THE TURNIP LYSOZYME*

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Received 10 March 1971

1. Introduction

Soon after his discovery of 'lysozyme' (EC 3.2.1.17), Fleming detected the presence of this enzyme in many vegetable tissues, especially in the flowers [1]. A number of roots and tubers were also examined [2]: of these the only one which showed any marked bacteriolytic effect was the turnip. In comparison to human tissues, the lysozyme content of turnip was weak, but a 1 in 10 extract completely cleared an opaque suspension of *Micrococcus lysodeikticus* in one hr [2]. Since Fleming's observations, only a very small number of studies have been devoted to plant lysozymes, and these all deal with the enzyme contained in latex. Meyer et al. [3] first reported lysozyme activity in crude proteolytic enzyme preparations from papaya and fig latex. In 1955, Smith et al. [4] prepared a crystalline lysozyme from papaya latex and quite recently Howard and Glazer [5, 6] studied such a lysozyme in detail. It was found that papaya lysozyme had a molecular weight of about 25,000, that glycine was its sole *N*-terminal as well as *C*-terminal residue, and that the molecule consisted of a single polypeptide chain. Papaya latex lysozyme lysed *M. lysodeikticus* cell walls at one third of the rate exhibited by hen egg-white lysozyme; whereas papaya lysozyme exhibited 200–400 times higher chitinase (EC 3.2.1.14) activity toward chitotetraose than hen egg-white lysozyme, with whole chitin as substrate, it was only 10 times as active as the egg enzyme. No involvement of tryptophan residues in the active site of papaya lysozyme could be demonstrated [5, 6].

In the present note, a first series of results concerning the purification and some properties of the turnip lysozyme is reported.

2. Materials and methods

The turnips (variety 'Navets de Paris') were supplied by M. Moser, Vevey, Switzerland. Hen egg-white lysozyme was obtained from Worthington (Freehold, N.J., U.S.A.) and from Tozai Soeki Kaisha (Japan). Chitotetraose and chitopentaose were prepared from partially hydrolyzed chitin [7]. All other special reagents were obtained from Merck or Prolabo.

The enzyme activity was measured according to the method of Jollès [8] and the proteins following the procedure of Lowry et al. [9] or by their absorption at 280 nm: hen egg-white lysozyme was used as a standard and its specific activity was taken as 1. Some of the procedures used in the purification of turnip lysozyme have been described by Jollès et al. [10] in their general method for the purification of mammalian lysozymes. Electrofocusing was performed as described in the preliminary instruction sheet and its addendum supplied by LKB Instruments [11]. An LKB 8101 electrofocusing column of 110 ml capacity was used. The carrier ampholyte was selected to give a pH gradient between pH 7 and pH 10. Acrylamide gel electrophoresis was performed at pH 2.3, 60 V, 5 mA, 2 hr.

* 77th communication on lysozymes; 76th communication: A. Faure and P. Jollès, Compt. Rend. Acad. Sci. Paris 271 (1970) 1916.

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Table 1
Assay of preparation of turnip lysozyme by ion-exchange chromatography on Amberlite CG-50.

282 kg turnips + 282 l water gave 500 l of extract + 40 kg of residue	Lysozyme content = 500 mg Protein content = 550 g Specific activity = 0.0009
↓	
Adsorption onto Amberlite CG-50 in 0.2 M phosphate buffer pH 6.5. Eluted with 0.8 M phosphate buffer, pH 6.5, after washing with water	Specific activity increased 167 times
↓	
40 fractions (1.2 l) containing significant lysozyme activity	Lysozyme content = 354 mg Protein content = 2400 mg Specific activity = 0.15
↓	
Filtration through Sephadex G-75 (in 0.05 M ammonium acetate buffer, pH 6.5) after dialysis and evaporation to 120 ml	Specific activity increased 8.6 times
↓	
30 fractions (290 ml) containing activity	Lysozyme content = 200 mg Protein content = 156 mg Specific activity = 1.3
↓	
Dialysis against distilled water, evaporation to 20 ml and chromatography on Amberlite CG-50, 0.2 M phosphate buffer pH 6.20	Specific activity increased 2.6 times
↓	
7 most active fractions bulked and dialysed to remove the salts (92 ml)	Lysozyme content = 124.8 mg Protein content = 37.3 mg Specific activity = 3.24
↓	
Purified turnip lysozyme preparation	

