PRELIMINARY NOTES

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Comparative behaviour of six different lysozymes in the presence of an inhibitor*

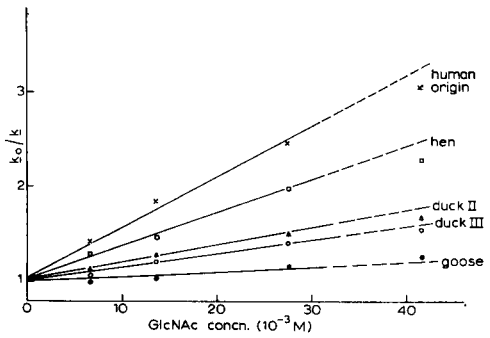
It was shown that hen egg-white lysozyme (EC 3.2.1.17) can be inhibited by *N*-acetylglucosamine (GlcNAc), GlcNAc–GlcNAc and some related compounds[1-3]. Recently SHARON[4] studied in detail the inhibitory action of GlcNAc on the lysis of *Micrococcus lysodeikticus* cells by hen lysozyme and indicated that the inhibition was accompanied by a corresponding change in the fluorescence of the enzyme. In their study devoted to lysozymes of different origins, JOLLÈS *et al.*[5] established that lysozymes from different sources have different structures; lysozymes from different organs or tissues of the same animal can be different (dog spleen and dog kidney enzymes) or seem identical (human lysozymes). Their heat stability and specific activity appear to be strongly correlated to their different cystine and tryptophan contents which were particularly low in the case of goose egg-white lysozyme[6]. As several tryptophan residues were demonstrated to be involved in the active site of hen egg-white lysozyme[3], it was of interest to study the behaviour of these different lysozymes in the presence of an inhibitor.

GlcNAc was obtained from Light and tetra-GlcNAc (chitotetraose) was prepared according to the method of RUPLEY[7]. Hen egg-white lysozyme was obtained from Armour, Kankakee, Ill.; duck egg-white lysozymes II and III, goose egg-white lysozyme, human milk lysozyme and lysozyme from human normal leucocytes were prepared by the procedures of JOLLÈS, SPOTORNO AND JOLLÈS[8], DIANOUX AND JOLLÈS[9], JOLLÈS AND JOLLÈS[10] and CHARLEMAGNE AND JOLLÈS[11], respectively. Dry *M. lysodeikticus* cells were purchased from Miles Laboratories. The lysis of these cells (300 μg/ml) by lysozyme (usually 9.1 μg/ml) at room temperature was followed with a Vitatron photometer (Dieren, The Netherlands) in the absence or in the presence of GlcNAc or tetra-GlcNAc; the absorbance, measured at 583 mμ, was automatically recorded. To 2.5 ml of a suspension of *M. lysodeikticus* cells in a 0.066 M phosphate buffer of pH 6.2 containing 0.1% NaCl were added 0.5 ml of a solution of GlcNAc in water and 0.3 ml of the enzyme solution in water. Apparent first order rate constants, k, could be calculated from the slopes of plots of log $A_{583\ m\mu}(t)$ – log $A_{583\ m\mu}(0)$ *versus* time, t, where $A_{583\ m\mu}(t)$ and $A_{583\ m\mu}(0)$ denote, respectively, the absorbances at time t and 0 sec. An increase in the concentration of the inhibitor led to a decrease in the rate constant for cell lysis, k; the inhibition ratio was defined as k_0/k, in which k_0 denotes the apparent first order rate constant of cell lysis in the absence of inhibitor.

The fluorescence was determined in 0.1 M ammonium acetate. The emission spectra were measured between 300 and 400 mμ in an Aminco Bowman spectrofluorimeter; an exciting wavelength of 285 mμ was used. The solution contained between 16.6 and 50 μg lysozyme per ml and between 0.2 and 10 mg GlcNAc per ml.

For each of the six lysozymes listed above and for various concentrations of GlcNAc, the inhibition ratio k_0/k was determined (Fig. 1). The two human lysozymes are more strongly inhitibed by GlcNAc than hen egg-white lysozyme; the concentra-

* 57th communication on lysozymes; 56th communication: see ref. 13.



