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Note

The enzyme-inhibitory properties of 1-α- and 1-β-D-glucopyranosylimidazoles

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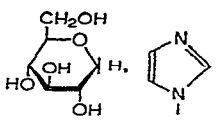
During the course of a study[1] of the synthesis and properties of the anomeric 1-D-glucopyranosylimidazoles (**1a,b**), it was noted that these compounds acted as inhibitors towards glycosidases. The exceptional stability of the glycosyl linkage in the 1-glycopyranosylimidazoles[1] makes them of particular value for studies of glycosidase–inhibitor interactions, and we now report the results of some inhibition experiments on yeast α-D-glucosidase, almond β-D-glucosidase, and lysozyme.



1a α-anomer
1b β-anomer

Yeast α-D-glucosidase

Reciprocal plots for the inhibition of the hydrolysis of methyl α-D-glucopyranoside catalysed by yeast α-D-glucosidase (Sigma) are shown in Figs. 1*a* and 1*b*. Re-plots of slopes *versus* inhibitor concentrations were linear in each case, thereby characterising the patterns of inhibition as linear competitive. The inhibition constants (K_i) obtained from the intercepts of the re-plots were 0.29mM for 1-α-D-glucopyranosylimidazole, and 190mM for 1-β-D-glucopyranosylimidazole. Thus, the yeast enzyme shows a high degree of inhibitor anomeric specificity. The K_i value for the α-compound is somewhat lower than the values obtained[2] for D-glucose (2.0mM) and 1L-1,2-anhydro-*myo*-inositol (6.9mM), and also for the Michaelis constant for methyl α-D-glucopyranoside (30 ±5mM).

Almond β-D-glucosidase

Reciprocal plots for the inhibition of the hydrolysis of cellobiose catalysed by almond β-D-glucosidase (Sigma) are shown in Figs. 2*a* and 2*b*. Inhibition was competitive except at high relative concentrations of 1-α-D-glucopyranosylimidazole which displayed non-competitive inhibition. In this case, the initial slope of the reciprocal plot was used in the re-plot. Re-plots were linear and gave values for the inhibitor constants of 33mM for 1-α-D-glucopyranosylimidazole and 50mM for 1-β-D-

