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BINDING OF *n*-ALKYL β-D-XYLOPYRANOSIDES AND *n*-ALKYL 1-THIO-β-D-XYLOPYRANOSIDES TO β-D-XYLOSIDASE FROM *Bacillus pumilus* PRL B12

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ABSTRACT

The binding of D-xylose and of a series of *n*-alkyl β-D-xylopyranosides and their 1-thio analogues to β-D-xylosidase from *B. pumilus* PRL B12 has been investigated. The binding constants and thermodynamic equilibrium parameters ΔH^0 and ΔS^0 have been determined. The enzyme does not distinguish between α- and β-D-xylopyranose. Although the enthalpy of binding of D-xylose is very favourable, the overall free-energy is small, due to a large decrease in entropy. Furthermore, all of the evidence available suggests that the aglycon group is bound by unspecific, hydrophobic forces. However, simple correlations between the binding parameters and the relative hydrophobicity of the compounds could not be found. Unexpectedly, no parallelism between binding of *n*-alkyl β-D-xylopyranosides and the corresponding 1-thio derivatives was found.

INTRODUCTION

In a previous paper[1], we reported on the binding of phenyl β-D-xylopyranosides and their 1-thio homologues to β-D-xylosidase (β-D-xyloside xylohydrolase, EC 3.2.1.37) from *Bacillus pumilus* PRL B12. This study indicated that the aglycon group binds by rather unspecific, hydrophobic forces, and that the influence of substituents on the phenyl ring is small and unpredictable. We now report on the binding of *n*-alkyl β-D-xylopyranosides and their 1-thio analogues to the same enzyme. These xylose derivatives are not hydrolysed by the enzyme and behave as competitive inhibitors. Since the aglycon group is very simple and can interact with the enzyme through hydrophobic forces only, it was hoped to gain more insight into the process by which this group is bound. In addition, the contribution of the glycon part of the substrate to the binding process was studied by determining the thermodynamic binding parameters for D-xylose.

EXPERIMENTAL

The following substrates and inhibitors were synthesised by literature proce-

dures: *p*-nitrophenyl β-D-xylopyranoside[15], α-D-xylose[16], alkyl β-D-xylopyranosides[17], alkyl 1-thio-β-D-xylopyranosides[18]. The isolation, purification, and standardisation of the β-D-xylosidase from *B. pumilus* have been described[19,20]. Hydrolysis of *p*-nitrophenyl β-D-xylopyranoside was followed continuously at 400 nm. All experiments were performed in 10mM phosphate buffer (pH 7.15), containing mM EDTA (to prevent spontaneous denaturation of the enzyme). As the enzymic reaction followed formal Michaelis–Menten kinetics, classical methods[3,4] for the calculation of the maximal rate V and the Michaelis–Menten constant K_m (or $K_a = 1/K_m$) could be used. For at least ten concentrations of the substrate (*p*-nitrophenyl β-D-xylopyranoside), the initial velocity (v_i) was measured, and V and K_m were then calculated by the method of Wilkinson[21] on a Wang 2200-S table-computer. Each value of V and K_a is the arithmetical means of 3–5 determinations. All values of V were calculated on the same enzyme-activity basis (1 unit). Throughout the text, all values of binding constants (real or apparent) are expressed as association constants ($1/K_m$).

The inhibition constants K_i were determined by measuring V and the apparent K_a' for *p*-nitrophenyl β-D-xylopyranoside, in the presence of a constant concentration of inhibitor. As all xylopyranosides tested behaved as competitive inhibitors, the inhibition constant K_i could be calculated from the classical formula[3,4] for competitive inhibition: $K_a = K_a'[1 + (I)K_i]$, with K_a' the value of $1/K_m$ with, and K_a the value of $1/K_m$ without, inhibitor. The process was then repeated with a different concentration of inhibitor. The K_i values given are the arithmetical mean of at least three determinations. When D-xylose is used as an inhibitor, K_i itself may be an apparent constant (see Text). The standard enthalpy of binding ΔH^0 was calculated from the K_i values at five different temperatures by the method of least squares; ΔS^0 was then calculated from $\Delta G^0 = \Delta H^0 - T\Delta S^0$.

