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Note

Inhibition of α-L-arabinofuranosidase (*Aspergillus niger*) and non-inhibition of α-L-arabinopyranosidase (almond emulsin and barley) by L-arabinono-1,4-lactone

KONRAD SCHWABE, ANNEMARIE GROSSMANN, BARBARA FEHRMANN, AND BRUNO TSCHIERSCH
Central Institute for Cancer Research, Academy of Sciences of the G.D.R., 1115 Berlin-Buch (G.D.R.)

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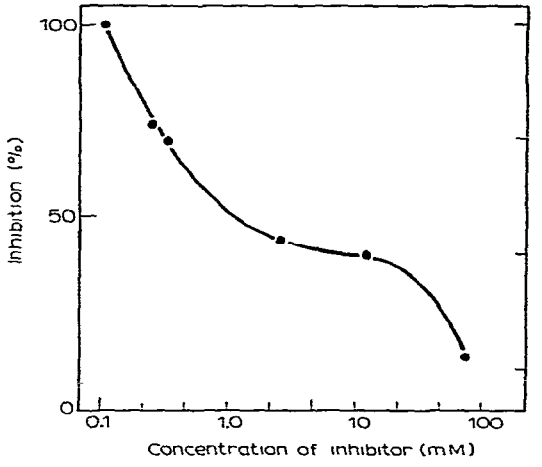
Since the studies of Levvy[1,2], it has been known that glycosidases are inhibited by aldonolactones. Bound to Sepharoses, the latter have been used for affinity chromatography of glycosidases[3]. By this method, numerous enzymes (such as β-D-galactosidase, β-D-glucosidase, and neuraminidase) have been highly purified. However, no inhibitors of α-L-arabinofuranosidase are yet known that could be used for affinity chromatography of this enzyme. Highly purified α-L-arabinofuranosidases are of interest in connection with a new concept of cancer chemotherapy[4–7]. The present paper deals mainly with inhibition of the α-L-arabinofuranosidase of *Aspergillus niger* K1 and non-inhibition of the α-L-arabinopyranosidase of emulsin or barley by L-arabinono-1,4-lactone.

Concentration of the inhibitor. — The dependence of the inhibition of α-L-arabinofuranosidase on the concentration of the inhibitor is shown in Fig. 1. The concentration of L-arabinono-1,4-lactone required for 50% inhibition of the α-L-arabinofuranosidase was approximately mM.

pH-Dependence. — The effect of pH on the inhibition of α-L-arabinofuranosidase was studied at constant concentration of the inhibitor. Whereas the α-L-arabinofuranosidase displayed optimal activity at pH 4.0, the strongest inhibitory effect of the L-arabinono-1,4-lactone was at pH 4.5 (Fig. 2).

Time of incubation. — Fig. 3 reveals that the inhibitory action of L-arabinono-1,4-lactone on α-L-arabinofuranosidase remains constant for up to 60 min of incubation at all inhibitory concentrations studied (0.2–100mM).

Specificity of inhibitory action. — In order to test the specificity of the inhibitory action of L-arabinono-1,4-lactone, the inhibition of α-L-arabinopyranosidase (almond emulsin) was studied. The ratio of β-D-glucopyranosidase to α-L-arabinopyranosidase in almond emulsin is known to be 20:1. *p*-Nitrophenyl α-L-arabinopyranoside was used as a substrate that is hydrolysed by almond emulsin[13]. Even at concentrations of L-arabinono-1,4-lactone of 20mM, there was no inhibition of α-L-arabinopyranosidase (almound emulsin). Similarly, the α-L-arabinopyranosidase obtained from barley



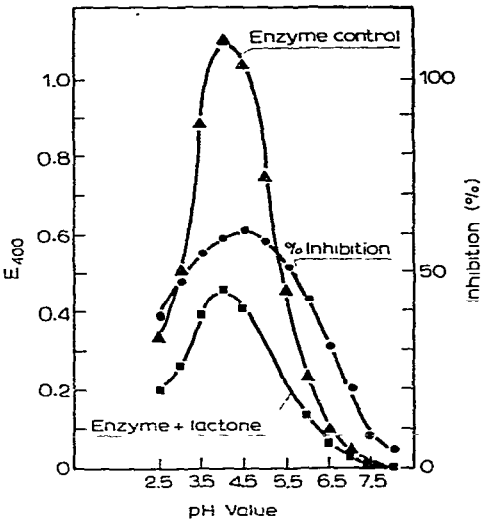


Fig. 1. Effect of the concentration of L-arabinono-1,4-lactone on the inhibition of α-L-arabinofuranosidase. The enzyme stock solution was diluted 1:1000. Time of incubation: 30 min.

Fig. 2. Effect of pH on the inhibition of α-L-arabinofuranosidase by 2mM L-arabinono-1,4-lactone. The enzyme stock solution was diluted 1:500. Time of incubation: 30 min.