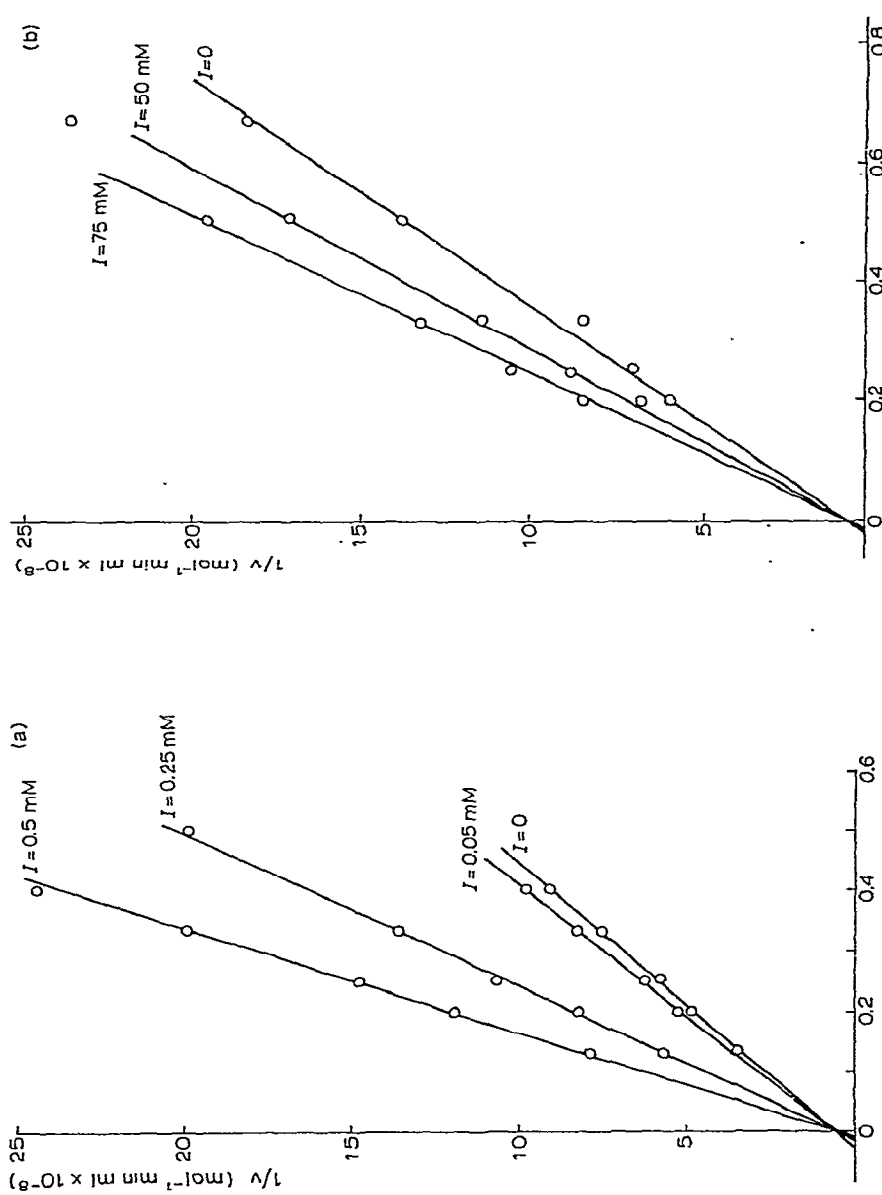


Fig. 1. Inhibition of yeast α-D-glucosidase by (*a*) 1-α-D-glucopyranosylimidazole and (*b*) 1-β-D-glucopyranosylimidazole. The reaction mixtures contained enzyme [Sigma, 0.002% w/v (*a*) or 0.0025% w/v (*b*)], methyl α-D-glucopyranoside [2–7.5mM (*a*) or 1–5mM (*b*)], and inhibitor [0–0.5mM (*a*) or 0–75mM (*b*)] dissolved in phosphate buffer (pH 6.8, 1 ml). After 120 min at 37°, the reactions were terminated by heating the mixtures for 2 min at 100°, and the D-glucose liberated was determined by using D-glucose oxidase[10].