RESULTS AND DISCUSSION

In aqueous solution, D-xylose occurs as an equilibrium mixture[2] of α (~36%) and β (~63%) pyranoid forms [$K_e \equiv (X_\beta)/(X_\alpha)$]. If β-D-xylosidase can distinguish between these two forms, two different enzyme–xylose complexes will be formed, according to the equilibria,

$$E + X_\alpha \rightleftharpoons EX_\alpha; \quad \text{with} \quad K_\alpha = [EX_\alpha]/[E][X_\alpha], \quad \text{and}$$
$$E + X_\beta \rightleftharpoons EX_\beta; \quad \text{with} \quad K_\beta = [EX_\beta]/[E][X_\beta].$$

If one of the binding constants is small enough compared to the other, one of the complexes may even become undetectable. When D-xylose is used as a competitive inhibitor, the classical equation[3,4] for competitive inhibition becomes

$$v_i = \frac{E_t K_{app} k_{cat} (S)}{1 + K_{app}(S) + K_\alpha(X_\alpha) + K_\beta(X_\beta)}. \qquad (I)$$

As the total concentration of D-xylose is always

$$(X)_t = (X_\alpha) + (X_\beta) = (X_\alpha)(1 + K_e) = (X_\beta)\left(\frac{K_e + 1}{K_e}\right),$$

equation *1* reduces to equation *2*.

$$v = \frac{E_t K_{app} k_{cat} (S)}{1 + K_{app}(S) + (X)_t \left(\frac{K_\alpha + K_\beta K_e}{1 + K_e}\right)} \qquad (2)$$

The experimentally attainable, inhibition constant K_i' (see Experimental) is thus a complex constant, and equals (expressed as association constant)

$$K_i' \equiv \frac{K_\alpha + K_\beta K_e}{1 + K_e} = \frac{(1 + \gamma K_e)}{(1 + K_e)} \cdot K_\alpha, \quad \text{with} \quad \gamma = K_\beta / K_\alpha.$$

Thus, it is necessary to determine to what extent the experimental K_i' is a true measure of the real binding-constants (K_α or K_β).

If the enzyme can distinguish ($\gamma \neq 1$) between the α and β forms, K_i' will depend on the ratio of the concentrations of these forms in the reaction mixture. However, if $\gamma = 1$, this ratio will have no influence on K_i' ($K_i' = K_\alpha = K_\beta$). For example, when the free energy of binding (ΔG^0) for β-D-xylose is 4.18 kJ.mol^{-1} lower than for α-D-xylose ($\gamma = 5.4$), K_i' will be $3.8K_\alpha$ when an equilibrium mixture is used, but $1.44K_\alpha$ when a 9:1 α,β-mixture is used. Such a difference (~2.6 times) can be experimentally detected.

These measurements can only be performed if the mutarotation of D-xylose, under the conditions used for the enzymic reactions, is slow enough compared to the enzymic hydrolysis of the substrate. Therefore, the change in optical rotation of a solution of pure α-D-xylopyranose (25mM) in 10mM phosphate buffer [pH 7.15; mM EDTA; mM *p*-nitrophenyl β-D-xylopyranoside (PNPX)] was followed at 15°. The final rotation (α_∞) was 0.073°. From the linear plot of $\ln(\alpha_t - \alpha_\infty)$, a pseudo-first-order rate coefficient $k = 0.073$ min^{-1} and a half-life time (until equilibrium) $t_h = 9.5$ min were calculated. Thus, if the rate of the enzymic reaction can be measured immediately after the addition of α-D-xylose, the reaction mixture will contain a high percentage of α-D-xylopyranose.