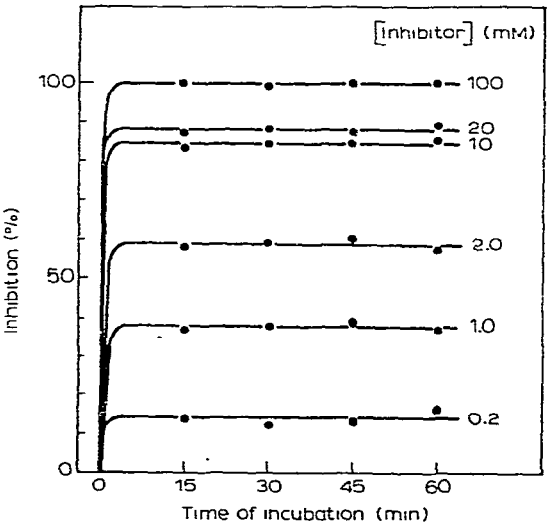


Fig. 3. Effect of time of incubation on the inhibition of α-L-arabinofuranosidase by various concentrations of L-arabinono-1,4-lactone. The enzyme stock solution was diluted 1:1000.

was not inhibited by 20mM L-arabinono-1,4-lactone. Furthermore, the inhibition of hydrolysis of *p*-nitrophenyl α-L-arabinofuranoside by α-L-arabinofuranosidase was studied by using D-glucono-1,5-lactone. No inhibitory effect could be observed, even at higher concentrations (2mM) of lactone. Finally, the enzymic cleavage of 10mM *p*-nitrophenyl β-D-glucopyranoside by β-D-glucopyranosidase (contained in the α-L-arabinofuranosidase preparation from *Aspergillus niger* K1) was not affected by L-arabinono-1,4-lactone.

The results show that L-arabinono-1,4-lactone is a moderate, but specific, inhibitor of the α-L-arabinofuranosidase of *Aspergillus niger* K1. In contrast, the same lactone has no inhibitory effect on the α-L-arabinopyranosidase of almond emulsin or the α-L-arabinopyranosidase of barley. Neither is the β-D-glucopyranosidase (contained in the α-L-arabinofuranosidase preparation from *Aspergillus niger* K1) inhibited by L-arabinono-1,4-lactone.

Compared with these findings, Levvy *et al.*[14], using 4.1mM and mM L-arabinono-1,5-lactone, found a 50% inhibition of α-L-arabinopyranosidase (almond emulsin) and α-L-arabinopyranosidase (barley), respectively. Hence, it may be concluded that the size of the lactone ring determines its specificity of inhibition. Whereas the 1,4-lactone is able to inhibit α-L-arabinofuranosidases exclusively, the 1,5-lactone of L-arabinonic acid inhibits α-L-arabino*pyrano*sidases. Whether or not the L-arabinono-1,5-lactone is also able to inhibit α-L-arabinofuranosidases could be clarified only with completely pure 1,5-lactone. This would difficult to accomplish, because aldono-1,4-lactones are more stable in aqueous solutions than aldono-1,5-lactones. From the fact that Levvy *et al.*[14] observed inhibition of α-L-arabino*pyrano*sidase (emulsin) by 0.11mM D-glucono-1,5-lactone, whereas we were unable to find any inhibition of α-L-arabino*furano*sidase (*Aspergillus niger* K1), even with 2mM D-glucono-1,5-lactone, it may be concluded that 1,5-lactones inhibit aldo*pyrano*sidases only.

The inhibitory effect of an aldono-1,4-lactone on an aldofuranosidase with simultaneous non-inhibition of the corresponding aldopyranosidase has not thus far been reported. The possibility that other enzyme pairs could be influenced in the same sense by 1,4- and 1,5-lactones should be examined.

EXPERIMENTAL

α-L-Arabinofuranosidase. — The enzyme was isolated from *Aspergillus niger* strain K1 as described by Kaji and Tagawa[8]. The enzyme was induced by addition of an alkaline sugar-beet extract and further ingredients. Fermentation and purification of the enzyme were performed by known procedures[7].

Enzyme assay. — The activity of α-L-arabinofuranosidase was assayed in a preparation containing 0.1 ml of enzyme solution, 0.2 ml of phosphate–citrate buffer according to McIlvaine, pH 4.0, 0.1 ml of L-arabinono-1,4-lactone, and 0.1 ml of *p*-nitrophenyl α-L-arabinofuranoside (10mM). Incubation was performed at 37°. The reaction was stopped by addition of 2.5 ml of 0.5M sodium carbonate solution, and the absorbance of the *p*-nitrophenol liberated was measured at 400 nm. The stock

solution of enzyme had an activity of 40 U/ml; its specific activity was 15 U/mg of protein.

α-L-*Arabinopyranosidase (almond emulsin).* — The preparation from sweet-almond meal was made as described by Conchie *et al.*[15]. The enzyme solution was diluted 1:2.

α-L-*Arabinopyranosidase (barley).* — The enzyme preparation from barley was made by ammonium sulfate fractionation of an aqueous extract, as described by Conchie *et al.*[15]. The solution was diluted 1:2.5.

L-*Arabinono-1,4-lactone.* — The preparation of L-arabinono-1,4-lactone was carried out by electrolytic oxidation of L-arabinose[9] and subsequent lactonization[10]. The lactone was paper chromatographically pure in a system (3:1:3 ethyl acetate–acetic acid–water, upper phase) that separates L-arabinonic acid, L-arabinono-1,4-lactone, and L-arabinono-1,5-lactone[11].

p-*Nitrophenyl* α-L-*arabinofuranoside and* p-*nitrophenyl* α-L-*arabinopyranoside**. — The glycosides were synthesized according to Fielding and Hough[12].

D-*Glucono-1,5-lactone and* p-*nitrophenyl* β-D-*glucopyranoside.* — These were commercial products from Koch–Light Laboratories Ltd., Colnbrook, U.K.

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