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Enzymatic Breakdown of Polymetaphosphate. II

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In a previous paper [1] we have shown that polymetaphosphates of very high molecular weight are broken down by enzymes from some microorganisms such as *Aspergillus niger*, *Aspergillus oryzae* and *Penicillium expansum*. We were able to show that the enzymatic breakdown of polymetaphosphate proceeds through the breaking of scattered -P-O-P- links in the polymetaphosphate chains, so that comparatively large fragments are at first formed; the hydrolysis does not occur by the removal of one or a few orthophosphoric acid groups at a time from the ends of the polymetaphosphate chains. We used preparations of polymetaphosphate having molecular weights of more than one million. Thus in our experiments a colloid of purely inorganic character was broken down by enzymatic means. (Concerning the earlier investigations on metaphosphatase see the references in our previous paper [1].)

We have continued our investigations by working out a convenient method of determining the enzyme activity of enzymes degrading polymetaphosphates. Hence it has become possible for us to investigate the influence of various factors on the velocity of the enzymatic breakdown of the polymetaphosphate with good accuracy. In these experiments we have used enzyme preparations from *A. niger*. We have also investigated the enzyme from *A. niger* regarding some of its physico-chemical properties.

Investigations on the occurrence of enzymes degrading polymetaphosphates in other organisms than those mentioned above are being carried out at present. The results will be published in a separate paper.

A non dialysable, high molecular substance of high phosphorus content has been isolated from *A. niger;* it has been described in a preliminary not [2]. The isolation of this substance, which is very like the polymetaphosphates, will be described in more detail in a future paper (where references will also be given to work dealing with the importance of the metaphosphate for micro-

organisms and substances of high phosphorus content from such micro-organisms).

The polymetaphosphates used as substrates in our investigations are designated as K 10, K 11, K 12, K 14 and K 15 of which all except K 15 has been described in previous papers [1, 3-6]. These polymetaphosphates are prepared by heating primary potassium orthophosphate. n in the formula has

$$n\,KH_2PO_4 \rightarrow (KPO_3)_n + n\,H_2O$$

magnitudes of the order 2,000—20,000. For the experiments the potassium metaphosphate is dissolved in buffer solutions containing an excess of sodium ions, whence the polymetaphosphate in solutions may be regarded as a sodium salt. (The potassium metaphosphate is insoluble in pure water.) Some data of the substances used, *viz.* the temperature of the synthesis, the intrinsic viscosity (defined as the limiting value $(\eta_{sp}/c)_{c\rightarrow 0}$) and the molecular weight have been collected in Table 1. The intrinsic viscosity was determined in 0.4 *N* NaCl. The molecular weights determined (in 0.4 *N* NaCl) by means of sedimentation in the ultracentrifuge and diffusion refer to the original $(KPO_3)_n$. The preparations are of course polydisperse. (Some of the molecular weight determinations were carried out some years ago [4], whereas the intrinsic viscosity measurements have been performed this year. It may happen that the polymetaphosphate preparations have altered a little during the storage.)

For the enzyme preparation we have used cultures of *A. niger* van Tieghem (no. 594 from the National Collection of Type Cultures maintained in Britain by the Medical Research Council) [cf. 7]. The mould was cultivated at 28° C on a medium of the following composition: 10 g glucose, 0.2 g K_2HPO_4, 0.4 g $NaNO_3$, 0.05 g $MgSO_4$, 7 H_2O and 100 ml water. The time of cultivation has varied but generally the enzyme preparation was begun after about 7 days growth.

The enzyme solutions have been prepared in the following manner: After addition of a suitable quantity of acetic acid — sodium acetate buffer (pH = 5.4; ionic strength = 0.1) the mould was ground up in a »Waring blender» and allowed to stand over night at a few degrees above zero. The mass was then filtered and the solution obtained dialysed against distilled water for some days. The solution was then evaporated at $\sim 35°$ *in vacuo* to about 1/20th of the original volume. After this the solution was dialysed against the buffer to be used in the experiments. Before being used the solution was centrifuged and filtered. In spite of a comparatively low nitrogen content these solutions have such a large enzyme activity that they must be diluted many times with a suitable buffer before being used for the study of the

Table 1. Some physico-chemical data of the polymetaphosphate preparations.

