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Note

Periodate oxidation of the non-reducing end-groups of substrates increases the rates of enzymic hydrolyses by chitinase and by lysozyme

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Chitin[1] [a (1→4)-linked 2-acetamido-2-deoxy-β-D-glucan] is hydrolysed by chitinase[2] (EC 3.2.1.14) and by lysozyme[3] (EC 3.2.1.17). However, little is known about the substrate specificity of chitinase, because of the lack of suitable derivatives of chitin. Of a series of *N*- and *O*-substituted derivatives[4,5] of chitosan, the *N*-acetyl derivative[6] was the most susceptible to chitinase and lysozyme. Furthermore, the gelled form of substrates increased the rates of enzymic hydrolyses. In extending our studies of the control of enzymic reactions by chemical and physical modifications of substrates[7], we now report on the increase in rates of hydrolyses by chitinase and by lysozyme after oxidation of the non-reducing end-groups of *N*-acetylchitosan and chitin (from crab shells) with sodium metaperiodate.

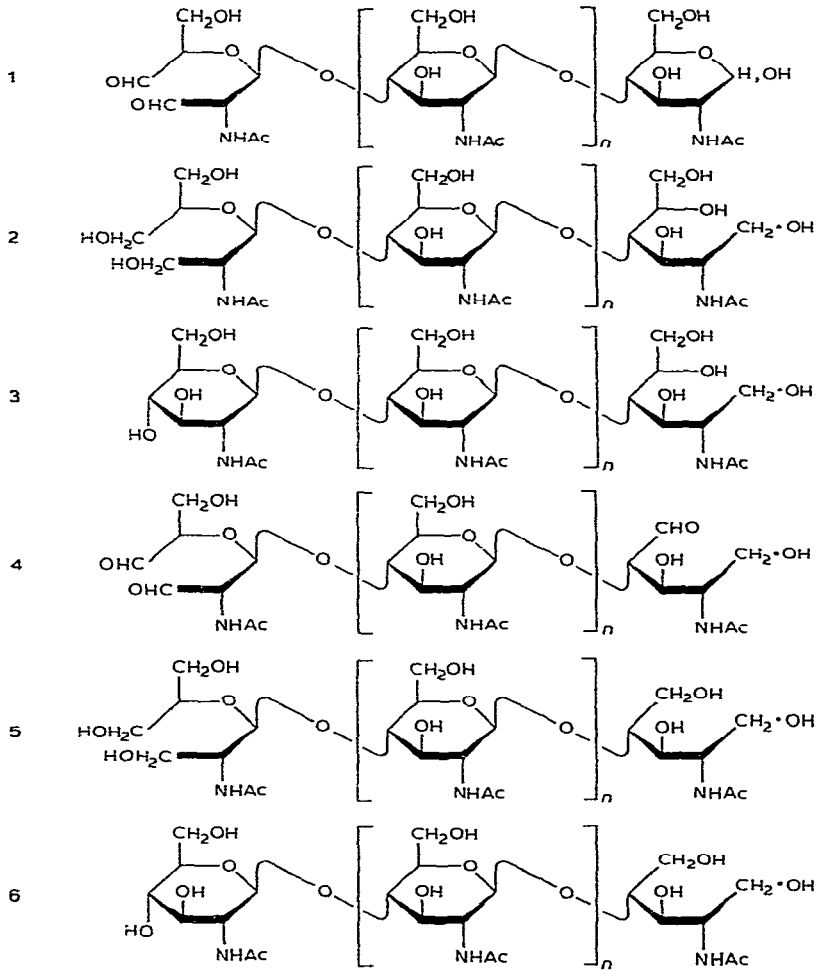
The derivatives **1**–**6** of *N*-acetylchitosan (d.s. 1.0) were prepared. Oxidation with periodate afforded **1**, which was reduced with borohydride[8] to give **2**. Reduction of *N*-acetylchitosan afforded **3**, which was oxidised with periodate to give **4**. Reduction of **4** afforded **5**, which was hydrolysed to give **6**.

The oxidation of **3** released 36.7 μmol of formaldehyde per hexosaminyl residue, corresponding to a d.p. of ~139.

Fig. 1 shows time courses of the hydrolyses of **1** and **3** by chitinase (as analysed by the increase of reducing power) and of *N*-acetylchitosan by chitinase and by lysozyme. Further data are given in Table I. Only for the chitin derivative **1** was the rate of hydrolysis by chitinase significantly enhanced. The modified, non-reducing end-group in **1** may increase hydrophilicity and accelerate the formation of enzyme–substrate complexes.

The *N*-acetylchitosan derivatives **1**–**6** were insoluble in water and in buffer solutions (pH 5.2 and 6.0), in contrast to *O*-(2-hydroxyethyl)chitin. That solubility affects the rates of enzymic hydrolysis is suggested by the fact that *O*-(2-hydroxyethyl)chitin was hydrolysed by chitinase and by lysozyme, respectively, at 4.5 and 110 times the rate for *N*-acetylchitosan.

