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BINDING OF SUBSTITUTED PHENYL 1-THIO-β-D-GALACTOPYRANOSIDES TO β-D-GALACTOSIDASE FROM *E. coli*

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ABSTRACT

The binding of a series of substituted phenyl 1-thio-β-D-galactopyranosides to the active site of β-D-galactosidase from *E. coli* has been investigated. The inhibition constants and the standard free energy of transfer $\Delta G°(K_i)$ were compared with the partition coefficients and $\Delta G°$ (1-octanol) values of these inhibitor molecules in a model 1-octanol–water system. All of the evidence available suggests that the aglycon group of the inhibitor is bound to the active site by unspecific, hydrophobic forces. Correlations between $\Delta G°$ (K_i) and $\Delta G°$ (1-octanol) could not be found for the whole series, but only for simpler sub-series. Probably, the octanol system is too incomplete a model for the hydrophobic region in the active site of β-D-galactosidase.

INTRODUCTION

In a previous paper[1], we reported on the binding of alkyl 1-thio-β-D-galactopyranosides to the β-D-galactosidase (EC 3.2.1.23) from *E. coli*. This study indicated that the aglycon group of these inhibitors binds to a sterically limited, hydrophobic micro-region on the active site. In general, only unspecific hydrophobic forces, rather than direct interactions with amino-acid side-chains, were involved. The present paper deals with the binding of substituted phenyl 1-thio-β-D-galactopyranosides to the same enzyme, and with the influence of substituent groups on the binding process.

RESULTS AND DISCUSSION

For a number of substituted phenyl 1-thio-β-D-galactopyranosides, the partition coefficient (P) between aqueous phosphate buffer (0.1M; pH 7.5; mM $MgCl_2$) and 1-octanol was determined at 25°. From these values, the standard free energy of transfer ($\Delta G°$) and the hydrophobicity coefficient π ($\pi = \log P_X - \log P_H$, where P_X refers to the substituted, and P_H to the unsubstituted phenyl galactopyranoside) were calculated. For comparison, some π values from Hansch[2], and P values for alkyl 1-thio-β-D-galactopyranosides[1] are also given (Table I).

Although too few data are available to allow a study of the effect of a substituent

TABLE I

PARTITION COEFFICIENTS OF ALKYL AND ARYL 1-THIO-β-D-GALACTOPYRANOSIDES IN WATER–1-OCTANOL (25°)

Compound	*Aglycon group*	P	*log* P	ΔG°(25°) (*kcal.mol*$^{-1}$)	π^a	π^b	π^c	ΣS^E
1	Phenyl	0.27	−0.56	+0.763	0	0	0	0.857
2	*p*-Chlorophenyl	2.33	+0.37	−0.504	0.93	0.70	0.93	2.863
3	*p*-Bromophenyl	3.55	+0.55	−0.749	1.11	1.02	1.13	3.083
4	*p*-Fluorophenyl	0.53	−0.28	+0.382	0.28	0.15	0.31	1.532
5	*p*-Methylphenyl	1.01	+0.01	−0.007	0.57	0.52	0.48	1.945
6	*p*-Ethylphenyl	2.96	+0.47	−0.641	1.04	0.97	—	—
7	*p*-Methoxyphenyl	0.30	−0.52	+0.70	−0.04	−0.04	−0.12	3.148
8	*p*-Nitrophenyl	0.82	−0.09	+0.120	0.48	0.24	0.50	—
9	*p*-Acetylphenyl	0.19	−0.73	+0.995	−0.16	−0.37	−0.11	3.193
	n-Propyl	0.066	−1.18	+1.40				
	n-Hexyl	7.03	+0.86	−1.19				
	n-Butyl	0.29	−0.54	+0.54				
	n-Pentyl	2.478	+0.39	−0.33				

[a]This work. [b]For phenoxyacetic acids, from Ref. 2. [c]For phenols, from Ref. 2.

on the relative hydrophobicity of these phenyl 1-thio-β-D-galactopyranosides, some relations are noteworthy.