Preparation	Temperature of synthesis °C	Intrinsic viscosity	Mol. wt.
K 10	290	0.48	460,000
K 11	445	1.13	2,000,000
K 12	260	0.73	280,000
K 14	495	0.80	1,300,000
K 15	500	0.92	1,200,000

polymetaphosphate degradation. The enzyme solutions contain not only the enzyme degrading polymetaphosphate but also, of course, other high molecular products from the mould, for instance an enzyme degrading cellulose; this enzyme can destroy the cellophane bags used for dialysis. Sometimes it has been necessary, therefore, to use bags of nitrocellulose for dialysis of the concentrated enzyme solutions. Several different enzyme preparations have been used in our experiments. We have started experiments to fractionate the impure enzyme solution in order to obtain the enzyme for the degradation of polymetaphosphate in a purer state.

Calculations of the enzyme activity from viscosity measurements

In our previous work we followed the enzymatic breakdown of the polymetaphosphate mainely by means of viscosity measurements. As these polymetaphosphate solutions have a comparatively high viscosity it is easy to follow the first phase of the breakdown by viscosity measurements. Therefore in this work we have used the same method; the viscosity measurements have been performed in a capillary viscosimeter according to Ostwald. The viscosity of the polymetaphosphate solution has been measured relative to that of the buffer solutions. From the relative viscosity (η/η_o) the specific viscosity has been calculated ($\eta_{sp} = \eta/\eta_o - 1$). All viscosity measurements in this work have been performed in a thermostat at 25.0°C.

When calculating the enzyme activity it is particularly valuable to determine the velocity of the breakdown during its first phase. However the derivative $d\eta_{sp}/dt$ at $t = 0$ is not easy to determine with sufficient accuracy, and is very dependent on the degree of polymerisation of the subtrate and the ionic strength; hence it can only be used as a measure of the breakdown velocity

under very well-defined conditions. Therefore we have used the method of calculation described below.

A somewhat analogous degradation, namely the breakdown of starch by α-amylase has been described by, among others, Hultin [8]. He has deduced and applied the following formula for the enzyme activity A:

$$A = c^2 \cdot \frac{d\left(\frac{1}{\eta_{sp}}\right)}{dt} \tag{1}$$

where c is the substrate concentration, η_{sp} the specific viscosity and t the time. We have tried to apply this formula for the polymetaphosphatase activity but in our case conditions are more complicated. The viscosity of a polymetaphosphate solution is not only dependent on the concentration of the colloid but also on the concentration and species of the low molecular salts in the solution. On addition of salt to a polymetaphosphate solution the viscosity decreases, at first very rapidly, then more moderately, but without approaching a limiting value before the salting out of the polymetaphosphate occurs [5]. Kern [9] has proposed to regard the specific viscosity of a colloidal electrolyte as the product of two factors, the ionic factor and the macromolecular factor allowing for the charge effect and the degree of polymerization of the colloid respectively

$$\eta_{sp}/c = J \cdot (\eta_{sp}/c)_m \tag{2}$$

where J is the ionic factor and $(\eta_{sp}/c)_m$ is the macromolecular factor. Kern's work concerns polyacrylic acid and its sodium salt, but assuming Eq. (2) to be valid for the polymetaphosphates also, it is suggested to write Hultin's formula (Eq. (1)) in the following form for the present.

$$A = J \cdot c^2 \cdot \frac{d\left(\frac{1}{\eta_{sp}}\right)}{dt} \tag{3}$$

However, it is very difficult to determine the ionic factor J. As an approximation we may assume J to be independent of the substrate concentration and constant during the first phase of the enzymatic breakdown and proportional to the spec. viscosity at $t = 0$ ($(\eta_{sp})_{t=0}$), but if we do so we must not forget that $(\eta_{sp})_{t=0}$ is dependent not only on the substrate concentration but also on the molecular size and shape. Hence it follows that if we replace J by $(\eta_{sp})_{t=0}$ the

measurements of the enzyme activities must be carried out on the same substrate and at the same substrate concentration if the activities are to be compared. Of course it is desirable to evaluate the absolute value of J, or of least a relative one, for all polymetaphosphate preparations and all concentrations and in different salt solutions but this work — if possible to perform — must be carried out later. As a relative measure of the enzyme activity in comparison experiments (the same substrate and substrate concentration) we use a quantity z defined by Eq. (4)

$$z = (\eta_{sp})_{t=0} \cdot \frac{d\left(\frac{1}{\eta_{sp}}\right)}{dt} \tag{4}$$

The z-values are calculated from the curves which are obtained when the values of $(\eta_{sp})_{t=0}/(\eta_{sp})_{t=t}$ are plotted against t.