The methods used to follow the digestion of chitotetraose and chitopentaose have been previously described by Charlemagne and Jollès [12]. The chitinase activity of the enzyme was also characterized by the digestion of colloidal chitin prepared according to Skujins et al. [13]; the appearance of reducing groups was determined by the procedure of Dygert et al. [14]. The inhibition of the turnip lysozyme by *N*-acetylglucosamine was measured by the procedure of Jollès et al. [15].

3. Results

3.1. *Assay of purification of the enzyme by ion-exchange chromatography on Amberlite CG-50*

Table 1 summarizes the method of preparation of a 'purified' turnip lysozyme. The main active fraction was consistently eluted very close to the dead volume during the ion-exchange chromatography on Amberlite CG-50. The active material had two absorption maxima at 275 nm and 405 nm and showed several bands when submitted to starch gel electrophoresis.

3.2. *Purification of the enzyme by electrofocusing*

The active material obtained after filtration on Sephadex G-75 (table 1) was submitted to 2 successive filtrations on Sephadex G-100 (90 × 2 cm) with 0.1 N acetic acid as eluent. The biologically inactive material, with an absorption maximum at 405 nm, was almost entirely eliminated by this treatment. However, the peak which was active against *M. lysodeikticus* cells, always gave rise to several bands when submitted to acrylamide gel electrophoresis. The lysozyme rich material was at this stage purified by electrofocusing between pH 7 and 10 (fig. 1). The active fractions were pooled and filtered on Sephadex G-25 (33 × 2.5 cm; 0.1 M phosphate buffer of pH 7.2) to eliminate the carrier ampholytes. The active material again gave rise to 2 or 3 bands by acrylamide gel electrophoresis. After a further treatment on Sephadex G-75 (138 × 1.2 cm; 0.01 M tris-HCl, 0.1 M NaCl buffer, pH 7.4) an electrophoretically pure material (starch-gel electrophoresis: insert fig. 1) was finally obtained. However, at this stage, the turnip lysozyme was very labile. The over-all yield did not exceed 10–15%.

3.3. *Molecular weight*

Filtrations on Sephadex G-75 (138 × 1.2 cm; 0.01 M tris-HCl, 0.1 M NaCl buffer, pH 7.4) in the presence of test substances (pepsin, trypsin, hen egg-white lysozyme) suggest a molecular weight of about 25,000 for the turnip lysozyme.