Binding of D-*xylose*

Pure, crystalline α-D-xylose (final concentration, 25mM) was added to reaction mixtures (15°) containing 10mM phosphate buffer (pH 7.15; mM EDTA), various concentrations of PNPX, and a known number of enzyme units. After dissolution of D-xylose (~45 sec), the release of *p*-nitrophenol was monitored at 400 nm. The rate of the reaction remained constant for at least 5 min. This is a first indication that the mutarotation does not influence the enzymic reaction. From the dependence

TABLE I

BINDING CONSTANTS AND EQUILIBRIUM PARAMETERS FOR D-XYLOSE[a]

Temperature (degrees)	K_i (M^{-1})	*Equilibrium parameters*
15	40.0 ± 4.0	
20	31.5 ± 0.8	$\Delta H°$ = − 51 ± 3 kJ.mol^{-1}
25	22.5 ± 1.0	$\Delta S°$ (25°) = − 142 ± 10 J.K^{-1}.mol^{-1}
28	19.0 ± 1.1	$\Delta G°$ (25°) = − 7.7 ± 1 kJ.mol^{-1}

[a]pH 7.15.

TABLE II

ALKYL β-D-XYLOPYRANOSIDES: BINDING CONSTANTS AND EQUILIBRIUM PARAMETERS

	Aglycon group	K_i (M^{-1})					$-\Delta G°$ *(25°)*	$-\Delta H°$	$-S°$ *(25°)*
		10°	*15°*	*20°*	*25°*	*28°*	*(kJ.mol^{-1})*	*(kJ.mol^{-1})*	*(J.K^{-1}.mol^{-1})*
1	Methyl	40	33	26	23	19	7.74	27.9 ±1.8	67 ±6
2	Ethyl	70	58	48	42	36	9.25	25.6 ±1.0	54 ±4
3	Propyl	435	345	285	235	198	13.5	30.1 ±1.1	54 ±4
4	Butyl	1650	1250	964	784	667	16.5	35.2 ±0.8	63 ±3
5	Pentyl	1400	1120	923	779	701	16.5	27.1 ±0.6	36 ±2
6	Hexyl	640	564	498	437	393	15.1	18.9 ±0.8	13 ±3
7	Heptyl	817	720	580	505	468	15.4	22.7 ±1.2	24 ±4
8	Octyl	1380	1095	956	854	713	16.7	24.0 ±2.0	25 ±6
9	Isopropyl	—	—	—	18	—	7.15	—	—
10	Methyl (α)	—	—	—	38	—	9.01	—	—

of the initial velocities on the concentration of PNPX, a maximal rate $V = (7.2 \pm 0.2) \times 10^{-7}$ mol.min^{-1}.u^{-1} and an apparent constant (see Experimental) $K_a' = 400 \pm 22 M^{-1}$ were calculated. Under identical conditions, but without addition of D-xylose, $V = (7.0 \pm 0.2) \times 10^{-7}$ mol.min^{-1}.u^{-1} and $K_a = 800 \pm 80 M^{-1}$. Thus, the apparent (experimental) inhibition constant $(K_i' = (K_a - K_a')/[K_a'(I)]$ is $40 \pm 5 M^{-1}$.

The measurements were repeated under the same conditions, except that D-xylose was added as an equilibrium mixture. The same value of $K_i'(40 \pm 4 M^{-1})$ was calculated. The above findings strongly indicate that the enzyme does not distinguish between α- and β-D-xylose, and that $K_\alpha = K_\beta = K_i = 40 M^{-1}$ is the true association constant of the enzyme–xylose complex. That the axial position of the hydroxyl (or other) group on C-1 of the D-xylopyranose moiety does not prevent binding is further evidenced by the fact that methyl α-D-xylopyranoside binds even better than the corresponding β anomer (Table II). However, the axial position of the aglycon group does prevent the catalytic effect of the enzyme, since neither alkyl nor aryl α-D-xylopyranosides are hydrolysed.

Using an equilibrium mixture of D-xylose, the binding constant K_i was determined at four temperatures. The mean values from three determinations, the estimated standard deviations, and the thermodynamic equilibrium parameters, as calculated by weighted regression analysis, are given in Table I. Although the estimated errors are large, it is nevertheless clear that the favourable enthalpy of binding of the xylose molecule is partly neutralised by the decrease in entropy resulting from the formation of the enzyme–xylose complex. According to Jencks[5], the loss of entropy required to bring two molecules to the proper position to form a product amounts to 142 $J.K^{-1}.mol^{-1}$ and thus agrees with ΔS^0 for D-xylose. It may also be noted that the free energy of binding for D-galactose, binding to β-D-galactosidase from *E. coli*[6], is -9.4 $kJ.mol^{-1}$.