(*1*) The $\Delta G°$ values of the unsubstituted and of the alkyl-substituted phenyl 1-thiogalactosides are linearly related to the cavity surface area $(Å)^2$, calculated by Hermann[3] for the respective benzene derivatives (benzene, 240.7; toluene, 273.9; ethylbenzene, 302.3) according to the regression function, $\Delta G° = 6.278 - 0.023(Å)^2$, with standard error of the estimate 0.01, standard error of the slope $s_b = 0.0003$, and correlation coefficient $r = 0.9999$. This linear relationship indicates that, for phenyl galactosides having simple substituent groups, the relative hydrophobicity increases with increasing size of the aglycon group. The major driving force for the transfer of the aglycon group to the octanol phase would then be the increasing number of water molecules returning from the lower entropy state around the solute molecule to the less-organized bulk-water.

(*2*) The $\Delta G°$ values of the unsubstituted and halogen-substituted phenyl 1-thiogalactosides are linearly related to the Van Der Waals[4] radii (R) of the substituents according to the equation:

$$\Delta G° = 3.137 - 2.008R, \text{ with } s_{y/x} = 0.051, s_b = 0.083, \text{ and } r = 0.998.$$

However, for these substituents, a dependence on size alone would be too simple, as the electronic effects of the substituents can also be expected to influence the relative hydrophobicity. This is indeed the case, as $\Delta G°$ for this sub-series is linearly related to the Hammett substituent constant[5] σ, according to the equation:

$$\Delta G° = 0.766 - 6.07\sigma, \text{ with } s_{y/x} = 0.11, s_b = 0.54, \text{ and } r = 0.992.$$

The electronic influence seems to be caused by the relative polarizability ΣS^E. Using the ΣS^E values obtained by Cammarata and Rogers[6], the following equation is calculated for the halogen subseries:

$$\Delta G^\circ = 1.374 - 0.672\ \Sigma S^E, \text{ with } s_{y/x} = 0.06,\ s_b = 0.03, \text{ and } r = 0.998.$$

(*3*) The ΔG° values for the other derivatives (nitro, methoxyl, and acetyl) could not be fitted to the above equations, and we were unable to correlate them with known substituent parameters.

From the above results, it may be concluded that, even in the simple 1-octanol–water system, the relative hydrophobicity of aryl 1-thio-β-D-galactopyranosides can be correlated with known substituent parameters only when simple sub-series (*e.g.*, alkyl or halogen substituted) are considered. Within these sub-series, the mode of interaction between solute and solvent molecules remains essentially the same, and the relative hydrophobicity changes in a regular manner. However, if a substituent is introduced which drastically changes the mode of interaction, the regularity will be lost. With nitro, methoxyl, and acetyl groups, besides the induction forces due to the polarization of the solvent by the solute, hydrogen bonding between the substituent and the water molecules will produce strong solvent–solute interactions. Consequently, it may be expected that the effect of such substituent groups on the transfer of these phenyl 1-thiogalactosides to a protein molecule will be still more complex.

Binding of substituted phenyl 1-thio-β-D-galactopyranosides to β-D-galactosidase

With *o*-nitrophenyl β-D-galactopyranoside as substrate, the substituted phenyl

TABLE II

INHIBITION CONSTANT K_I OF ALKYL AND ARYL 1-THIO-β-D-GALACTOPYRANOSIDES (pH 7.5)

Compound	*Aglycon group*	$K_I(20°)$ (M^{-1})	$K_I(30°)$ (M^{-1})	$\Delta G°(20°)$ (*kcal. mol*$^{-1}$)	*log* $K_{I,2}$	*log* $10^3 M_{50}$	*log 1/*P	*log 1/*c
1	Phenyl	8,670	6,770	—5.28	1.90	0.770	3.32	4.80
2	*p*-Chlorophenyl	12,000	7,990	—5.47	2.43	0.643	4.07	5.09
3	*p*-Bromophenyl	12,550	9,930	—5.50	2.61	0.617	4.29	5.12
4	*p*-Fluorophenyl	9,430	7,720	—5.33	2.02	0.739	3.70	—
5	*p*-Methylphenyl	4,840	3,700	—4.94				
6	*p*-Ethylphenyl	5,230	4,140	—4.99				
7	*p*-Methoxyphenyl	5,370	3,690	—5.00				
8	*p*-Nitrophenyl	7,830	5,720	—5.22				
9	*p*-Acetylphenyl	3,220	2,610	—4.70				
10	Methyl	690	540	—3.81				
11	*n*-Propyl	10,990	9,320	—5.41				
12	*n*-Pentyl	18,400	17,600	—5.72				
13	*n*-Heptyl	37,200	33,100	—6.13				
14	Benzyl	380,200	234,000	—7.48				
15	2-Phenylethyl	1,314,000	811,000	—8.20				