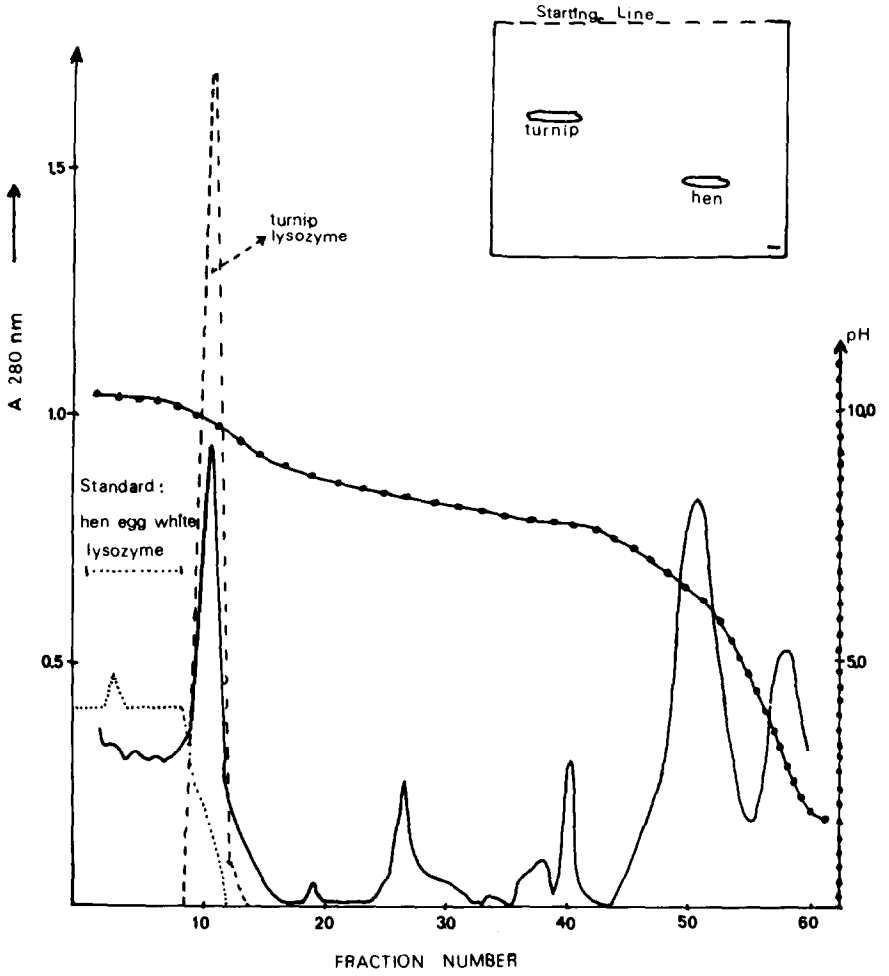

Fig. 1. Electrofocusing between pH 7 and pH 10 of a turnip lysozyme rich fraction (2 ml fractions, 300–500 V, 45 hr, 12°). Insert: starch gel electrophoresis (pH 3.8 formic acid–NaOH buffer, 5 M urea, 250 V, 26 mA, 8 hr).

3.4. *pH optimum for the lysis of M. lysodeikticus cells by turnip lysozyme. Influence of the temperature*

The pH activity profile for *M. lysodeikticus* cells is shown in fig. 2 and the relationship between temperature and activity in fig. 3.

3.5. *Comparison of the digestion of chitopentaose and colloidal chitin by turnip lysozyme and hen egg-white lysozyme*

0.5 mg chitopentaose was digested at 37° with 4 or 16 μg of turnip or hen lysozyme in 0.05 M ammonium acetate for varying periods of time (0, 1, 4, 24 hr). The digestion products are shown in fig. 4. The action of turnip lysozyme is very rapid. The main hydrolysis products are chitobiose and chitotriose; no chitotetraose was found and only traces of undigested chitopentaose could be observed. The substrate was not attacked by 4 μg of hen lysozyme though it was hydrolyzed by 16 μg after a reaction time of at least 4 hr. In this case, chitotetraose was observed among the digestion products and a significant amount of chitopentaose remained undigested.

Preliminary assays to test the activity of turnip lysozyme on colloidal chitin showed that the enzyme had a high chitinase activity. The turnip lysozyme was twelve times more active in this respect than hen egg-white lysozyme (fig. 5).

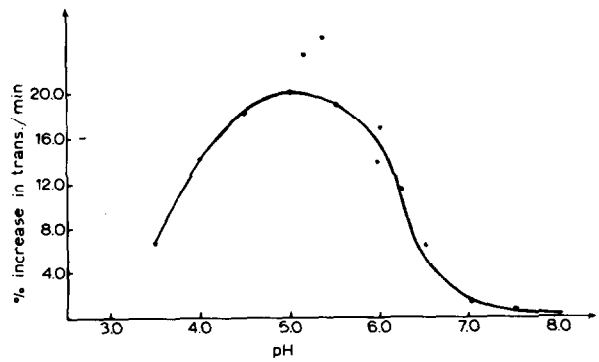

Fig. 2. pH activity profile for turnip lysozyme. Suspensions of acetone dried *Micrococcus lysodeikticus* cells were made in 0.066 M phosphate buffer (25 mg/100 ml) at the following pHs: 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.2, 6.5, 7.0, 7.5. Each suspension contained 0.1% NaCl and had an initial transmission of 20–25% at 650 nm. To 3 ml substrate at 25° was added 0.3 ml of enzyme (40 μg) and the increase in transmission was measured continuously. The percentage increase in transmission per minute was calculated from the steepest part of the curve.