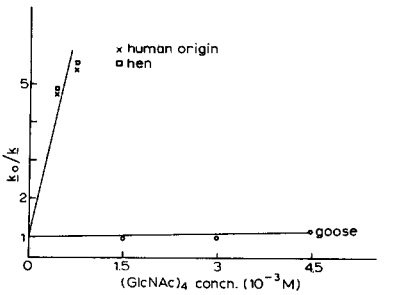


Fig. 1. Inhibition of the action of different lysozymes ($0.63 \cdot 10^{-6}$ M) on *M. lysodeikticus* cells by GlcNAc. For conditions, see the text.

Fig. 2. Inhibition of the action of different lysozymes ($0.63 \cdot 10^{-6}$ M) on *M. lysodeikticus* cells by tetra-GlcNAc.

tions of GlcNAc necessary for 50% inhibition were, respectively, $1.8 \cdot 10^{-2}$ M and $2.8 \cdot 10^{-2}$ M. It was not possible under the conditions described above to observe 50% inhibition for the two duck egg-white lysozymes. Furthermore, duck egg-white lysozymes II and III had a slightly different behaviour in the presence of GlcNAc (Fig. 1); this result was in accordance with recent observations by JOLLÈS *et al.*[12] who established that these two duck lysozymes have slightly different chemical structures. Goose egg-white lysozyme, however, was practically uninhibited at low GlcNAc concentrations and only to a small extent at higher GlcNAc concentrations: once more its behaviour seemed different from that of other lysozymes as already indicated by JOLLÈS *et al.*[6]; it is worth adding that its specificity was also quite different when low molecular weight substrates were used: they do not seem to be easily split by this enzyme[13].

Similar results were obtained with tetra-GlcNAc. According to SHARON[4] the concentration of this sugar for 50% inhibition of hen lysozyme action on cells of *M. lysodeikticus* was around 10^{-3} M. Under the conditions indicated above, hen egg-white and human milk lysozymes were more strongly inhibited, the concentration for a 50% inhibition being around $0.15 \cdot 10^{-3}$ M for both enzymes. Goose lysozyme, however, was not inhibited by tetra-GlcNAc until a concentration of $4.5 \cdot 10^{-3}$ M (Fig. 2).

As noted by SHINITZKY *et al.*[14], strong inhibitors of hen egg-white lysozyme, such as GlcNAc–GlcNAc, lead to a 10 mμ blue shift of the emission maximum of the enzyme under the conditions of the fluorescence experiments; the observed changes may be ascribed to changes in the environment of the indole rings of the active site upon binding with the inhibitor[15]; the weaker inhibitors, such as GlcNAc, do not cause any shift. Our experiments with different lysozymes lead us to the conclusion that, as in the case of hen egg-white lysozyme, GlcNAc does not cause any shift in the emission maximum. This method does not allow one to study the binding of GlcNAc at the active sites of these enzymes. Furthermore, as could be expected from the absence of a blue shift, no strong enhancement of the fluorescence was observed. The change of the height of the emission maximum of the different lysozymes in the presence of GlcNAc does not exceed $100 \pm 5\%$, taking as 100% that of an enzyme in the absence of GlcNAc.

In order to find some other tests of the binding of GlcNAc at the active site of goose lysozyme, this substance and hen egg-white lysozyme were submitted to heat

denaturation at 75° (different time intervals) in the presence or in the absence of GlcNAc. Even at low GlcNAc concentrations ($10 \cdot 10^{-3}$ M) the hen enzyme was markedly stabilized while no marked difference was observed with the goose enzyme. At higher GlcNAc concentrations ($25 \cdot 10^{-3}$ M), some protective effect could be observed with goose lysozyme while the hen enzyme was naturally strongly stabilized. Thus only high GlcNAc concentrations seem to be able in part to inhibit the goose enzyme and to protect it against heat denaturation.

Preliminary experiments allow us to add that other inhibitors of hen lysozyme such as histamine or L-histidine methyl ester[14] also inhibit goose lysozyme at concentrations similar to those used for the hen enzyme.

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