1-thio-β-D-galactopyranosides were used as fully competitive inhibitors of the enzymic reaction at pH 7.5 and various temperatures. The inhibition constant K_i (calculated as an association constant) is thus the equilibrium constant for the process E + I → EI, *i.e.*, the transfer of the free inhibitor molecule from the aqueous phase to the active site of the enzyme, and $\Delta G°$ (K_i) is a measure of the standard free energy of transfer to the enzyme phase. Table II shows the values of K_i (20° and 30°) and $\Delta G°$ (K_i) at pH 7.5. For comparison, some values for alkyl 1-thio-β-D-galactopyranosides are included.

The transfer of the 1-thio-β-D-galactopyranose moiety of the substrates to the active site of the enzyme is an exergonic process, for which a standard-free-energy change ($\Delta G°$ at 25°) of −2.86 kcal.mole^{-1} was calculated[1]. From Table II, it therefore follows that an aryl aglycon group in an equatorial position does contribute significantly to the binding of the whole substrate molecule.

However, several facts indicate that, in general, the aryl 1-thio-β-D-galactopyranosides bind less strongly than would be expected from their octanol-hydrophobicity. The increase in $\Delta G°$ (1-octanol) from the *p*-acetylphenyl (+0.995) to the *p*-bromophenyl derivative (−0.749) is ∼1.74 kcal.mol^{-1}, whereas $\Delta\Delta G°$ (K_i) for the same compounds is 0.80 kcal.mol^{-1}. The octanol-hydrophobicity of the aryl 1-thio-β-D-galactopyranosides lies somewhat between the hydrophobicity of the *n*-hexyl and *n*-propyl derivatives, yet the K_i values of most of the aryl 1-thio galactosides are lower than that for *n*-propyl 1-thio-β-D-galactopyranoside. For the transfer of the 1-thio-β-D-galactopyranose moiety of the substrates to the active site, $\Delta G°$ (transfer) was calculated[1] to be ∼ −2.86 kcal.mol^{-1} (25°). Since $\Delta G°$ (K_i) for phenyl 1-thio-β-D-galactopyranoside is −5.34 kcal.mol^{-1} [Table III; from K_i (25°) = 8.340 M^{-1}], this leaves −2.48 kcal.mol^{-1} for the transfer of the phenyl ring, if the contributions are additive. However, this is far less than the value of

TABLE III

VALUES OF $\Delta H°$ AND $\Delta S°$ FOR BINDING OF ALKYL AND ARYL 1-THIO-β-D-GALACTOPYRANOSIDES[a]

Compound	*Aglycon group*	K_i (M^{-1})			$\Delta H°$	$\Delta S°(20°)$
		15°	*25°*	*35°*	(*kcal.mol*$^{-1}$)	(*cal.degree*$^{-1}$.*mol*$^{-1}$)
1	Phenyl	9,800	8,340	5,240	−5.3	0
2	*p*-Chlorophenyl	12,810	10,760	7,330	−5.4	−1
5	*p*-Methylphenyl	5,070	4,260	2,810	−5.1	0
8	*p*-Nitrophenyl	8,530	7,320	5,460	−4.3	−3
9	*p*-Acetylphenyl	3,740	2,880	2,160	−4.6	0
10	Methyl				−3.6	0
11	*n*-Propyl				−3.7	+5.7
12	*n*-Pentyl				−3.7	+7.0
13	*n*-Heptyl				−2.6	+12.0
14	Benzyl				−8.0	−2.0
15	2-Phenylethyl				−11.0	−8.0

[a]Statistical standard deviation on $\Delta H°$, ∼ ±0.6 kcal.mol^{-1}; on $\Delta S°$, ±2 cal.degree^{-1}.mol^{-1}.