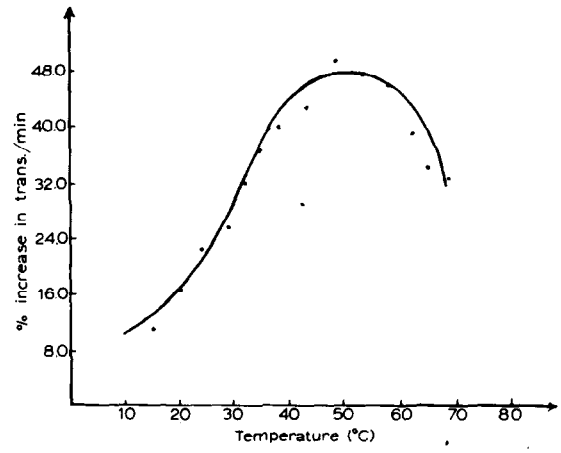

Fig. 3. The relationship between temperature and activity was done as indicated in legend to fig. 2, using a suspension of cells at pH 5.0.

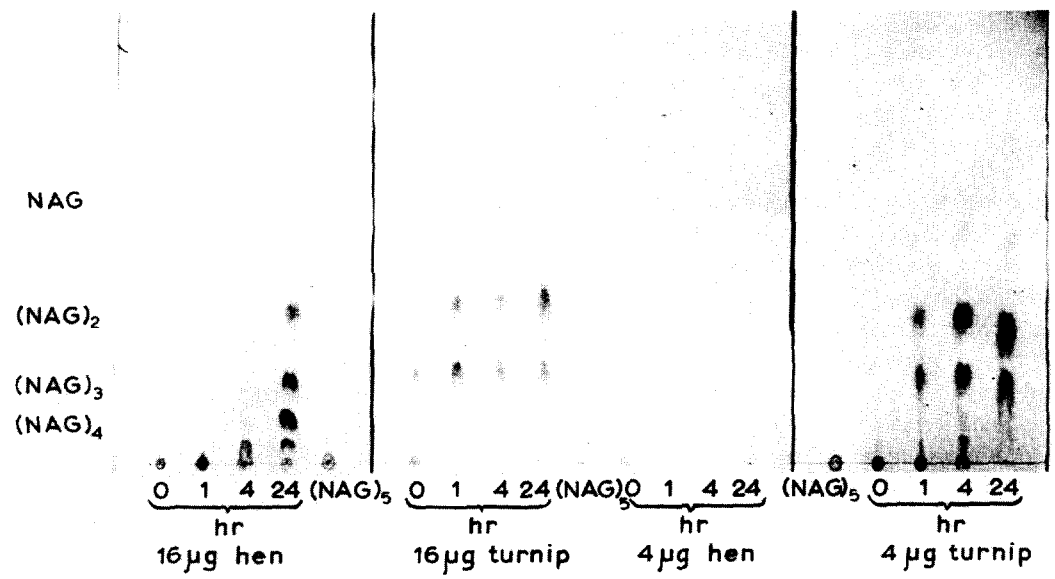

Fig. 4. Digestion at 37° of 0.5 mg chitopentaose by 4 or 16 μg turnip or hen egg-white lysozyme (0.05 M ammonium acetate solution). NAG stands for *N*-acetylglucosamine.

3.6. *Inhibition of turnip lysozyme by N-acetylglucosamine.*

Turnip lysozyme like many other lysozymes [15] was inhibited at pH 6.2 by *N*-acetylglucosamine (0.066 M phosphate buffer). The inhibition was of the same order as that already described for the duck lysozymes and less pronounced than for hen or human lysozymes.