(It may be pointed out that if Eq. (2) is valid and the ionic factor can be regarded constant during the first phase of the breakdown in a certain experiment we may write

$$\frac{(\eta_{sp})_{t=0}}{(\eta_{sp})_{t=t}} = \frac{[(\eta_{sp})_m]_{t=0}}{[(\eta_{sp})_m]_{t=t}} \tag{5}$$

The factor J cancels out by division of the equations corresponding to the times $t = 0$ and $t = t$ respectively. The quantities $(\eta_{sp})_{t=0}$ and $[(\eta_{sp})_m]_{t=0}$ are constants for each experiment. However, it must be emphasized that the application of the equation of Kern and the assumption that the ionic factor J is constant during the first phase of the breakdown have rather weak foundations.)

The z-values are usually suitable as a relative measure of the enzyme activity (the substrate and the substrate concentration being the same), as the z-values are rather independent of the variations in the salt concentrations which may occur. At varying salt concentrations and ionic strengths, but other factors such as substrate concentration and enzyme concentration pH etc. being constant, the $d\ (1/\eta_{sp})/dt$-values differ very much whereas the z-values differ but slightly. This is illustrated by experiments described below. (See Table 2). However, the determinations ought to be carried out at salt concentrations as high as possible but not too near the point of salting out.

When plotting $1/\eta_{sp}$ or $(\eta_{sp})_{t=0}/\eta_{sp}$ against t we usually get a linear relation. This is a support for the assumption that the ionic factor is rather constant during the first phase of the breakdown. Some typical curves are shown in Figs. 1 and 2 illustrating the change of the spec. viscosity with time for various

enzyme concentrations and the corresponding $(\eta_{sp})_{t=0}/\eta_{sp}$-values as a function of t. (t is the time that has elapsed from the addition of the enzyme to the beginning of the viscosity measurements + half the time of outflow). The experiments in question are described below.

Experiments at different ionic strengths

As has been mentioned already the viscosity of a polymetaphosphate solution is very dependent on the ionic strength and the species of the low molecular salts present. However, even if the original η_{sp} values and the decrease of η_{sp} with time differ very much with the ionic strength, almost the same z-values are obtained within the μ-interval convenient for these experiments (if enzyme and substrate concentration, pH, etc., are constant). This is illu-

Table 2. Experiments at different ionic strengths.

Substrate	Ionic strength	$(\eta_{sp})_{t=0}$	$d\left(\frac{1}{\eta_{sp}}\right)/dt \cdot 10^4 \text{min}^{-1}$	$z \cdot 10^4 \text{min}^{-1}$
K 11	0.30	0.62	416	258
in acetate	0.20	1.62	159	258
pH = 4.6	0.10	3.72	67	250
	0.05	7.14	31	220
K 15	0.22	2.20	133	292
in phosphate	0.15	3.00	97	292
pH = 6.4	0.09	5.20	51	263

strated by the following experiments. To samples of 5 ml of 0.5 % polymetaphosphate solutions in buffers of different ionic strengths was added 1 ml of enzyme solution. (Different enzyme preparations in the two series.) The measurements were made at 25.0° C. The results are collected in Table 2.

As is seen from the table the z-values are rather constant in each series, whereas the $d(1/\eta_{sp})/dt$-values vary greatly with the ionic strength. This is a good support for the use of the z-values in comparison experiments where the salt concentration may vary a little. However, there seems to be a tendency for the z-values to decrease with decreasing ionic strength. For our purposes this fact has no importance as our measurements are generally carried out at ionic strengths where z varies very little with μ. Wheather the equation of Kern is valid or not, the z-values have proved to be useful in comparison measurements of the enzyme activity at varying salt concentrations.

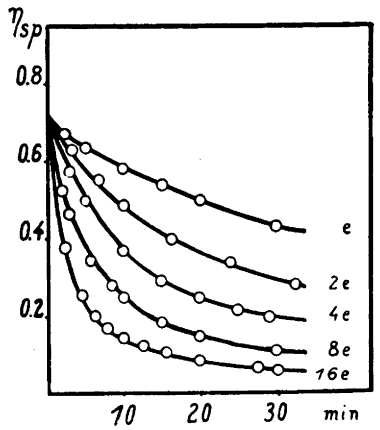

Fig. 1. Variation of η_{sp} with time during the enzymatic breakdown. Different concentrations of the enzyme. The curves refer to experiment no. I in Table 3.