Binding of n-*alkyl* β-D-*xylopyranosides*

With PNPX as substrate, eight *n*-alkyl β-D-xylopyranosides were used as fully competitive inhibitors at pH 7.5 and at five temperatures. The inhibition constant K_i (association) is thus the equilibrium constant for the process $E + I \rightleftharpoons EI$. As the plots of log K_i *versus* 1/T were always linear up to 28°, the thermodynamic equilibrium parameters ΔH^0, ΔS^0, and ΔG^0 could be calculated (Table II).

At 25°, the value of K_i ($\sim\Delta G^0$) for methyl β-D-xylopyranoside equals that for D-xylose. Replacement of the H-atom at HO-1 by a methyl group thus had no effect on the overall free-energy of binding. However, if ΔH^0 and ΔS^0 are compared, it follows that, whereas the enthalpy becomes less negative ($\Delta\Delta H^0 \sim +23$ $kJ.mol^{-1}$), the entropy becomes more favourable ($\Delta\Delta S^0 \sim 75$ $J.K^{-1}.mol^{-1}$). Both effects cancel each other, so that $\Delta\Delta G^0 \sim 0$.

These findings suggest a hydrophobic effect of the methyl group. When this group is transferred from the aqueous phase to a more hydrophobic, enzyme phase, at least part of the highly structured water molecules around the methyl group return to the less-structured bulk-water phase. This process is characterised[7] by positive values for both $\Delta H(CH_3)$ and $\Delta S(CH_3)$. Consequently, the contribution of the methyl group will make the overall $\Delta H^0(K_i)$ and $\Delta S^0(K_i)$ of binding less negative.

However, if the effect of the methyl group were purely hydrophobic, methyl β-D-xylopyranoside should bind more strongly [$\Delta\Delta G^0(K_i)$ at least -3.4 $kJ.mol^{-1}$], since $\Delta\Delta G^0$ (hydrophobic) for a CH_2 group[7] is ~ 3.4–4.2 $kJ.mol^{-1}$. For the transfer of alkyl glycosides from water to an inert organic phase, values of ΔH^0 and ΔS^0 are not available for comparison. For the transfer of a methane molecule from water to an inert phase[8], $\Delta G^0 = -11.7$ $kJ.mol^{-1}$, $\Delta H^0 = +11.3$ $kJ.mol^{-1}$, and $\Delta S^0 = +75$ $J.K^{-1}.mol^{-1}$. Introduction of the methyl group into D-xylose yielded $\Delta\Delta H^0(K_i) = +23$ $kJ.mol^{-1}$ and $\Delta\Delta S^0(K_i) = 75$ $J.K^{-1}.mol^{-1}$. Whereas the $\Delta\Delta S^0$ values are comparable, $\Delta\Delta H^0 = +23$ $kJ.mol^{-1}$ is too large to be accounted for by hydrophobic effects. Replacement of the H-atom of HO-1 of xylose by a methyl group seems to cause some sort of "hindrance", resulting in an extra, unfavourable (positive) enthalpy term. It is noteworthy that isopropyl β-D-xylopyranoside, although its relative

