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Note

N-Acyl derivatives of chitosan and their hydrolysis by chitinase

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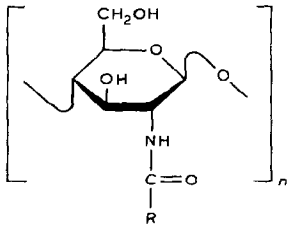
Chitinase (EC 3.2.1.14) hydrolyses chitin, a (1→4)-linked 2-acetamido-2-deoxy-β-D-glucan, to afford oligosaccharides. We now report some novel structural variants of chitin and their hydrolysis by chitinase, in continuation of our interest in the substrate specificity[1] of the enzyme.

N,O-Acylation[2] of chitosan (*N*-deacetylated chitin) and *O*-deacylation of the products gave, in yields of 42–59%, *N*-glycoloyl (**1**, d.s. 0.84), *N*-(DL-lactoyl) (**2**, d.s. 0.85), *N*-(*O*-methylglycoloyl) (**3**, d.s. 1.0), *N*-thioglycoloyl (**4**, d.s. 0.62), *N*-(3-mercaptopropionyl) (**5**, d.s. 0.68–0.73), and *N*-thioglycoloyl-*N*-decanoyl [**6**, d.s. 0.77 (0.54/0.28 for thioglycoloyl/decanoyl)] derivatives, of which only **1** and **4** were soluble in water. The d.s. values for *N,O*-acyl groups in the products were 1.8–1.9. *O*-Deacylation was accompanied by some *N*-deacylation even under mild conditions, and this accounts for the variation of d.s. for *N*-acyl group (d.s. 0.6–1.0) in **1**–**6**.



1 R = CH_2OH **2** R = $CHOHCH_3$
3 R = CH_2OCH_3 **4** R = CH_2SH
5 R = CH_2CH_2SH
6 R = $CH_2SH/(CH_2)_8CH_3$

The rates of hydrolysis of **1**–**6** by chitinase are shown in Table I. The rates of hydrolysis with respect to R of the RCONH group are summarised in the sequence CH_2OH (**1**) > $CH(OH)CH_3$ (**2**) > CH_2CH_3[1] > CH_3 (crab-shell chitin)[1] > H[1] >

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CH_2OCH_3 (**3**) > $CH_2CH_2CH_3$[1] > $CH(CH_3)_2$[1] > CH_2NH_2[1] > CH_2Cl[1]; derivatives where R is CH_2SH (**4**), CH_2CH_2SH (**5**), CF_3[3], and $(CH_2)_nCH_3$ (**6**, $n > 3$) were not hydrolysed, presumably because formation of the ES complex was sterically hindered. Compound **1** (R = CH_2OH) was hydrolysed at a rate higher than that of *N*-acetylchitosan xerogel[1], but **4** (R = CH_2SH) was not hydrolysed, indicating an inhibition of the enzymic reaction by the SH group.

TABLE I

RATES OF HYDROLYSIS OF *N*-ACYLATED DERIVATIVES OF CHITOSAN BY CHITINASE[a]

N-*Acylchitosan*	*Increase in reducing sugar value (μmol/2 h)*[b]	*Relative rate*[c]
Glycoloyl (**1**)	2.68	10.0
DL-Lactoyl (**2**)	0.52	2.0
Chitin (crab shell)[d]	0.26	1.0
O-Methylglycoloyl (**3**)	0.17	0.7

[a]*N*-Thioglycoloyl (**4**), *N*-(3-mercaptopropionyl) (**5**), *N*-thioglycoloyl-*N*-decanoyl (**6**), and *N*-trifluoroacetyl[3] derivatives were not hydrolysed. [b]The value was calculated as 2-acetamido-2-deoxy-D-glucose. [c]Relative to the reducing value of natural chitin (crab shell). [d]Ref. 1.

EXPERIMENTAL

D.s. for *N* and *O*-acyl groups was calculated from C/N and S/N values in the elemental analyses. Enzymic hydrolysis was performed, as reported[1], in 0.05M citric acid–0.01M Na_2HPO_4 buffer (pH 6.6) by chitinase from *Streptomyces griseus* (Sigma, 3 U/mg). Increase of reducing-sugar value was analysed by a modified Schales' method[4], and expressed as μmol of 2-acetamido-2-deoxy-D-glucose. The other analytical methods have been reported[1].

Acylation of chitosan. — (*a*)[2] Chitosan {0.16 g, $[\alpha]_D^{17}$ −9° (*c* 0.5, aqueous 2% acetic acid); C/N, 6.01} was stirred severally with 7 g each of glycolic, DL-lactic, *O*-methylglycolic, thioglycolic, and 3-mercaptopropionic acids at 70–80° for 40 h.

(*b*)[3] A solution of chitosan (0.16 g) in a mixture of the carboxylic acid (7 g) and decanoic anhydride (3 g) was stirred at 70–80° for 40 h. The product obtained as in (*a*) and (*b*) was poured into acetone (~100 mL). The precipitate was collected, washed with ethanol and ether, and dried to afford the *N,O*-acyl derivative, which had i.r. absorptions at ~1750 and ~1210 (C=O and C–O of *O*-acyl), ~1650 and ~1550 cm^{-1} (C=O and NH of *N*-acyl). The product was *O*-deacylated by treatment with 0.01M NaOH at room temperature for 18 h to afford the *N*-acyl derivative, which was isolated directly or after the addition of ethanol (3 vol.). The product had no i.r. absorption for *O*-acyl group. Compound **1** (59% yield; C/N, 7.69), $[\alpha]_D^{18}$ −11° (*c* 1, water), obtained as in (*b*), gave glycolic acid (R_F 0.64); and **2** (42% yield; C/N, 8.57), obtained as in (*b*), gave DL-lactic acid (R_F 0.78) on hydrolysis (2M HCl,

100°, 20 h), as indicated by p.c. (13:3:1 ether–acetic acid–water). Compounds **3** (50% yield; C/N, 8.98) and **5** (53% yield; C/N, 8.05; and S/N, 0.73) were obtained as in (*a*); **4** {51% yield; C/N, 7.25; and S/N, 0.62; $[\alpha]_D^8$ −11° (*c* 0.5, water)} was obtained as in (*a*); and **6** (55% yield; C/N, 9.38; and S/N, 0.54) was obtained as in (*b*).

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