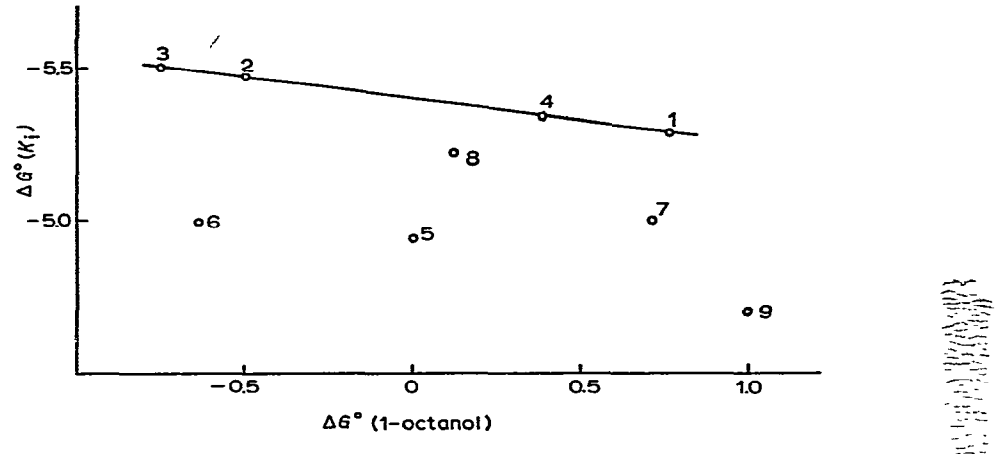

Fig. 1. Plot of $\Delta G°$ (K_i) *versus* $\Delta G°$ (1-octanol); $\Delta G°$, kcal.mol^{-1}. Numbers refer to Table 1.

-4.65 kcal.mol^{-1} calculated for the transfer of a phenyl ring from water to benzene[7]. These findings indicate that, if the binding of the aglycon groups occurs through hydrophobic forces, the phenyl ring is only partially transferred out of the water phase to the active site of the enzyme. This is in accordance with our earlier suggestion[1] that the transfer of the aglycon occurs to a sterically limited, hydrophobic micro-region on the enzyme surface, and that this micro-region can accept only the first three CH_2 (or CH_3) groups of an unbranched alkyl chain. In a similar way, this region would then accept only three atoms (one side) of the phenyl ring.

As illustrated in Fig. 1, and in contrast to what was found for a series of alkyl 1-thio-β-D-galactopyranosides, a simple relation between $\Delta G°$ (K_i) and $\Delta G°$ (1-octanol) or π parameters cannot be demonstrated. All attempts to correlate $\Delta G°$ (K_i) with known substituent parameters were unsuccessful. However, the absence of a simple relation between $\Delta G°$ (K_i) and $\Delta G°$ (1-octanol) does not prove the absence of hydrophobic interaction, as the octanol system is too imperfect a model for the enzymic system to be valid in all circumstances. Since, in the octanol system, the substrate molecule leaves the water phase to become completely surrounded by octanol molecules, a simple relation between log K_i and π-parameters could be expected when the aglycon part of the substrate is completely buried in a hydrophobic pocket ("oil-drop" active site). If a large part of the aglycon group, unlike in the octanol system, remains in contact with the bulk-water phase, a simple relation is still possible when the substituent has no effect on the extent to which the aglycon group remains in contact with the water phase, or on the magnitude of the interaction forces. If, however, the substituent itself alters the extent of the remaining contact, *e.g.*, through aspecific interactions of polar substituents with polar groups on the enzyme surface, steric hindrance, *etc.*, the analogy with the octanol system is invalid. In such cases, the proportionality between the substituent effect in the octanol system and the effect in the enzyme system no longer exists, and linear free energy relationships (LFER) will not be found, even when hydrophobic forces are still involved.

Such complex behaviour can be expected with substituted-phenyl aglycon groups, since the properties (electronic, steric, *etc.*) of the substituents differ widely. It is less likely to occur with simple alkyl aglycon groups, which would explain why a simple relation between $\Delta G°$ (K_i) and $\Delta G°$ (1-octanol) was found for alkyl 1-thio-β-D-galactopyranosides, but not for the corresponding aryl derivatives.

If one considers the more simple sub-series consisting of the unsubstituted and halogen-substituted phenyl 1-thio-β-D-galactopyranosides (Fig. 1), a perfect LFER between $\Delta G°$ (K_i) and $\Delta G°$ (1-octanol) can be calculated:

$\Delta G°$ (K_i) = −5.39 + 0.148 $\Delta G°$ (1-octanol), with $s_{y/x}$ = 0.004, s_b = 0.003, and r = 0.999.

Consequently $\Delta G°$ (K_i) for this sub-series will be linearly related to the Van Der Waals radii (R), the polarizability (ΣS^E), and σ, as follows:

$\Delta G°$ (K_i) = −4.835 − 0.357R, with $s_{y/x}$ = 0.02, and r = 0.98;
$\Delta G°$ (K_i) = 5.187 − 0.10ΣS^E, with $s_{y/x}$ = 0.01, and r = 0.997;
$\Delta G°$ (K_i) = −5.277 − 0.90σ, with $s_{y/x}$ = 0.01, and r = 0.995.