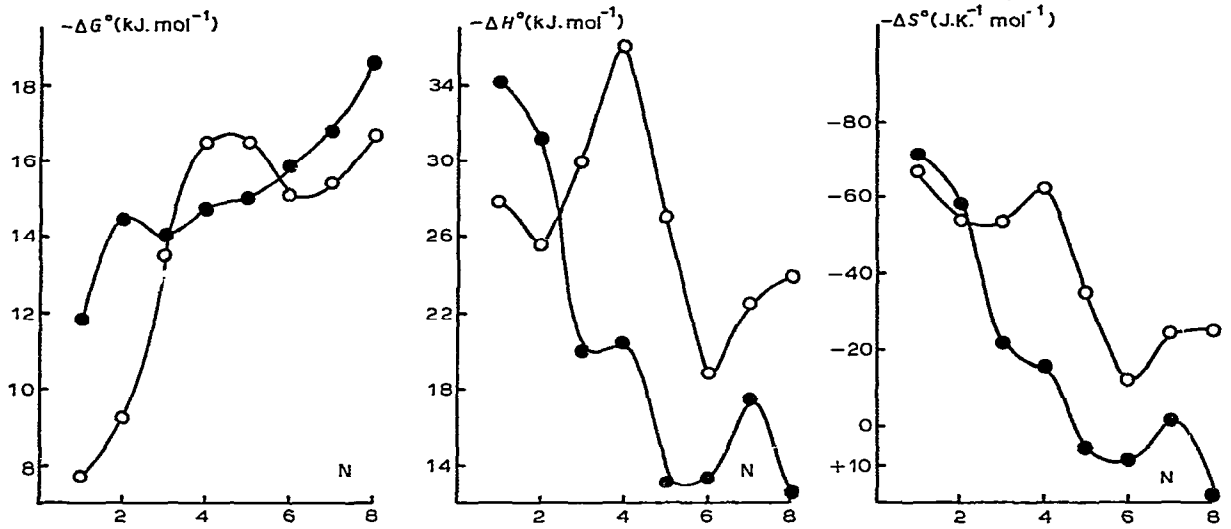

Fig. 1. Thermodynamic parameters $\Delta G°$, $\Delta H°$, and $\Delta S°$ *versus* the number of carbon atoms (N): ○, *n*-alkyl β-D-xylopyranosides; ●, *n*-alkyl 1-thio-β-D-xylopyranosides.

intrinsic hydrophobicity is higher, has a lower binding-constant (or ΔG^0) than the methyl derivative. The exact nature of this "hindrance" is unknown, but it is probably not steric (see below). A hypothetical explanation would be that the positioning of the methyl group on the enzyme surface would require the breaking up of a layer of highly structured water molecules around a hydrophobic group (or in a hydrophobic cleft) of the protein. This would result in an extra, positive enthalpy term.

The data in Table II and Fig. 1 illustrate the complex influence of elongation of the aglycon chain upon the binding parameters. The same pattern (*cf.* K_i) is found at each temperature, and thus the complexity is not due to experimental errors. Log K_i (or $\Delta G^0(K_i)$) is not a linear function of the number of carbon atoms in the chain. Consequently, linear free-energy relationships between $\Delta G^0(K_i)$ and the classical hydrophobicity parameters [π, log P (alcohols), *etc.*] will not be demonstrable.

Up to the butyl derivative, the decrease in $\Delta G^0(K_i)$, although not linearly related to the number of carbon atoms, is rather regular. In model systems, the decrease in free energy of transfer ($\Delta\Delta G^0$) is −3.4 to −4.2 kJ.mol^{-1} per CH_2 group[7] (up to at least 8 carbon atoms). However, $\Delta\Delta G^0(K_i)$ between the ethyl and methyl xyloside is ∼−1.5 kJ.mol^{-1} and thus too small. For the transfer of alkanes from water to a hydrocarbon phase[9], $\Delta\Delta H^0(CH_2)$ is ∼−3.4 kJ.mol^{-1}; for transfer to an organic micelle[9], $\Delta\Delta H^0(CH_2)$ is ∼−4.1 kJ.mol^{-1}. In each case, the enthalpy of transfer becomes more negative (or less positive) with increasing length of the chain. However, $\Delta H^0(K_i)$ for the ethyl derivative is less negative than $\Delta H^0(K_i)$ for the methyl xyloside. Thus, the second carbon atom of the aglycon chain also causes an unfavourable enthalpy effect. The effect of the second carbon atom on the entropy of binding is favourable [$\Delta\Delta S^0(K_i) \sim +12$ J.K^{-1}.mol^{-1}]. For the transfer of alkanes[9]

