ACTA CHEMICA SCANDINAVICA 3 (1949) 157—162

Enzymatic Breakdown of Polymetaphosphate. III

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In previous papers [1,2] it has been shown that polymetaphosphates of very high molecular weight are broken down by enzymes from moulds such as *Aspergillus niger*, *Aspergillus oryzae* and *Penicillium expansum*. In these experiments preparations of polymetaphosphate were used having molecular weights of more than one million. Thus in the latter experiments a colloid of purely inorganic character was broken down by enzymatic means. (Concerning the earlier investigations on metaphosphatases see the references in our previous articles.)

During the course of continued investigation attention has also been paid to the occurrence of polymetaphosphate degrading enzymes in other organisms than those mentioned above. In this paper some experiments carried out on enzyme extracts from some different kinds of fungi and bacteria will be described.

As substrate for these experiments a high molecular polymetaphosphate $(KPO_3)_n$ has been used; the preparation is designated K 15. The substance, the molecular weight of which was determined, as 1,200,000, has been described in a previous paper [2]. When preparing the substrate solutions this polymetaphosphate has been dissolved in solutions containing an excess of sodium ions.

With some exceptions (see below) the organisms have been cultivated under sterile conditions on suitable nutrient solutions or agar-plates. The enzyme extracts have been prepared by grinding up the organisms in water or in a suitable buffer. (For more details concerning the enzyme preparation reference is made to [1].) In some cases the organisms have been treated with acetone for a short time before the enzyme extraction in order to remove lipoid substances. The extracts have been dialyzed in cellophane bags against water at some degrees above zero for some days. Before being used for experiments the extracts have been centrifugated and filtered.

As previously the breakdown has been studied at 25 °C by means of viscosity measurements in a capillary viscosimeter according to Ostwald. The experiments have been performed in acetate and phosphate buffers of different pH-values in order to determine the pH-optima of the enzymes. As a measure of the enzyme activity for comparing experiments the z-values defined in an earlier paper have been used [2]

$$z = (\eta_{sp})_{t=0} \cdot \frac{d(1/\eta_{sp})}{dt} \qquad (1)$$

(η_{sp} = specific viscosity, t = time)

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 i the salt concentrations which may occur. (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.)

The experiments have been carried out in the following manner: 1 ml of the enzyme solution has been added to 5 ml of a 0.5 % polymetaphosphate (K 15) solution in a suitable buffer and the decrease of the viscosity with time has been recorded. (The ionic strength of the buffers generally has been 0.3 of which 0.2 is due to NaCl). $1/\eta_{sp}$ was plotted as a function of time and from the slopes of the straight lines obtained, the z-values were calculated.

EXPERIMENTS WITH FUNGI

These organisms were cultivated under sterile conditions with the exception of *Collybia velutipes* and *Tricholoma equestre*, found in the forest (in the month of October). The extracts of *Saccharomyces cerevisiae* were prepared from commercial baker's yeast. The mould *Penicillium chrysogenum* was obtained in a frozen state from the penicillin plant of Kärnbolaget, Stockholm.

In the cases of inactive extracts (extraction with water or buffer) the process was generally repeated after undergoing treatment with acetone, yet without obtaining active preparations. In the case of baker's yeast the extracts showed a much greater activity if treatment with acetone preceded the extraction. The *A. niger* extract used for these experiments was from the same culture as that used during a previous work [2], but this time the mycelium was treated with acetone. However, the pH-optimum was not affected by this modified method of preparation.

For the sake of brevity the results have been collected in Table 1. As is seen from the table the *Ascomycetes* (hitherto investigated) have enzymes which break down the high molecular polymetaphosphate.

Table 1. Experiments with enzyme extracts from fungi.

	Organism	pH-interval investigated	Activity	pH-optimum
Phyco-mycetes	*Phycomyces Blakesleeanus*	4.4 — 8.0	—	
	Rhizopus nigricans	4.4 — 8.0	—	
Asco-mycetes	*Penicillium expansum*	3.6 — 5.8	+	4.5
	Penicillium chrysogenum	3.6 — 6.3	+	4.8
	Penicillium funiculosum	3.7 — 5.4	+	4.5
	Aspergillus niger	4.0 — 7.0	+	5.7
	Aspergillus oryzae	6.6	+	—
	Saccharomyces cerevisiae	5. 2— 9.0	+	7.2
Basidio-mycetes	*Marasmius graminum*	4.4 — 7.3	—	
	Marasmius ramealis	4.2 — 6.2	—	
	Merulius domesticus	4.2 — 6.2	—	
	Polyporus betulinus	4.2 — 6.2	—	
	Collybia velutipes	3.9 — 8.0	—	
	Tricholoma equestre	3.9 — 8.0	—	

Neither from the *Basidiomycetes* nor from the *Phycomycetes* has it been possible to obtain extracts of polymetaphosphate degrading enzymes.

In Figs. 1 and 2 the z-values obtained have been plotted as a function of pH.

EXPERIMENTS WITH BACTERIA

Extracts from a number of different bacteria have also been investigated in a similar way. The organisms were cultivated on agar plates under sterile conditions. After being removed from the plates the bacteria were treated with acetone, dried at room temperature and extracted by being ground up with sand in water or a suitable buffer.

Hitherto it has only been possible to obtain active extracts from *Proteus vulgaris*.

The results have been collected in Table 2. In Fig. 3 the z-values have been plotted as a function of pH (*Proteus vulgaris*). As seen the enzyme activity has its optimum at pH $\sim$ 4.7.