The above equations indicate that the effect of these substituents on $\Delta G°$ (K_i) is proportional to their effect on $\Delta G°$ (1-octanol), and they suggest that the binding of the aglycon group occurs merely through hydrophobic forces. The low value of the slope (0.148), and the small influence of the substituent on $\Delta G°$ (K_i), as compared to its influence on $\Delta G°$ (1-octanol), show that the aglycon group is only partially buried in a hydrophobic micro-region, and in such a way that the halogen atoms remain in contact with the water phase. The hydrophobic "bonding" is thus very unspecific and must occur through the phenyl ring-system itself. Further evidence for the above conclusion follows from the finding that the binding of these halogen-substituted phenyl 1-thio-β-D-galactopyranosides to the active site of β-D-galactosidase can be correlated with the binding of analogously substituted phenyl glycosides (or other substituted phenyl derivatives) to totally different proteins. For that purpose, we compared our values of K_i (derivatives **1** to **4** in Table II) with (*a*) the inhibition constant[8] ($K_{i,2}$) for the same thiogalactosides binding on the active site of β-D-glucosidase from *Stachybotrys atra*, (*b*) the M_{50} values[9] of the corresponding phenyl β-D-glucopyranosides binding to Concanavalin A, (*c*) the reciprocal of the concentration[10] ($1/P$) of para-substituted phenols necessary to produce a one-to-one phenol–protein complex with serum albumin, and (*d*) the reciprocal of the concentration[11] ($1/c$) of para-substituted benzyl isothiocyanates ($XC_6H_4CH_2NCS$) required to produce a standard antifungal effect towards *Aspergillus niger*. In each set, the variable ($K_{i,2}$; $1/M_{50}$; $1/P$; $1/c$) is a measure of the effect of the substituent on the "binding" of a substrate to a protein. For the last two sets, Hansch *et al.*[10,11] have shown that the binding occurs through hydrophobic forces. For each of these sets, a highly significant relation between our log K_i values (derivatives **1** to **4**) and the above-mentioned variables could be calculated, as follows:

$\log K_i = 3.55 + 0.211 \log K_{i,2}$, with $s_{y/x} = 0.066$, and $r = 0.998$;
$\log K_i = 4.76 - 1.064 \log M_{50}$, with $s_{y/x} = 0.004$, and $r = 0.999$;
$\log K_i = 3.33 + 0.180 \log(1/P)$, with $s_{y/x} = 0.02$, and $r = 0.977$;
$\log K_i = 1.56 + 0.496 \log(1/c)$, with $s_{y/x} = 0.003$, and $r = 0.999$.

It is very difficult to see how these linear relations between log K_i and such widely differing binding parameters would be possible if specific interactions of the aryl groups, especially from the substituents, were involved.

Consequently, we must assume that the "binding" of the aglycon groups (within this sub-series) to the active sites is very unspecific, and arises mainly from the return of water molecules into the less-ordered bulk-water when the aglycon approaches the protein surface.

The analysis of the effect of other substituents on $\Delta G°$ (K_i) is more difficult, as no correlations could be found between $\Delta G°$ (K_i) and $\Delta G°$ (1-octanol) or any other substituent constant. However, there is other evidence that, for these substituents, the binding of the aglycon group is unspecific.

For several substituted-phenyl 1-thio-β-D-galactopyranosides, K_i was determined at different temperatures. The values of $\Delta H°$ and $\Delta S°$ are the same within experimental error (Table III). In the previous study[1] on the binding of alkyl 1-thio-β-D-galactopyranosides, it was found that the direct interaction of the aglycon group with a side chain of an amino acid on the active site gave rise to a decrease in $\Delta H°$ of ~5 to 7 kcal.mol^{-1}, and to negative $\Delta S°$ values (up to −8 cal.degree^{-1}.mol^{-1}). Consequently, the values in Table III do not suggest a direct interaction of the phenyl aglycon with the active-site side-chains. The finding that none of the values of $\Delta S°$ is negative (except for derivatives **14** and **15**; see later Discussion), notwithstanding the fact that $\Delta S°$ represents the change in entropy accompanying the formation of an enzyme–inhibitor complex, strongly suggests that the decrease in entropy of complex formation is compensated for by the increase in entropy resulting from the return of water molecules to the bulk-water.

