74	2.67	5.34
0.40	2.5	4.00	2.50	10.00
0.50	2.0	cca 3,70	—	—

the substrate concentration (Fig. 3), according to the definition, $K_m = \frac{V_{max}}{2}$. From the diagram $K_m = 0.058$ can be read off.

According to HOFFMANN—OSTENHOF [18], the Michaelis constant can be more precisely determined if the known LINEWEAVERBURK [19] equation is brought to the following form:

$$\frac{C_s}{V} = \frac{C_s}{V_{max}} + \frac{K_m}{V_{max}} \tag{10}$$

and the experimental results are illustrated in the diagram $\frac{C_s}{V} - C_s$. The

experimental data are shown in Fig. 4. The straight line in Fig. 4. is determined by the following equation:

$$\frac{C_s}{V} = 2{,}120 \cdot C_s + 130$$

of which $K_m = 0.0613$ (g/100 g). It can be seen that the K_m value determined by the two method shows fairly good agreement. The inaccuracy of the diagrams and the errors in the readings are the cause of the K_m values slightly deviating from each other.

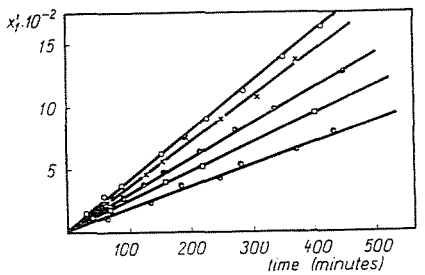

Fig. 2. Determination of enzymic hydrolysis rate of pectine solutions with different concentrations

—●—●— with Na-pectate concentration of 0.05%
—□—□— ,, ,, ,, ,, 0.075%
—●—●— ,, ,, ,, ,, 0.1%
—×—×— ,, ,, ,, ,, 0.2%
—○—○— ,, ,, ,, ,, 0.4%
measured milli-equivalent bonds ($x_2' \cdot 10^{-2}$).

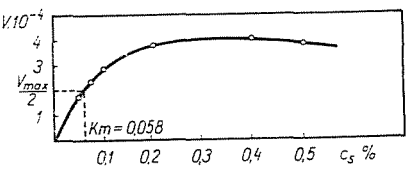

Fig. 3. Graphic determination of the Michaelis-constant, K_m of *PG*
C_s = concentration of Na-pectate in per cent
V = reaction velocity

The effect of enzyme concentration on the reaction rate

A 0.3% Na-pectate solution containing 0.125 M NaCl was hydrolysed with a PG preparation of different concentrations. From the decrease of viscosity, the change in the degree of polymerization was calculated by means of formula 2, while the values of the reaction rate constants k_1 and k_2 were determined with formulae 7 and 9. The results are summarized in Tables V. and VI.

When illustrating the reaction rate constants, which had been measured with the various enzyme concentrations, in the function of the enzyme concentration, a straight line will be obtained *(Fig. 5)* meaning that the reaction rate is in ordinal relation with the enzyme concentration.

Tables V—VI

Values of reaction rate constants ($k_1 \cdot 10^{-4}$) as measured at different times in the hydrolysis of 0.3% Na-pectate solution with different enzyme concentrations (C)

Incubation time (min.)	$C = 0.025$ g/l $k_1 \cdot 10^{-4}$	$C = 0.05$ g/l $k_1 \cdot 10^{-4}$	$C = 0.1$ g/l $k_1 \cdot 10^{-4}$	$C = 0.25$ g/l $k_1 \cdot 10^{-4}$
0	—	—	—	—
60	—	—	—	0.49
120	0.034	0.059	0.15	0.48
240	0.041	0.079	0.15	0.48
360	0.044	0.077	0.15	0.45
480	0.044	0.082	—	—

Incubation time (min.)	$C = 0.5$ g/l $k_1 \cdot 10^{-4}$	Incubation time (min.)	$C = 1$ g/l $k_1 \cdot 10^{-4}$
0	0	0	—
65	0.80	35	1.46
130	0.81	70	1.87
250	0.77	100	1.89
370	0.60	130	1.66
		190	1.48
		250	1.31

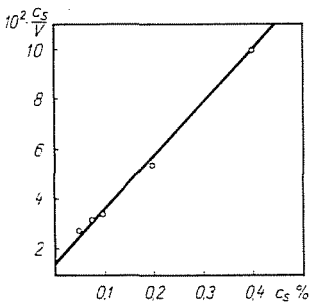

Fig. 4. Determination of the Michaelis-constant with the Lineweaverburk reciprocal plot
C_S=concentration of the Na-pectate in per cent
V=reaction velocity