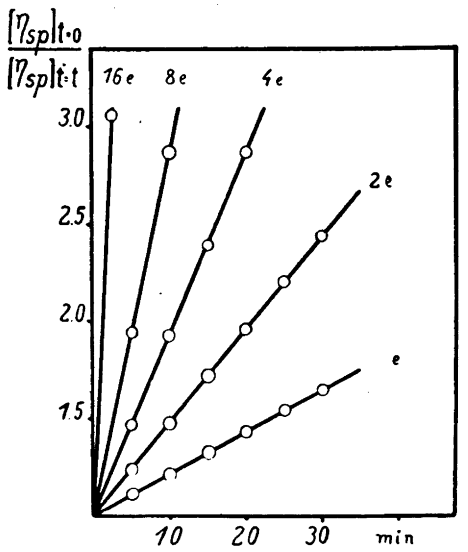

Fig. 2. Variation of $(\eta_{sp})_{t=0}/(\eta_{sp})_{t=t}$ with time. Different concentrations of the enzyme. The curves refer to experiment no. I in Table 3.

Experiments at different enzyme concentrations

If the enzymatic breakdown of the polymetaphosphate occurs in the same manner as assumed in the deduction of Hultin's formula, one can expect a direct proportionality between the z-values obtained and the enzyme concentrations if all other factors such as pH, substrate concentration, etc., are constant. We have performed some series of measurements in order to investigate if there exists such a relation under the experimental conditions used by us. These measurements were made at 25.0° C in an acetate buffer of ionic strength 0.3 and pH 5.4. To samples of 5 ml of a 0.5 % polymetaphosphate solution in this buffer was added 1 ml of enzyme solutions of different dilutions in the same buffer.

The curves of one such typical series are shown in Figs. 1 and 2 where the solution of the lowest enzyme concentration is designated as e and the solutions of higher enzyme concentrations as multiples of e. The z-values of this series are obtained from the slopes of the straight lines in Fig. 2. The results of the measurements of some series are collected in Table 3. Different enzyme stock solutions have been used in the three series. In the table are given the

Table 3. Experiments at different enzyme concentrations.

Expt. no.	Relative enzyme concentration	$z \cdot 10^4$ min^{-1} (experimental value)	Method of least squares $z \cdot 10^4$ min^{-1}
I	16 e	3690	3680
	8 e	1800	1840
	4 e	945	920
	2 e	480	460
	e	220	230
	6 e′	190	187
	4 e′	123	125
II	2 e′	61	62
	e′	27	31
	24 e″	1600	1590
III	12 e″	768	794
	2 e″	134	132
	e″	60	66

determined z-values as well as the z-values calculated from the experimental data according to the method of least squares on the assumption that z is a linear function of the enzyme concentration.

As is seen from the table the values agree rather well and it may be justified to regard the z-values as a relative measure of the enzyme concentration under similar conditions (the same substrate and the same substrate concentration etc.). Of course, in this case, the $d(1/\eta_{sp})/dt$-values might be used as well as the z-values, for these differ only by the constant factor $(\eta_{sp})_{t=0}$ provided that the saltconcentration is exactly the same. However, comparatively small changes in the ionic strength generally have so great an influence on the specific viscosity that the z-values are to be preferred in comparison measurements of the activity. Thus we have shown that if the concentrations of the polymetaphosphate and the enzyme are chosen in an interval convenient for measurements, as in our case, z is proportional to the enzyme concentration.

EXPERIMENTS AT DIFFERENT SUBSTRATE CONCENTRATIONS

In Eq. (1) the concentration of the substrate is involved as a squared factor. However, Eq. (1) is derived on the assumption that Staudinger's formula is valid, which does not seem to be strictly true for the polymetaphosphates.