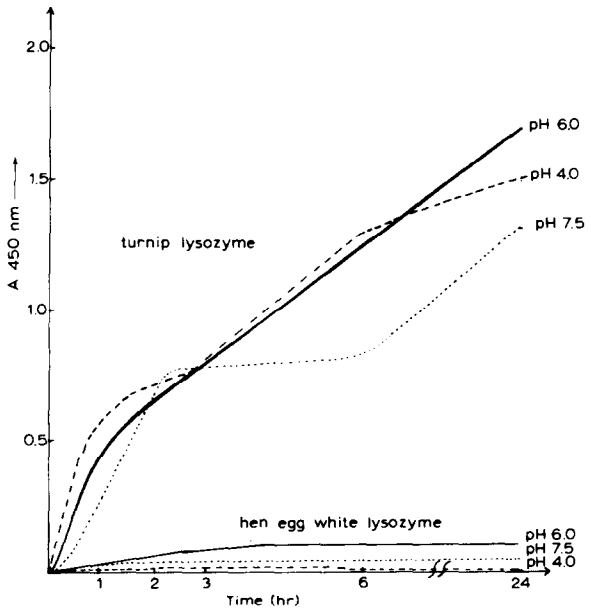

Fig. 5. Increase in reducing groups at different pHs and constant ionic strength (0.1) during the action at 20° of turnip and hen egg-white lysozymes (40 μg) on 0.8 mg colloidal chitin determined by the method of Dygert et al. [14].

4. Conclusion

The 'classical' hen egg-white lysozyme and turnip lysozyme both hydrolyse β(1–4)glycosidic bonds in *M. lysodeikticus* cells. However, the results presented here show that the two lytic enzymes differ in the following properties: molecular weight, electrophoretic behaviour, optimum pH, relative activity toward various substrates and mode of attack upon these substrates. Under certain conditions the turnip lysozyme seems to act more as a chitinase than a lysozyme. It thus resembles papaya latex lysozyme [5, 6] and further investigation of its properties should establish whether the same differences (structure, specificity, speed of action etc.) exist among plant lysozymes as those described in the case of vertebrate lysozymes [16].

Acknowledgement

One of the authors (I.B.) is Chargée de Recherches à l'I.N.S.E.R.M.

References

[1] A. Fleming, Proc. Roy. Soc. (London), Ser. B 93 (1922) 306.
[2] A. Fleming, Proc. Roy. Soc. Med. 26 (1932) 71.
[3] K. Meyer, E. Hahnel and A. Steinberg, J. Biol. Chem. 163 (1946) 733.
[4] E.L. Smith, J.R. Kimmel, D.M. Brown and E.O.P. Thompson, J. Biol. Chem. 215 (1955) 67.
[5] J.B. Howard and A.N. Glazer, J. Biol. Chem. 242 (1967) 5715.
[6] J.B. Howard and A.N. Glazer, J. Biol. Chem. 244 (1969) 1399.
[7] C. Bedell and L.N. Johnson (1968) private communication.
[8] P. Jollès, Methods in Enzymology 5 (1961) 137.
[9] O.H. Lowry, N.J. Roseborough, A.L. Farr and R.J. Randall, J. Biol. Chem. 196 (1951) 265.
[10] P. Jollès, H. Zowall, J. Jauregui-Adell and J. Jollès, J. Chromatog. 8 (1962) 363.
[11] LKB Instruments Inc., LKB 8102 Electrofocusing column – Preliminary instruction sheet and addendum, Rockeville, Md. (1967).
[12] D. Charlemagne and P. Jollès, Bull. Soc. Chim. Biol. 49 (1967) 1103.
[13] J.J. Skujins, H.J. Potgieter and M. Alexander, Arch. Biochem. Biophys. 111 (1965) 358.
[14] S. Dygert, L.H. Li, D. Florida and J.A. Thoma, Anal. Biochem. 13 (1965) 367.
[15] P. Jollès, J. Saint-Blancard, D. Charlemagne, A.-C. Dianoux, J. Jollès and J.L. Le Baron, Biochim. Biophys. Acta 151 (1968) 532.
[16] P. Jollès, Angew. Chem. Intern. Ed. Engl. 8 (1969) 227.