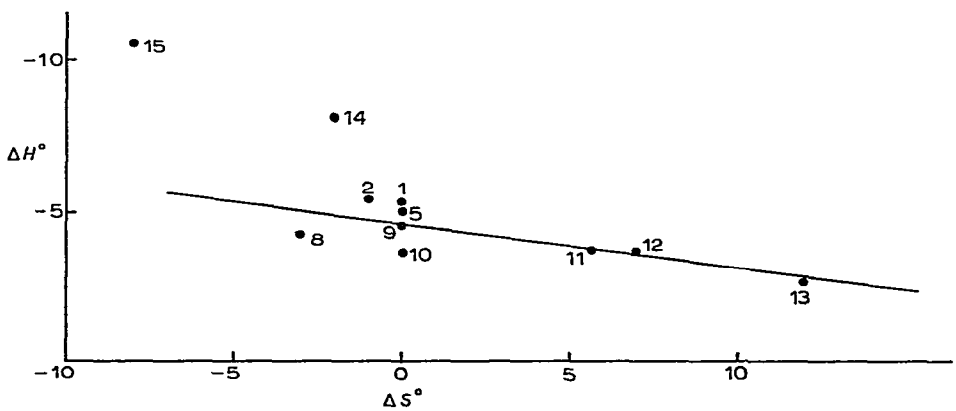

Fig. 2. Leffler Plot. Numbers refer to Table III.

If, according to the Leffler[12] method, $\Delta H°$ (Table III) is plotted against $\Delta S°$, the result (Fig. 2) indicates the possible existence of an isokinetic relationship of the form $\Delta H° = \text{constant} + \beta\Delta S°$. The line in Fig. 2 is the most probable function-line, calculated by regression analysis and using the values for derivatives **1** to **9** in Table III. Since the Leffler plot is far from convincing, more evidence was sought for the existence of the isokinetic relation by using the Exner[13,14] method. A plot of log K_i (30°) against log K_i (20°) (Table II) yields Fig. 3. Regression analysis using derivatives **1** to **13** (Table II) yields the equation:

$$\log K_i\ (30°) = -0.172 + 1.02 \log K_i\ (20°),$$
$$\text{with } s_{y/x} = 0.04,\ s_b = 0.02, \text{ and } r = 0.996.$$

When log K_i (30°) is recalculated from this equation, the recalculated values agree with the experimental values (within the standard error), except for derivatives **14** and **15**. The calculated K_i (30°) values are: 330,750 for benzyl, and 1,172,000 for 2-phenylethyl 1-thio-β-D-galactopyranoside. Consequently, the two points which possibly deviate from the isokinetic relation are those for the same two alkyl derivatives for which a direct binding of the aglycon with an amino-acid side-chain of the enzyme was postulated[1]. Although the Exner and Leffler plots, together with the significant log K_i regression function, do not *prove* the existence of a real, isokinetic relationship, they strongly suggest that all 1-thiogalactosides (except derivatives **14** and **15**) bind by the same basic mechanism. According to the Hinshelwood–Exner classification[13], all derivatives would belong to a compensation series, so that a change in $\Delta S°$, induced by the aglycon group, will be accompanied by a change in $\Delta H°$, proportional

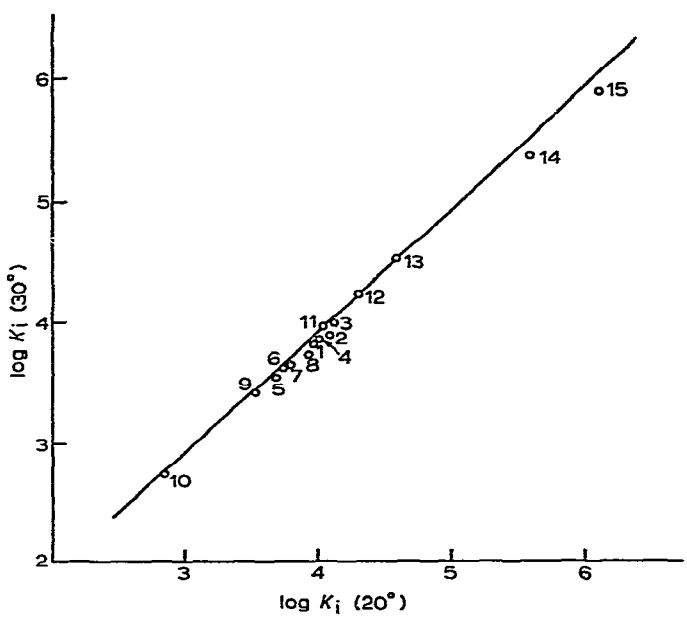

Fig. 3. Exner Plot. Numbers refer to Table III.