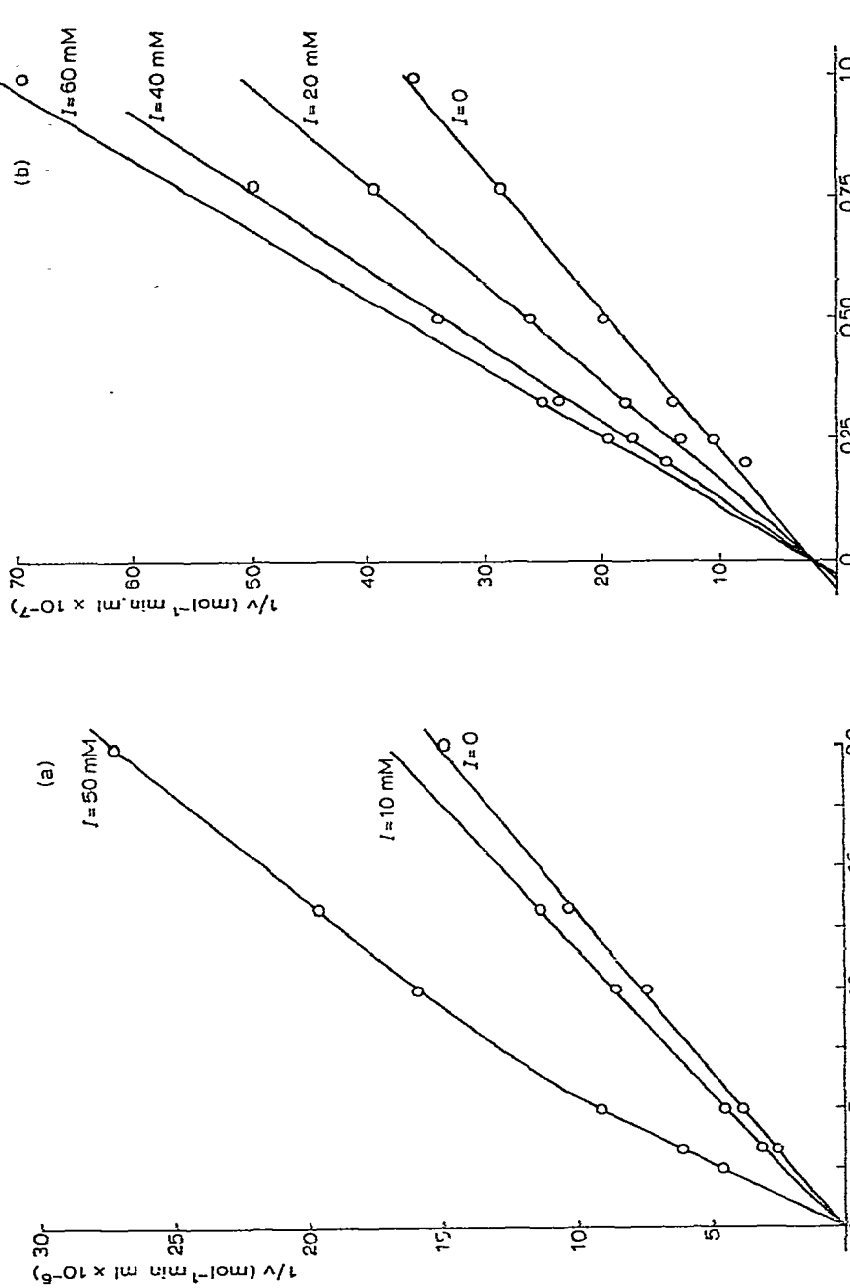


Fig. 2. Inhibition of almond β-D-glucosidase by (*a*) 1-α-D-glucopyranosylimidazole and (*b*) 1-β-D-glucopyranosylimidazole. The reaction mixtures contained enzyme [Sigma, 0.05% w/v (*a*) or 0.0125% w/v (*b*)], substrate cellobiose [0.05–0.4mM (*a*) or 1–5mM (*b*)], and inhibitor [0–50mM (*a*) or 0–60mM (*b*)] dissolved in citrate buffer (pH 5.3, 1 ml). After 120 (*a*) or 32 min (*b*) at 37°, the reactions were terminated by heating the mixtures for 2 min at 100°, and the D-glucose was determined by using D-glucose oxidase[10]. A blank correction was made for D-glucose derived non-enzymically from cellobiose.

glucopyranosylimidazole. These substrate-analogue inhibitors give K_i values which are of the same order of magnitude as the K_m value for cellobiose (24 ±2mM), but are much less effective than the potential transition-state-analogue inhibitors nojirimycin and D-glucono-1,5-lactone[3]. The lack of anomeric specificity towards the D-glucopyranosylimidazole inhibitors by this enzyme is somewhat surprising, but not unique. Nitta *et al.* reported[4] a similar phenomenon in a study of the inhibition of Taka-amylase A by various glycosides.

Lysozyme

1-β-D-Glucopyranosylimidazole did not inhibit the degradation of *Micrococcus lysodeikticus* cells by lysozyme at inhibitor concentrations of up to 69mM. This result was not unexpected since, although small molecules such as 2-acetamido-2-deoxy-D-glucose and the methyl and ethyl glycosides thereof are known to be inhibitory[5], it is believed that the 2-acetamido[6] or other 2-amino[7] group is necessary for binding. However, somewhat surprisingly, 1-α-D-glucopyranosylimidazole at 50mM concentra-

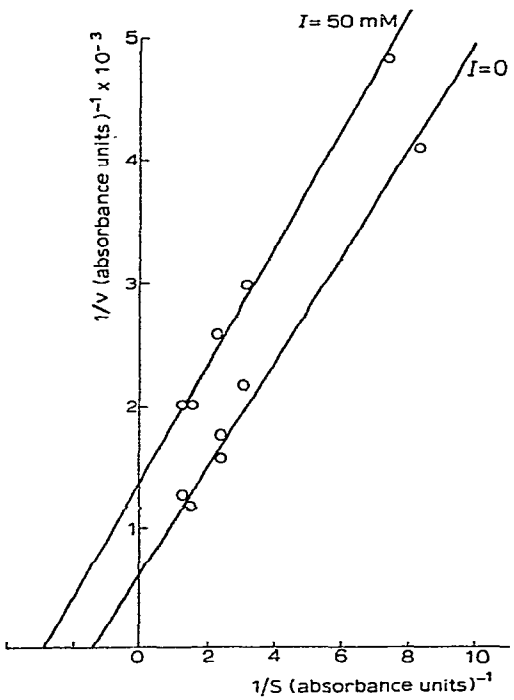


Fig. 3. Inhibition of lysozyme by 1-α-D-glucopyranosylimidazole. The reaction mixtures contained enzyme (Sigma, 1 μgml^{-1}), substrate *Micrococcus lysodeikticus* cells (Sigma, 22–180 μg.ml^{-1}), and inhibitor (0 and 50mM) dissolved in phosphate buffer (pH 6.2) (ionic strength 0.067[5], 2.6 ml) at 20°. The absorbance of the mixtures compared to a blank not containing cells was monitored at 450 nm by using a Pye Unicam SP1800 spectrophotometer. Cell suspensions containing no enzyme showed no change in absorbance during the time over which the reaction was followed (*ca.* 2 min). Initial rates were computed by using a least-squares curve-fitting programme kindly provided by Mr. K. Freeman of this Department.

tion behaved as an uncompetitive inhibitor, as shown by a reciprocal plot (Fig. 3). Previous workers[5,8] have shown that imidazole and imidazole derivatives in their protonated forms inhibit lysozyme, possibly by the formation of charge-transfer complexes with tryptophan residues at the enzyme active site. This proposal has received recent support from an X-ray crystallographic study[9] of complexes of lysozyme with histamine and histidine. However, these compounds were proposed to act as competitive inhibitors, although the kinetic data given do not seem to warrant this. The greater inhibitory power of 1-α-D-glucopyranosylimidazole than that of the β anomer may be illustrative of a general phenomenon whereby the anomeric configuration is not of over-riding importance except for specific substrates. Thus Davies *et al.* reported[5] that at pH 6.2 ethyl 2-acetamido-2-deoxy-α-D-glucopyranoside is a stronger inhibitor of lysozyme than the β anomer.

ACKNOWLEDGMENTS

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