A partially *O*-acetylated (d.s. ~0.9) derivative of *N*-acetylchitosan was hydro-



lysed by chitinase and by lysozyme, respectively, at 0.3 and 0.9 times the rate for *N*-acetylchitosan. The action of chitinase, but not of lysozyme, was inhibited by *O*-substitution of hexosaminyl residues. Substitution at O-3 and O-6 of 2-acetamido-2-deoxy-β-D-glucopyranosyl residues has little effect on the formation of enzyme–substrates complexes in Phillips' model[3].

EXPERIMENTAL

Materials. — Chitinase from *Streptomyces griseus* (3 units/mg, Sigma), lysozyme from chicken egg-white (57,200 units/mg, Grade III, Sigma), *O*-(2-hydroxyethyl)chitin, and chitin from crab shells were commercial products (Nakarai Chemi-

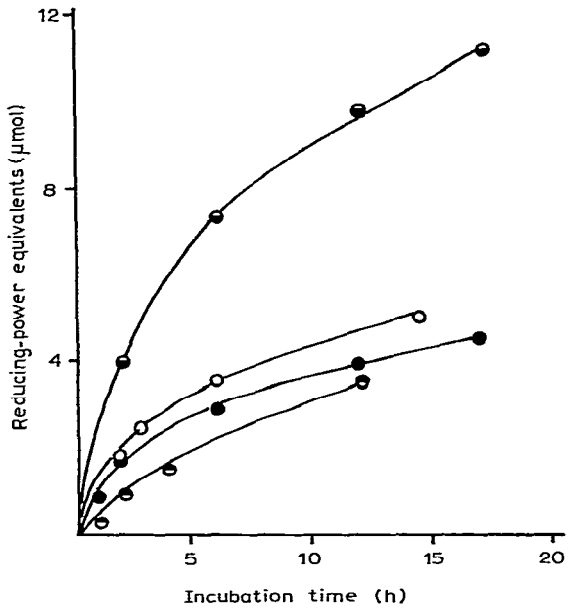


Fig. 1. Enzymic hydrolyses of *N*-acetylchitosan (○), **1** (◒), and **3** (●) by chitinase (*Streptomyces griseus*), and of *N*-acetylchitosan (◓) by lysozyme (chicken egg-white).

TABLE I

THE EFFECTS OF MODIFICATION OF THE REDUCING AND NON-REDUCING END-GROUPS OF *N*-ACETYLCHITOSAN AND CHITIN ON THE RATES OF HYDROLYSES BY CHITINASE (*Streptomyces griseus*) AND BY LYSOZYME (CHICKEN EGG-WHITE)

Substrate	*Increase in reducing sugar values*[a]		*Relative rates*[b]	
	Chitinase	*Lysozyme*	*Chitinase*	*Lysozyme*
1	3.69	3.82	186	189
2	2.04	2.71	103	134
3	1.64	1.72	83	85
4	2.55	n.d.	129	n.d.
5	1.31	n.d.	66	n.d.
6	1.41	n.d.	71	n.d.
Oxidised chitin	0.34	n.d.	17	n.d.
Reduced chitin	0.17	n.d.	9	n.d.
Partially *O*-acetylated *N*-acetylchitosan[c]	0.63	1.89	32	94
N-Acetylchitosan (Amor.)[d]	1.98	2.02	100	100
Chitin	0.24	0.19	12	9
O-(2-Hydroxyethyl)chitin	8.96	216	453	10,700

[a] μMol/2 h for chitinase and μmol/5 h for lysozyme, respectively. [b] The hydrolysis rate for *N*-acetylchitosan is regarded as 100. [c] D.s. for *O*-acetyl, ~0.9. [d] The gelled form of *N*-acetylchitosan was lyophilised and powdered.

cals, Kyoto). *N*-Acetylchitosan[4] (d.s. 1.0) and its *O*-acetyl derivative[5] (d.s. 0.9 for OAc) were prepared as described previously.

Derivatives of N-*acetylchitosan and chitin.* — Suspensions of samples (1 g) of *N*-acetylchitosan or **3** in 15mM $NaIO_4$ (250 ml) were stirred at 5° in the dark for 3 days. Excess of oxidant was decomposed with ethylene glycol, and the products were collected, resuspended in distilled water, collected, washed with water, and dried to afford **1** or **4**, respectively. Suspension of samples (1 g) of **1**, *N*-acetylchitosan, or **4** in 0.01M $NaBH_4$ (250 ml) were stirred at room temperature overnight. Excess of reductant was decomposed with dilute acetic acid. The products were collected, methanol was repeatedly evaporated therefrom, and the products were suspended in distilled water, collected, washed with water, and dried to afford **3**, **2**, and **5**, respectively. A suspension of **5** (1 g) in 0.5M HCl (250 ml) was stirred at room temperature overnight, and then neutralised with M NaOH. The product was collected, resuspended in distilled water, collected, washed with water, and dried to afford **6**. The yields in these reactions were almost quantitative.

Methods. — Enzymic hydrolyses by chitinase and by lysozyme were performed as described previously[6].

D.p. of **3**. — A suspension of **3** (500 mg) in 15mM $NaIO_4$ (100 ml) was stirred at 5° in the dark. Periodate consumption was monitored on aliquots by the spectrophotometric method[9], and the formaldehyde liberated was determined by the chromotropic acid method[10]. Oxidation was complete after 5 days.

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