In order to investigate whether Eq. (3) is valid for the enzymatic breakdown of polymetaphosphate we write Eq. (3) in the more common form represented by Eq. (6).

$$A = J \cdot c^n \cdot \frac{d\left(\frac{1}{\eta_{sp}}\right)}{dt} \tag{6}$$

Writing this equation in a logarithmic form we have

$$\log \frac{A}{J} = \log \left(\frac{d\left(\frac{1}{\eta_{sp}}\right)}{dt} \right) + n \log c \tag{7}$$

At different substrate concentrations in the same buffer the contribution to the ionic strength from the dissociated polymetaphosphate cannot be expected to be the same. For the present we neglect this contribution to the ionic strength and assume J constant at different substrate concentrations for a certain polymetaphosphate preparation when the salt concentration is constant. Under this assumption we have determined the exponent n in Eq. (6) by measuring the $d(1/\eta_{sp})/dt$-values for solutions of various substrate concentrations, the enzyme concentration and the salt concentration etc. being constant. The experiments were performed in acetate buffers of ionic strength 0.3 and pH 5.4. Four series of measurements have been carried out on different polymetaphosphate preparations. Different enzyme preparations of varying concentrations have been used in these four series, but of course the same preparation within a given series. The experimental c- and $d(1/\eta_{sp})/dt$-values have been put in Eq. (7) and n has been calculated according to the method of least squares. The results of these measurements are collected in Table 4.

The n-values obtained are less than 2. However, it must be remembered that in some respects there are large differences between the properties of starch and polymetaphosphate. As is seen from Table 4 n is about 1.7 in all the series. The variation in the n-values is perhaps to be ascribed to differences in the molecular shape and size and polydispersity of the substrates (in addition to experimental errors). If the contribution of the polymetaphosphate to the ionic strength at varying substrate concentration cannot be neglected, the difference of the n-values from 2 may be a consequence of the dependence of J on the substrate concentration rather than a deviation from Eq. (3). However, the experimental material does not yet permit the drawing of final conclusions.

Table 4. Experiments at different substrate concentrations.

Substrate	Substrate concentration (%)	$d(1/\eta_{sp})/dt \cdot 10^4$ min^{-1}	n
K 10	0.125	13370	1.64
	0.250	3940	
	0.500	1270	
	0.750	860	
	1.000	380	
K 11	0.167	960	
	0.333	320	1.75
	0.667	86	
K 12	0.500	2200	1.62
	0.750	1180	
	1.000	710	
K 15	0.104	3380	
	0.208	1010	1.85
	0.416	260	
	0.624	120	
	0.832	75	
		mean value: $n = 1.71$	

Experiments with different polymetaphosphate preparations

Although the reaction mechanism of the enzymatic breakdown of the polymetaphosphate is probably the same irrespective of the polymetaphosphate preparation used, the $d(1/\eta_{sp})/dt$-values of various preparations differ somewhat, even if the experimental conditions such as pH, substrate and enzyme concentrations, ionic strength, etc., are identical. This is illustrated by some experiments, the data of which are collected in Table 5. These investigations were carried out in acetate buffer of pH 5.4 and ionic strength 0.3 (expt. I) and phosphate buffer of pH 5.7 and ionic strength 0.3 of which 0.2 was from NaCl (expt. II). The solutions contained 0.5 % of polymetaphosphate. To 5 ml of each of these solutions was added 1 ml enzyme solution (different enzyme preparations in the two series) and $d(1/\eta_{sp})/dt$ was determined in the usual manner.

Table 5. Experiments with different polymetaphosphate preparations.

Preparation		$(\eta_{sp})_{t=0}$	$d\left(\frac{1}{\eta_{sp}}\right)/dt \cdot 10^4$ min^{-1}
Expt. I	K 12	0.49	667
	K 14	0.55	291
	K 15	0.74	337
	K 11	0.82	306
Expt. II	K 10	0.42	298
	K 12	0.80	430
	K 14	0.87	258
	K 15	1.13	282
	K 11	1.24	311

As is seen from the Tables 5 and 1, it is difficult to find any relation between the molecular constants and the $d(1/\eta_{sp})/dt$-values. In neither of the two series are these values constant, but they do not differ very much with the exception of the value for K 12. It is possible that in addition to experimental errors the molecular size, shape, and polydispersity of the different preparations are responsible for the variation in the values, because the ionic factor J might depend not only on the salt concentration but also on the molecular properties mentioned above. K 12 seems to give somewhat higher $d(1/\eta_{sp})/dt$-values than the other preparations, which might be due to its polydispersity. K 12 probably contains a fraction of relatively low molecular polymetaphosphate, which fraction does not satisfy the assumptions in the derivation of the Hultin formula, where all the substrate is considered to be sufficiently high molecular. That would mean that the »effective concentration» is less than the analytical one and therefore the experiments with K 12 cannot be compared with the others.

