

Studies on the Bacteriolytic Activity of *Streptomyces albus* Culture Filtrates. 3. Affinity for Chitin

L. AKSNES and A. GROV

The University of Bergen, School of Medicine, The Gade Institute,
Department of Microbiology, N-5000 Bergen, Norway

The enzymes of *Streptomyces albus* G culture filtrates were adsorbed to chitin and deaminated chitin, and desorbed quantitatively under mild conditions. The affinity was dependent on low ionic strength, whereas variation in pH between 4.5 and 9.0 had no effect. The similarity to the results obtained with carboxymethyl cellulose suggests a binding to substrate-analogous structures.

Earlier studies^{1,2} have shown that culture filtrates of *Streptomyces albus* G contain a muramidase, an amidase, and several endopeptidase and caseinolytic activities. The two former activities were isolated in apparently pure states on carboxymethyl cellulose, whereas the latter activities were eluted together without detectable amidase and hexosaminidase activities. Further separation was not obtained either on gel filtration or electrophoresis.

Since the first report by Berger and Weiser³ on degradation of chitin by egg-white lysozyme, the binding of this and other muramidases (E.C. 3.2.1.17) has been extensively studied using chitin as substrate.⁴⁻⁸ This property of muramidases, *i.e.* reversible binding to a structural analog of its substrate, has also been utilized in isolation and purification of this type of enzyme.⁹

The intention of the present study was to test chitin as a possible adsorbent for the endo-*N*-acetylmuramidase of *Streptomyces albus* G culture filtrates.

MATERIALS AND METHODS

Chitin, a polymer of essentially non-branched chains of β -(1-4)-*N*-acetyl-D-glucosamine units,

was obtained from Sigma Chemical Co., Mo. U.S.A. All other materials and chemicals used were described previously,^{1,2} including the test bacteria abbreviated: Sa (*Staphylococcus aureus* Copenhagen), Sp (*Streptococcus pyogenes* A), Pc (*Planococcus* 2389), M-144 (*Micrococcus l. teus* 144), M-84 (*M. conglomeratus* 84), M1 (*M. lyso-deikticus* NCTC 2665), S1 (*Sarcina lutea*), and Bm (*Bacillus megaterium*).

The methods for testing of lytic activities, dialysis, concentration, determination and quantitation of free groups and *N*-acetyl amino sugars, and estimation of enzymatic specificities were those described or referred to earlier.^{1,2}

Preparation of chitin. Chitin was prepared and treated according to the description of Jensen and Kleppe⁵. Deamination was performed as described by Cherkasov and Kravchenko,¹⁰ suspending chitin (10 ml) in a mixture of 1.27 ml 12 N HCl and 1 g NaNO₂ in 30 ml of water, incubating for 6 h at 0°C with continuous stirring, followed by washing in 1 % NaCl, 1 % acetic acid and finally distilled water.

Column fractionation. Columns (1.2 × 2 cm) of chitin and deaminated chitin, 50–100 mesh, were packed and equilibrated with buffer. Enzyme solutions were applied at a rate of about 5 ml/h followed by washing with buffer (100 ml). The columns were eluted with either (a) M acetic acid or (b) 0.01 M Tris-HCl pH 8.0, and a linear NaCl-gradient (200 ml) from 0 to 0.2 M. The elution rate was approximately 5 ml/h. Fractions (2 or 5 ml) were collected, dialyzed against 0.01 M Tris-HCl pH 8.0, and subjected to activity tests. All fractionation experiments were carried out at 4°C.

RESULTS

The various activities of crude enzyme preparation (CEP)¹ were apparently adsorbed to chitin. Only a slight caseinolytic activity was demonstrated in the effluent, but due to low activity there was uncertainty with respect to

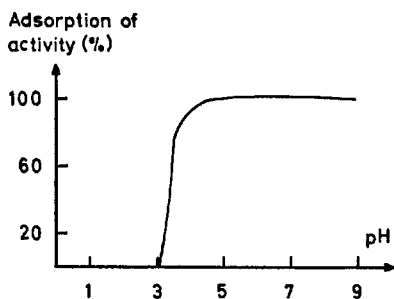


Fig. 1. Percentage adsorption to chitin of bacteriolytic activity (tested on *B. megaterium*) at various pH-values.