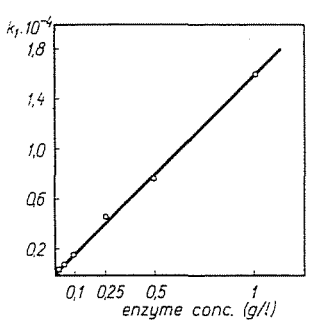

Fig. 5. Relation between the enzyme concentration and the reaction rate constant (k_1)

The effect of temperature on the reaction rate

ARRHENIUS [19] established a relationship between reaction rate and temperature, from which equation the activation energy of reaction can also be determined.

$$E = \frac{4.575 \cdot T' \cdot T''}{T'' - T'} \cdot \log \frac{k''}{k'} \tag{11}$$

where E is the activation energy of the process, k' and k'' are the reaction rate constants measured at temperatures T' and T''.

We hydrolysed a 0.4% Na-pectate solution containing 0.125 M NaCl with 0.125 g/litre PG preparation at 25 and 30 °C. From the viscosity measuring data we computed as described above and by means of formula 7, the reaction rate constants k' and k'' belonging to 25 and 30 °C, respectively; k' proved to be 0.21, while k'' was 0.329 and from this the activation energy of the enzymic pectin hydrolysis was (by using the formula 11.): 16 065 cal M^{-1}. The activation energy computed with the values $k' = 0.143$, and $k'' = 0.163$ that had been determined by way of formula 9, was: 16 020 cal M^{-1}.

Discussion

The intrinsic viscosity of pectin cannot be determined in aqueous solutions by using a graphical method because with small pectin concentration the value of the reduced viscosity increases abruptly. It may be assumed that the strengthening ionization changes the form of the chain of molecules and this results in an increased viscosity. This distorted curve with minimum becomes straight on the addition of an electrolyte, and thus, the intrinsic viscosity can be determined with the MARTIN-equation or the usual graphical method. The solution of the MARTIN-equation as well as the value of the intrinsic viscosity that was extrapolated to $C_s = 0$, showed good agreement. The max. deviation of the values $[\eta]$ determined with the different pectin concentrations, from the mean $[\eta]$ is $\pm 3.6\%$, and the max. deviation of the polymerization degrees calculated from these intrinsic viscosity values, from the mean degree of polymerization is $\pm 2.9\%$. As will be seen the method gives reproduceable results, therefore, the formulae can be applied for kinetic tests.

The enzymic hydrolysis rate measured with different pectin concentrations does not change perceptibly, — at least, not in the examined time-interval, at a given pectin concentration. From this it follows that the affinity of PG to the substrate does not change though the polymerization degree of pectin has, in certain cases, decreased from 200 to even 50—60. With most experiments the reaction rate constant computed from the equation

of the first order reaction was steady, which means, that the hydrolysis of the Na pectate of Type PG follows the laws of the first order reaction. With some tests, however, it seems as if the value of k showed a monotonous decrease in the function of time. This can be explained by presuming that, with the calculations, we have committed such neglections that were not permissible or because the hydrolysis was not a first order reaction. (E.g. in the course of hydrolysis, we have considered the k_2 of OWENS to be a constant though it is known that k_2 depends on the molecule weight. Unfortunately, the relation between k_2 and the molecule weight is not measured; thus, these neglections were made out of necessity.) In spite of this, with certain enzyme and pectin concentrations, it seems that the enzyme hydrolysis of Na pectate — at least in the initial 6—7 hours of the reaction — may be considered as a first order reaction.

The value of $K_m = 0.0613$ as determined by us is considerably less than the $K_m = 0.3$ value measured by JANSEN [3] in the first stage of pectin hydrolysis; much greater than the value $3 \cdot 10^{-4}$ for the MICHAELIS constant determined by WEITNAUER [8], however, in the order of magnitude, it agrees with the 0.032 value as established by SLAVIČKOVA [6]. SLAVIČKOVA has worked with a pectin of a higher esterification degree than the mean, but in her tests she measured the resultant of the joint effect of PE, and Types I—II. PGs. The great deviation from the K_m value of WEITNAUER is quite understandable because in measuring the viscosity he started out from a principle that is quite erroneous. He determined the viscosity of pectin solutions of various concentrations. For the calculations he availed himself — and this was fundamentally wrong — of the pectin concentrations readable from the calibration diagram, instead of viscosity values measured at the enzymic pectin decomposition. This, however, is not right because during the enzymic pectolysis the pectin concentration does not change, it is only the polymerization degree of pectin that decreases.