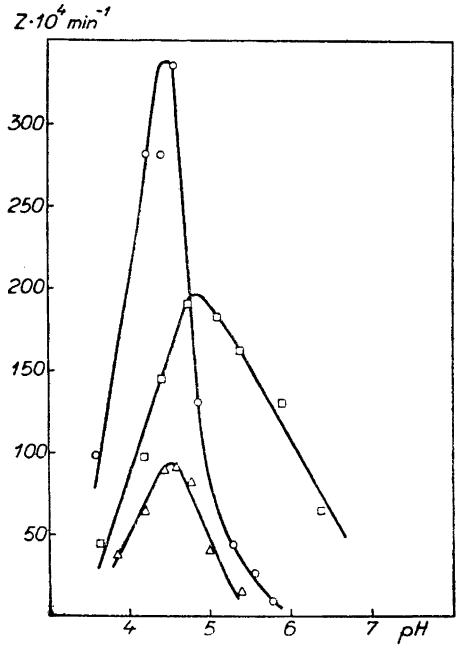

Fig. 1. The influence of pH upon the activity.
- ○ *Pencillium expansum*
- △ *Pencillium funiculosum*
- □ *Penicillium chrysogenum*

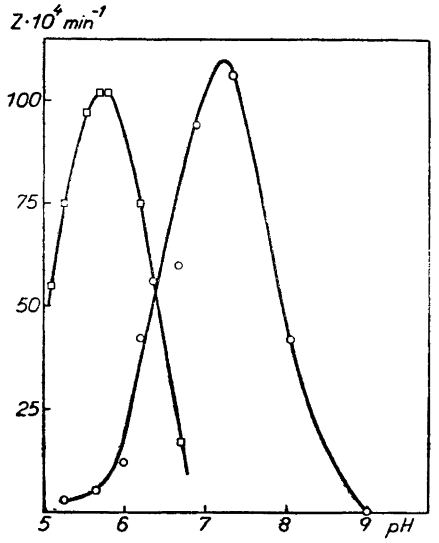

Fig. 2. The influence of pH upon the activity.
- □ *Aspergillus niger*
- ○ *Saccharomyces cerevisiae*

Table 2. Experiments with enzyme extracts from bacteria.

Organism	pH-interval investigated	Activity	pH-optimum
Proteus vulgaris	3.9 — 5.8	+	4.7
Bacillus subtilis	4.5 — 8.0	—	
Micrococcus lysodeiticus	4.2 — 8.0	—	
Pseudomonas aeruginosa	4.2 — 7.3	—	
Sarcina lutea	4.2 — 8.0	—	
Cellvibrio fulva	4.5 — 8.0	—	
Escherichia coli	4.2 — 7.3	—	
Bacillus mesentericus	4.2 — 6.9	—	
Bacillus prodigiosus	4.2 — 7.3	—	

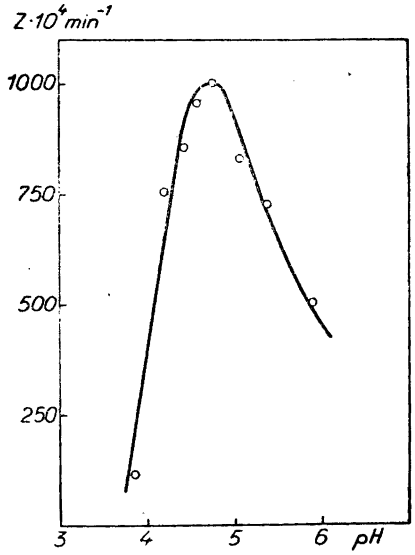

Fig. 3. The influence of pH upon the activity. Proteus vulgaris.

DISCUSSION

As seen from the tables enzyme extracts degrading high molecular polymetaphosphate have been obtained from some fungi belonging to the *Ascomycetes* and from one bacterium *viz. Proteus vulgaris.* In the cases of inactive extracts it may of course be difficult to decide whether the organism does not possess such an enzyme or whether the enzyme preparation has failed for some reason. However, it must be pointed out that in many cases the experiments have been repeated and the results have still been negative. However, low molecular metaphosphate, *e.g.* sodiumtrimetaphosphate $Na_3(PO_3)_3$, considered to be of cyclic structure, can be broken down by extracts from some organisms which do not seem to possess enzymes capable of breaking down the high molecular colloid polymetapohsphate. Thus, for instance, the authors have studied the degradation of sodiumtrimetaphosphate to orthophosphate by enzyme extracted from liver of rabbit and cow. These experiments will be published in another paper.

Perhaps an investigation of the presence or absence of polymetaphosphate degrading enzymes may be of value in classifying microorganisms. The position of the pH-optimum or other physical constants may perhaps also be of importance in this connection.

SUMMARY

Investigations of the occurrence of enzymes degrading colloid polymetaphosphates have hitherto shown that such enzymes are found in some fungi belonging to *Ascomycetes* and in the bacterium *Proteus vulgaris*. The enzymatic breakdown has been studied by means of viscosity measurements and the pH-optima of the enzymes have been determined.

The authors wish to thank Prof. A. Tiselius and Prof. T. Svedberg for the privilege of carrying out this work in their laboratories. We are grateful for the valuable technical assistance rendered by Miss A. Karlsson and Mrs M. Hårsta. The investigation was supported by a grant from *Statens Naturvetenskapliga Forskningsråd*.

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Received January 24, 1949.