the amidase. The effect of pH on the binding to chitin was studied over a pH-range of 2.6–9.0 using the buffer systems: 0.01 M Na-citrate-HCl (pH 2.6–5.0), 0.01 M Na-phosphate (pH 5.0–8.0), and 0.01 M Tris-HCl (pH 8.0–9.0). CEP (10 ml), dialyzed against the appropriate buffer, was applied to the chitin column equilibrated with the same buffer. After washing with buffer, the activity adsorbed was eluted with M acetic acid and dialyzed against 0.01 M Tris-HCl, pH 8.0. Washings and eluates were tested for lytic activity on heat-killed cells of *B. megaterium* and for caseinolytic activity. The percentage of total lytic activity applied to the column which was bound at various pH-values is illustrated in Fig. 1. Above pH 4.2 complete adsorption of bacteriolytic activity was obtained. An abrupt drop in adsorption occurred below pH 4.2 and at approximately pH 3.2 the affinity of lytic enzymes for chitin was zero. Upon digestion of *Sa* mucopeptide, *Bm* cell wall, and casein with the eluates, free amino and reducing groups showed an increase in the same pH-range, as did also lysis of casein. The use of deaminated chitin as column material gave the same results as for chitin, and rubbed paper (Whatman No. 1 chromatography paper) at pH 8.0 (0.01 M Tris-HCl) behaved similarly as far as adsorbing capability is concerned.

Elution of enzyme-loaded (15 ml of the CEP) column with the NaCl-gradient and testing of every second fraction for bacteriolytic and caseinolytic activity gave the results shown in Figs. 2 and 3. To some extent the lytic activity of the material applied to the column seems, ac-

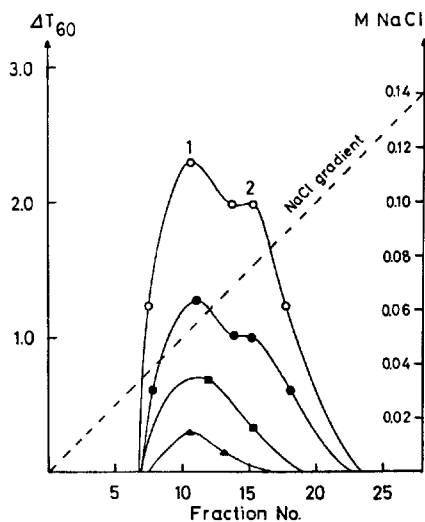


Fig. 2. Lytic activity in fractions (5 ml) from chitin column eluted with a linear NaCl-gradient, as tested on heat-killed *Bm* (O), *Sa* (●), *Ml* and *Sl* (■), and *M-144*, *M-84* and *Sp* (▲). ΔT_{60} (reduction in turbidity after 60 min) = $(\Delta T/t) \times 60$.

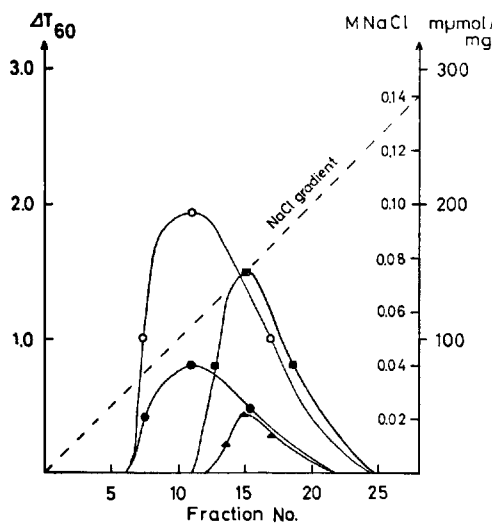


Fig. 3. Caseinolytic activity (●) and the increase in free amino groups on *Sa* mucopeptide (O), reducing groups (■) and *N*-acetylamino sugars (▲) of *Bm* cell wall upon digestion with fractions from the chitin column eluted with a linear NaCl-gradient. ΔT_{60} : See legend Fig. 2.

ording to the tests on Bm and Sa cells, to be divided into two peaks (Fig. 2). One of these peaks corresponds to the increase in free amino groups (tested on Sa mucopeptide) and the other to the increase in reducing groups (tested on Bm cell wall) (Fig. 3). The fractions showed no absorbance at 280 nm. Of the two activity peaks indicated, only the first showed lytic activity on all test bacteria and on casein within a digestion period of 90 min. The second peak lysed only Sa and Bm cells and increased the amount of reducing groups and *N*-acetyl amino sugar. The recovery of bacteriolytic and caseinolytic activity was calculated to be about 80 % and 30 %, respectively. Deaminated chitin gave a similar elution pattern.