The unit of the enzyme activity will not be given yet, as we cannot determine the ionic factor J in Eq. (3). In future perhaps it will be possible to choose an arbitrary J_o value for a certain preparation under well defined conditions and then measure J relative to this J_o under other conditions.

Experiments at different pH-values

In our preliminary investigations it was demonstrated that the enzyme from *A. niger* degrading polymetaphosphate, has its optimal activity at an acid pH. However, a more accurate determination of the influence of the pH

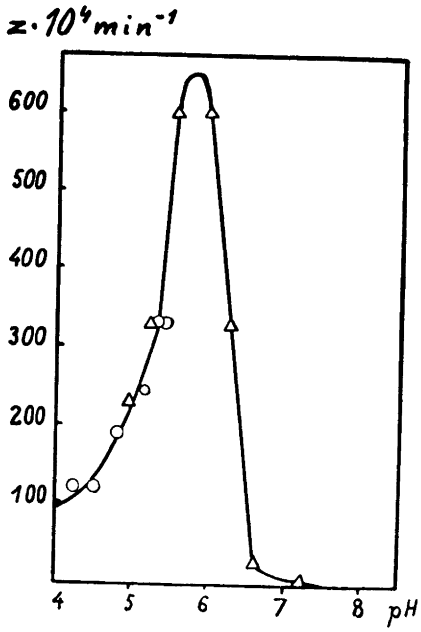

Fig. 3. The influence of pH upon the activity. ○ = *acetate buffers.* △ = *phosphate buffers.*

on the activity was not made at that time and therefore we have now carried out a series of measurements on the variation of z with the pH. The experiments have been performed in the following manner. The polymetaphosphate (preparation K 14) was dissolved in sodium acetate-acetic acid and sodium phosphate buffers of different pH but of the same ionic strength, 0.3 (0.1 from the buffer substances and 0.2 from NaCl). The solutions contained 0.5 % of the polymetaphosphate. To samples of 5 ml of these solutions was added 1 ml of an enzyme solution and the breakdown was followed as usual. The enzyme used in these measurements was taken from a freshly prepared stock solution which was diluted 10 times with water. As the determinations were performed in a rather short time the enzyme activity of the stock solution may be considered constant. (The activities of our freshly prepared enzyme solutions usually remain constant for several days if the solutions are stored in a refrigerator.) In very acid medium the z-values are to be corrected for the spontaneous breakdown of the substrate, but in the pH-range of our measurements these corrections are mostly rather negligible and have only been applied at the lowest pH values. In Fig. 3 the z-values obtained are plotted against the pH. As is seen from the figure the maximum at about pH 5.7 is rather pronounced. It may also be pointed out that the z-values in acetate and phosphate buffers of the same pH agree very well, whereas the $d(1/\eta_{sp})/dt$-values in dif-

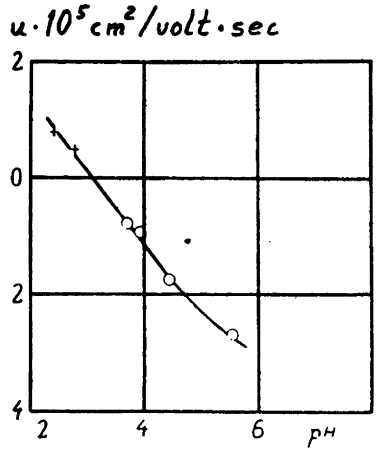

Fig. 4. Variation of the electrophoretic mobility of the enzyme with pH at a temperature of 0°.0 C. ○ = *acetate buffers.* + = *citrate buffers.*

ferent buffers of the same pH may differ very much. A curve where $d(1/\eta_{sp})/dt$ is plotted as a function of pH shows irregularities depending on the fact that the contribution of the ionic factor to the viscosity is not the same in different buffers.

SOME PHYSICO-CHEMICAL PROPERTIES OF THE ENZYME

Electrophoretic mobility and the isoelectric point

The electrophoretic mobility of the enzyme has been studied in the Tiselius apparatus (provided with two pairs of short cells [10]). However it was not possible to use the Svensson optical method of registering the electrophoretic movement because the concentration of the enzyme was too low to give a sufficient increment of refractive index. Therefore we measured instead the enzyme activity of the solutions in the different cells of the Tiselius apparatus after the electrophoretic runs as well as the activity of the original enzyme solution used to fill the apparatus. Thus, for instance, if after the experiment the activity of the solution in the upper cell on the ascending side (the activity measured after stirring) is half the activity of the original enzyme solution, the mobility was calculated on the assumption that the boundary had moved half the length of the upper cell, the dimensions of which are known.