from water to the hydrocarbon phase, $\Delta\Delta S^0$ is +4.1 J.K^{-1}.mol^{-1} per CH_2 group. However, the transfer of propanol[9] from water to the pure alcohol is characterised by a ΔS^0 value that is 11.3 J.K^{-1}.mol^{-1} more positive than that for the transfer of ethanol. Thus, $\Delta\Delta S^0(CH_2)$ strongly depends on the nature of the hydrophilic group attached to the alkane chain. It may therefore be purely fortuitous that $\Delta\Delta S^0(K_i)$ is of the same order of magnitude ($\sim +12$ J.K^{-1}.mol^{-1}). However, the positive sign of $\Delta\Delta S^0$ (ethyl/methyl) and the order of magnitude are in accordance with a hydrophobic binding of the aglycon group. Moreover, both the positive $\Delta\Delta H^0(K_i)$ and positive $\Delta\Delta S^0(K_i)$ suggest that the binding of the first two carbon atoms of the aglycon chain is rather loose and unspecific, occurring mainly through the return of water molecules to the bulk-water phase. Therefore, the above-mentioned "hindrance" is believed not to be of steric nature.

For the propyl and butyl β-D-xylosides, $\Delta\Delta G^0(K_i)$ per CH_2 is −4.25 and −3.0 kJ.mol^{-1}, respectively, and $\Delta\Delta H^0 \sim -5$ kJ.mol^{-1}. These values are in accordance with the expected, normal hydrophobic contribution of a CH_2 group. No further "hindrance" is observed.

For the four higher members of the series, $\Delta\Delta G^0(CH_2)$ is small and very irregular. When $\Delta H^0(K_i)$ and $\Delta S^0(K_i)$ values are compared, it becomes clear that elongation of the chain has a significant effect on both $\Delta H^0(K_i)$ and $\Delta S^0(K_i)$, but that these effects partially cancel each other. The result is a small and irregular overall effect on $\Delta G^0(K_i)$. For *n*-alkyl β-D-galactopyranosides[6] in water–octanol, it was found that the relative hydrophobicity of these compounds increased linearly with increasing chain-length [$\Delta\Delta G^0(CH_2) \sim 3.35$ kJ.mol^{-1}][10] up to at least eight carbon atoms. Consequently, the complex influence of the length of the chain on $\Delta G^0(K_i)$ is not due to some peculiar behaviour of long alkane chains in aqueous solutions, but to the fact that the chain binds to a system far more complex than the water–octanol model-system.

At present, further analysis of the binding parameters seems impossible, because too many unknown factors can be involved. However, the experimental observation that the complex pattern starts when a fifth carbon atom is added to the chain is perhaps noteworthy. It seems logical to assume that xylobiose is the natural substrate of the enzyme[11]. In this substrate, the aglycon group is in fact the second xylopyranose ring, and the active site of the enzyme must be built up in such a way that it can accept this ring structure. If, during the process of binding, the carbon atoms of the alkyl chain try to occupy the positions normally occupied by the carbon atoms of the second xylopyranose ring, the carbon atoms of a chain up to butyl can do this in a simple way, since short alkyl chains in glycosides do not coil[6] or form dimers[12] in aqueous solution. Fig. 1 shows that, up to the butyl derivative, the effect of lengthening the chain is indeed rather regular. However, major conformational changes will be necessary to bring the fifth and sixth carbon atoms into the required position. Fig. 1 shows that ΔG^0, and especially ΔH^0, increase (become less favourable) when the fifth and sixth carbon atoms are added. When still more carbon atoms (which are no part of the ring structure) are added, both ΔG^0 and ΔH^0 decrease again.

Another possibility would be that the binding site for the alkyl chain is built up from two binding areas, as suggested by Rodrigues De Miranda[13] for albumin. However, no evidence in favour of this possibility is available.

n-Alkyl 1-thio-β-D-xylopyranosides

In Table III, the K_i values and thermodynamic binding parameters for a series of alkyl 1-thio-β-D-xylopyranosides have been collected. From a previous study[6], it is known that the relative intrinsic hydrophobicity of alkyl 1-thiogalactopyranosides is higher than that of their oxygen analogues. It was further demonstrated[6] that *n*-alkyl β-D-galactopyranosides and their 1-thio analogues bind to β-D-galactosidase from *E. coli* in essentially the same way, the better binding of the thio derivatives being caused merely by their higher intrinsic hydrophobicity. Inspection of Fig. 1 reveals that, for alkyl β-D-xylopyranosides, there is no parallelism between the thio and oxygen series, and no constant difference between $\Delta G^0(K_i)$ values for corresponding thio and oxygen derivatives. Some oxygen derivatives bind even better than their thio analogues.