DISCUSSION

All the bacteriolytic enzymes of *Streptomyces albus* cultures seem to bind reversibly to chitin. The binding is apparently dependent on low ionic strength since elution of lytic material starts below 0.04 M NaCl (Fig. 2), but shows high affinity at pH-values above 4.2. The abrupt drop in binding affinity below pH 4.2, decreasing to zero at pH 3.2, is in accord with data reported for lysozymes.^{7,9} In these reports the carboxyl groups localized to the "active sites" of the enzymes were supposed to be essential for binding to chitin. No difference between chitin and deaminated chitin was observed in the adsorption and elution pattern. Purified chitin contains some free amino groups revealed by deacetylation. These groups have been shown to exhibit ion-exchange effect,⁵ but seem, according to the results with deaminated chitin, to be of no importance for adsorption.

Both endopeptidase and the endo-*N*-acetylmuramidase activity were demonstrated in the eluates from the chitin materials, the elution pattern being very similar to that of the carboxy methyl cellulose columns.² The binding of the muramidase to chitin is most probably due to a real enzyme-substrate affinity on account of analogy in the structures of glycan chains of mucopeptide and chitin, a specific binding of the endopeptidases being more doubtful. It has, however, previously been shown¹¹ that the endopeptidase activities of *Streptomyces albus* G to a certain degree depend on an intact glycan chain, and this may have a

Acta Chem. Scand. B 28 (1974) No. 2

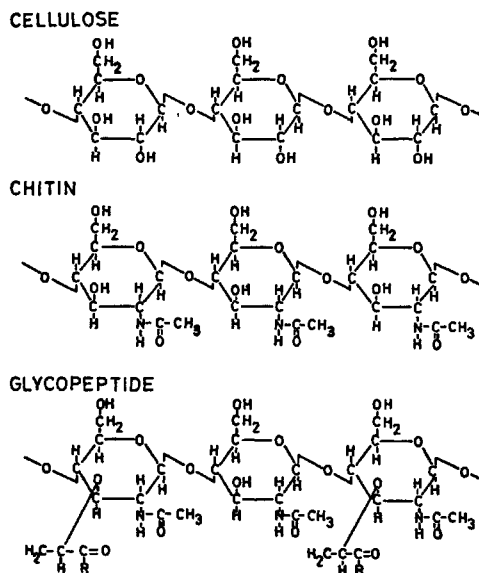


Fig. 4. The glycan chain of cellulose, chitin and mucopeptide (glycopeptide). R = OH or peptide.

connection with a possible binding site of endopeptidases to the glycan chain.

The results of the present experiments with chitin are comparable with those obtained with carboxy methyl cellulose, and cellulose also with regard to adsorbing ability. This points to the possibility that binding of the lytic enzymes to the glycan chains is effected similarly in mucopeptides, cellulose, carboxy methyl cellulose and chitin (see Fig. 4 for a structural comparison), and that the introduced carboxyl groups have only a minor influence on this binding.

Acknowledgement. The authors wish to express their gratitude to Dr. H. B. Jensen, The Biochemical Institute, The University of Bergen, for advice and help in preparation of the chitin materials.

REFERENCES

1. Aksnes, L. and Grov, A. *Acta Chem. Scand. B* 28 (1974) 185.
2. Aksnes, L. and Grov, A. *Acta Chem. Scand. B* 28 (1974) 193.
3. Berger, L. R. and Weiser, R. S. *Biochim. Biophys. Acta* 26 (1957) 517.

4. Cherkasov, I. A., Kravchenko, N. A. and Kaverzneva, E. D. *Dokl. Akad. Nauk. SSSR* 170 (1966) 213.
5. Cherkasov, I. A. and Kravchenko, N. A. *Mol. Biol.* 1 (1967) 381.
6. Cherkasov, I. A. and Kravchenko, N. A. *Biochim. Biophys. Acta* 206 (1970) 289.
7. Imoto, T., Heyashi, K. and Funatsu, M. *J. Biochem. (Tokyo)* 64 (1968) 387.
8. Heyashi, K., Fujimoto, N., Kugimiya, M. and Funatsu, M. *J. Biochem. (Tokyo)* 65 (1969) 401.
9. Jensen, H. B. and Kleppe, K. *Eur. J. Biochem.* 26 (1972) 305.
10. Cherkasov, I. A. and Kravchenko, N. A. *Biokhimiya* 34 (1969) 1089.
11. Petit, J. F., Munoz, E. and Ghuysen, J.-M. *Biochemistry* 5 (1966) 2764.

Received October 13, 1973.