The decrease in viscosity and the increase in the reducing material in the course of enzymic pectin decomposition was studied by KERTÉSZ [20]. He established that a viscosity decrease change of about 80% corresponds with 1.25% split bonds. This establishment agrees with the experimental results made known in this paper. JANSEN [3] had ascertained that 2% reducing material becomes freed with a viscosity decrease of 50%, this being for more than could be expected from the scission of glycosidic linkages resulting from the effect of liquefying PGs. Most probably, JANSEN worked with an enzyme preparation Type III. PG, having high activity and the considerable releasing of the reducing material may be attributed to the effect of the latter PG-component.

In Fig. 3 we might observe a remarkable thing. The reaction rate reaches the max. value with a pectin concentration of 0.4%, and with a substrate

concentration of 0.5% the rate decreases perceptibly. According to the Michaelis—Menten theory, after reaching the max., the reaction rate should remain at the value V_{max}, however, in the case of PG the substrate and the reaction rate are in relationship, that can be illustrated with a curve having a maximum. AFANASYEV [21] has reported on a similar observation in the case of the invertase. For discussing the present problem more thoroughly, more experimental data would be necessary, therefore, we do not wish to tackle this problem now.

With the aid of the deduced relations we made use of the viscosity measuring data that had been obtained during pectolysis, for the determination of the Michaelis constant of the reaction. On basis of similar principles, with the inhibition tests of the "liquefying" PG components it is possible to determine from the viscosity measuring data the competitive or non-competitive inhibition, too.

The value of the activation energy of the enzymic pectolysis determined with the aid of the Arrhenius formula (about 16000 cal M^{-1}) agrees with the value of 14.400 determined by VAS [22] but it deviates considerably from the 28,000 cal M^{-1} of MERILL [23]. The activation energy measured in our experiments make KERTÉSZ' [20] concept on the structure of pectin questionable; according to this concept there occurs "secondary" bonds in pectin too. The activation energy computed by us is much greater than the greatest activation energy value experienced with the secondary bonds (9,000 cal M^{-1}). As already shown above, there is no contradiction between the high decrease of viscosity observed by KERTÉSZ and the low increase of the measured reducing material; i.e. according to our experiments the number of split bonds calculated from the change of the degree of polymerization shows perfect agreement with KERTÉSZ' data on viscosity and reducing material determination. Therefore, there is no need to presume that the splitting of the secondary, "super" structure of pectin, with which there is no increase in the reducing material, should bring about the abrupt decrease in viscosity.

From the greatness of activation energy we may draw conclusions regarding the temperature coefficient of the enzymic hydrolysis of Na-pectate, the Q_{10} [22]. The activation energy 16,000 cal M^{-1} corresponds with $Q_{10} = 2.5$ showing suitable agreement with the 2.3 Q_{10} value established by VAS [7]. On the basis of the present measuring data, no final attitude can be taken in reference to this problem as to what kind of mechanism the enzymic hydrolysis of Na-pectate occurs. Does the splitting happen at random, statistically or is the "halving" splitting the prevailing mechanism. This problem will be finally solved when the individual PG components will be separated from each other. Our experiments performed on tricalcium-phosphate gels bid fair to succeed.

Summary

1. When kinetically testing the pectin decomposition of Type I. PG we availed ourselves of viscosimetric data on basis of the following consideration: with the enzymic hydrolysis of pectin the decrease in viscosity is a result of the decrease in the degree of polymerization. With the aid of the deduced formulas, the degree of polymerization can be computed from the viscosity and from the change of the degree of polymerization also the number of the split linkages can be determined.

2. The intrinsic viscosity value of the pectin solution, needed for the polymerization degree-calculations, can be determined only in a 0.125 M NaCl solution with the usual graphical method.

3. The enzymic hydrolysis of Na-pectate — at least in the first 6—8 hours of the reaction — follows the regularity of the first-order reaction; the reaction rate constant does not change in the course of hydrolysis.

4. From the reaction rates measured with different substrate concentrations, we have determined the Michaelis constant with the aid of two methods, the results being 0.058 and 0.0613.

5. The activation energy of the pectin hydrolysis of Type I PG is 16065 cal M^{-1} and the temperature coefficient of the hydrolysis reaction: $Q_{10} = 2.5$.

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