A series of electrophoretic experiments has been performed at different pH-values in acetate and citrate buffers of ionic strength 0.1. (The enzyme solutions dialysed against citrate buffers and that dialysed against acetate buffer of pH 4.5 were made 0.3 % with respect to saccharose in order to get a

more stable boundary, as the enzyme concentration is very low.) The electrophoretic experiments were carried out at 0.0° C; the current strength was 15—20 mA. The activity of the samples taken out after the experiments has been determined at 25° C under identical conditions, which in this case means that 0.5 % solutions of polymetaphosphate in an acetate buffer of pH 5.4 and ionic strength 0.3 were used for the activity measurements, which were performed as described above. As electrophoretic mobility u we have here adopted the mean value of the mobilities of the ascending and the descending side. In the experiments in citrate buffers the mobility has been corrected for the rather high viscosity of these buffers. Fig. 4 shows the mobility as a function of the pH. As is seen this enzyme has its isoelectric point at a rather acid pH, about 3.1. Thus the isoelectric point differs by more than 2 pH-units from the pH of the optimal activity.

Determination of the sedimentation constant

We have determined the sedimentation constant of the enzyme in the Svedberg ultracentrifuge. However, it has not been possible to use the ordinary optical method (»the scale method») for recording the sedimentation, as the enzyme was not pure and the concentration too low to use such a method. These difficulties have been overcome by the use of a separation cell instead of the ordinary ultracentrifuge cell and we have succeeded in determining the sedimentation constant s with a fairly good accuracy. (Concerning the construction and the theory of the separation cell see [11, 12]). After the centrifugation runs the solutions in the upper and the lower parts of the cell were taken out separately and the activity of both solutions was measured, and, of course, also that of the original solution. From these data s was calculated. For details concerning the sedimentation in the ultracentrifuge we refer to the book of Svedberg and Pedersen [12]. It may be mentioned here that it is rather difficult to take out all the substance sedimented to the bottom part of the cell. Hence the s value obtained from the upper part of the cell is to be regarded more reliable than that calculated from the increase of the concentration in the lower part of the cell. Some runs were carried out in acetate buffer of pH 5.4 and ionic strength 0.3; centrifugal field 250,000 times gravity (59,000 r. p. m.). Four of the s-values obtained (upper part of the cell) agree very well: 3.18, 3.11, 3.15 and 3.22 S (Svedberg units). (In two cases we get lower s-values because of convections during the stopping of the rotor.) As a mean value we adopt $s_{20} = 3.2$ S. (We are indebted to Mr. E. Hellman for carrying out the numerical calculations of the sedimentation constant.)

Determination of the diffusion constant

The diffusion constant of the enzyme has been determined according to »the porous-disk method»[13, 14] as being the most convenient in this case. As »porous-disk» we have used a »Jena glass filter G4». The apparatus was calibrated by means of a 0.1 *M* KCl solution the diffusion coefficient of which is known [15]. The experiments were performed at 0.1° C in acetate buffer of pH 5.4 and ionic strength 0.1. The activity measurements necessary for the calculations were carried out as described before. The diffusion constant thus obtained was corrected to pure water of 20.0° C. $D_{20} = 8.8 \times 10^{-7}$ cm²/sec.

The molecular weight of the enzyme

In Svedberg's wellknown formula of the molecular weight [12] the partial spec. volume V of the substance is also involved. It has not been possible to make a direct determination of V but as the partial spec. volume of proteins in most cases is about 0.73 within a small range of variation we assume this value to be valied for this enzyme also. On this assumption the molecular weight was calculated to be about 33,000.

SUMMARY

The enzymatic breakdown of polymetaphosphate of very high molecular weight with an enzyme extract from *Aspergillus niger* has been studied by viscosity measurements. A convenient method of determing the enzyme activity has been worked out. The influence of enzyme concentration, substrate concentration, ionic strength and pH on the breakdown velocity has been studied. Some physico-chemical properties of the enzymes degrading polymetaphosphate has also been investigated.

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