With the exception of the ethyl derivative, the influence of the chain length is more regular. Highly significant correlations with the Hansch[14] π-parameter and the free energy of transfer for the corresponding alkyl β-D-galactopyranosides in water–octanol[6] can be calculated. However, these equations are not linear free-energy relationships:

(*1*) $\Delta G^0(K_i) = -11.5 - 1.62(\pi)$, with standard error of the estimate $s_{y.x} = 0.69$, correlation coefficient $r = 0.95$, and confidence limits CL >99.5;

(*2*) $\Delta G^0(K_i) = -15.3 + 0.22\,\Delta G^0$ (octanol), with $s_{y.x} = 0.71$, $r = 0.95$, and CL >99.5.

The contribution of the hydrophobic forces to the binding of thioxylosides is much

TABLE III

ALKYL 1-THIO-β-D-XYLOPYRANOSIDES: BINDING CONSTANTS AND EQUILIBRIUM PARAMETERS

	Aglycon group	K_i (M^{-1})					$-\Delta G°$ (25°) ($kJ.mol^{-1}$)	$-\Delta H°$ ($kJ.mol^{-1}$)	$\Delta S°$ (25°) ($J.K^{-1}.mol^{-1}$)
		10°	15°	20°	25°	28°			
1	Methyl	242	187	147	116	102	11.8	34.1 ±0.4	−71 ±1
2	Ethyl	669	524	428	336	303	14.4	31.3 ±0.6	−58 ±2
3	Propyl	439	382	318	284	266	14.0	20.1 ±0.9	−21 ±3
4	Butyl	623	522	442	391	376	14.8	20.3 ±1.4	−17 ±4
5	Pentyl	523	504	452	421	372	15.0	13.0 ±1.8	+ 6 ±3
6	Hexyl	774	708	629	601	541	15.9	13.4 ±1.1	+ 8 ±3
7	Heptyl	1409	1241	1063	989	891	16.8	17.6 ±1.0	− 2 ±3
8	Octyl	2367	2172	1979	1853	1699	18.9	12.6 ±0.8	+21 ±3
9	Isopropyl				202		13.1		

smaller than could be expected from their normal octanol-hydrophobicity. $\Delta\Delta G^0(K_i)$ between the octyl and methyl 1-thio derivative is -6.9 kJ.mol^{-1}, whereas $\Delta\Delta G^0$ in water–octanol is -25 kJ.mol^{-1}, as measured for the corresponding 1-thiogalactopyranosides[6]. For the latter derivatives, binding to β-D-galactosidase from *E. coli*, $\Delta\Delta G^0$ (octyl/methyl)[6] is -11 kJ.mol^{-1}. The most simple explanation is that the alkyl chain is only partially desolvated, so that the decrease in free energy per CH_2 group is only a fraction of the value for the model octanol–water system. Because of the rather regular character (Fig. 1) of the decrease in $\Delta G^0(K_i)$, steric factors and/or conformational changes are less probable.

The effect of the chain length on $\Delta H^0(K_i)$ and $\Delta S^0(K_i)$ is more regular in the thio than in the oxygen series (Fig. 1). Comparison of the two series shows that, for the lower members, the patterns are different, whereas they are somewhat analogous for the higher members. Again the parameters ΔH^0 and ΔS^0 partially compensate each other.

The most important conclusion is that *n*-alkyl 1-thio-β-D-xylopyranosides (and especially the lower members of the series) bind to β-D-xylosidase in a way that differs significantly from that of their oxygen analogues. By replacing the glycosidic oxygen atom by a sulfur atom, bond angles and bond lengths are altered. The position of the carbon atoms next to the glycosidic bond will thus be different in the two series. These differences are small, but nevertheless they are sufficient to alter significantly the interactions of the alkyl chain with the protein. It is noteworthy that isopropyl 1-thio-β-D-xylopyranoside, in contrast to its oxygen analogue, has a binding constant in agreement with its relative hydrophobicity, and that the "hindrance" is not observable in the thio series. Although further explanation of these observations cannot be given, all experimental data indicate that it is the structure of the micro-region around the glycosidic bond (containing the catalytically active groups) which is responsible for the unexpected and subtle effects of the alkyl chain on the binding. Further investigations, with xylosides having branched chain and cyclic aglycon groups, are now in progress.

CONCLUSIONS

It may be concluded that the aglycon moiety of *n*-alkyl β-D-xylopyranosides binds to the active site of β-D-xylosidase by hydrophobic forces. However, the influence of the chain length on the binding is far more complex than can be accounted for by physical-organic hydrophobicity parameters. Furthermore, replacement of the exocyclic oxygen atom of the inhibitor molecule by a sulfur atom seems sufficient to induce a different way of binding of the inhibitor. These observations clearly indicate that even small changes in the aglycon group may have such large and unexpected effects on the binding parameters as to make further analysis of these effects very difficult. The reason is, of course, the very specific structure of the active site of the enzyme.

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REFERENCES

1 H. KERSTERS-HILDERSON, E. VAN DOORSLAER, AND C. K. DE BRUYNE, *Carbohydr. Res.*, 65 (1978) 219–227.
2 W. PIGMAN AND E. F. L. ANET, in W. PIGMAN AND D. HORTON (Eds.), *The Carbohydrates*, Vol. IA, Academic Press, New York, 2nd edition, 1972, p. 170.
3 M. DIXON AND E. C. WEBB, *Enzymes*, Longmans Green, London, 2nd edition, 1964, pp. 120–150.
4 K. J. LAIDLER, *The Chemical Kinetics of Enzyme Action*, Clarendon Press, Oxford, 1958, pp. 117-143.
5 W. P. JENCKS, *Adv. Enzymol.*, 43 (1975) 275–278.
6 C. K. DE BRUYNE AND M. YDE, *Carbohydr. Res.*, 56 (1977) 153–164.
7 C. TANFORD, *The Hydrophobic Effect*, Wiley, New York, 1973, pp. 16–21.
8 W. P. JENCKS, *Catalysis in Chemistry and Biochemistry*, McGraw-Hill, pp. 421–425.
9 C. TANFORD, *The Hydrophobic Effect*, Wiley, New York, 1973, pp. 18–20 and p. 38.
10 J. T. EDSALL AND H. A. MCKENZIE, *Adv. Biophys.*, 10 (1978) 196.
11 H. KERSTERS-HILDERSON, E. VAN DOORSLAER, C. K. DE BRUYNE, AND KEI YAMANAKA, *Anal. Biochem.*, 80 (1977) 41–50.
12 E. O. ARVIDSSON, F. A. GREEN, AND S. LAURELL, *J. Biol. Chem.*, 246 (1971) 5373–5379.
13 J. F. RODRIGUES DE MIRANDA, T. D. EIKELBOOM, AND G. A. J. VAN OS, *Mol. Pharmacol.*, 12 (1976) 454–462.
14 A. C. HANSCH AND E. W. DEUTSCH, *Biochim. Biophys. Acta*, 126 (1966) 117–128.
15 F. G. LOONTIENS AND C. K. DE BRUYNE, *Naturwissenschaften*, 51 (1964) 359.
16 C. S. HUDSON AND E. YANOVSKY, *J. Am. Chem. Soc.*, 39 (1917) 1013–1038.
17 C. K. DE BRUYNE AND F. G. LOONTIENS, *Nature (London)*, 209 (1966) 396–397.
18 M. CLAEYSSENS AND C. K. DE BRUYNE, *Carbohydr. Res.*, 22 (1972) 460–463.
19 H. KERSTERS-HILDERSON, F. G. LOONTIENS, M. CLAEYSSENS, AND C. K. DE BRUYNE, *Eur. J. Biochem.*, 7 (1969) 434–441.
20 M. CLAEYSSENS, H. KERSTERS-HILDERSON, J. P. VAN WAUWE, AND C. K. DE BRUYNE, *FEBS Lett.*, 11 (1970) 336–338.
21 G. N. WILKINSON, *Biochem. J.*, 80 (1961) 324–332.