TABLE IV

EQUILIBRIUM BINDING CONSTANT (K_i) OF SUBSTITUTED PHENYL α-D-GALACTOPYRANOSIDES AT 25° AND pH 7.5

Aglycon group	K_i (M^{-1})	$-\Delta G°(25°)$ (*kcal.mol*$^{-1}$)
Phenyl	172	3.05
p-Methylphenyl	146	2.95
p-Ethylphenyl	170	3.04
p-Ethoxyphenyl	150	2.97
p-Chlorophenyl	127	2.87
p-Bromophenyl	157	3.00
p-Nitrophenyl	26	1.92

to that in $\Delta S°$ (according to $\Delta H° =$ constant $+\beta\Delta S°$). However, from the values in Table III and from the Exner regression function, it is clear that β is very small.

Considering the large differences between the aglycon groups (alkyl and aryl), it is difficult to explain how these derivatives could form a single compensation series, when specific and direct protein–aglycon interactions were involved. On the other hand, the compensating effect can be understood more easily when it is assumed to arise from one single process, common to the binding of all derivatives, namely, the unspecific restructuring of the water phase.

In order to investigate the effect of the orientation of the aglycon group on the binding of the inhibitor, we determined the inhibition constant for a number of substituted phenyl α-D-galactopyranosides. These compounds behave as competitive inhibitors of the β-D-galactosidase-catalyzed reactions, but, unlike the corresponding β anomers, are not hydrolyzed by the enzyme. The values of K_i (Table IV), compared to the value of D-galactose itself (K_i ~45 M^{-1}; $\Delta G°$ (25°) ~ −2.2 kcal.mol^{-1}), clearly indicate that the contribution of an axially oriented aglycon group is very small. With the possible exception of the nitro group, the substituent has no effect on the binding. Consequently, it seems highly probable that an axially oriented phenyl-ring does not leave the water phase.

CONCLUSIONS

From the results reported above, it can be concluded that the aromatic ring in substituted phenyl D-galactopyranosides does contribute to the binding of these molecules to the active site of β-D-galactosidase when the ring is in the equatorial position [$^4C_1$(D)]. When the ring is axially oriented [$^4C_1$(D)], the contribution is very small or non-existent, and a substituent on the ring has no effect on the binding. With substituted phenyl 1-thio-β-D-galactopyranosides, the effect of a substituent group is small and rather unpredictable. For the β anomers, all of the available evidence indicates that the aglycon is bound by unspecific forces of hydrophobic

nature, mainly arising from the restructuring of the water phase which takes place when the whole substrate molecule is transferred to the active site. In this sense, the "binding" of the aglycon groups would occur merely as a consequence of the active binding of the glycon moiety of the substrate, rather than as a necessary part of the binding mechanism. The aglycon seems to be buried in a hydrophobic micro-region which is limited in size, so that only part of the phenyl ring can be accommodated.

With regard to the influence of substituent groups on the binding of the substrates, the conclusion seems to be that even a comparison with experimentally determined hydrophobic parameters in model systems is insufficient to allow an analysis of the effect of the substituent. Firstly, because a series of substituted phenyl derivatives is too complex in itself, too many different effects can play a role, so that more simple sub-series must be used. Secondly, because the homogeneous organic phase is an incomplete model system for a heterogeneous "enzyme-phase". Similar conclusions were also drawn from our previous study[9] on the binding of para-substituted phenyl glycosides to Concanavalin A.

EXPERIMENTAL

The substituted phenyl 1-thio-β-D-galactopyranosides and phenyl α-D-galactopyranosides were synthesized as previously described[15,16]. The β-D-galactosidase was a crystalline suspension from Boehringer. Partition coefficients were determined as described previously[1], except that the concentration of 1-thiogalactosides was determined by measuring the extinction of the aqueous solutions at the experimentally determined wavelength for which maximal absorption occurred.

Standardization of the enzyme and measurements of the rate of hydrolysis of *o*-nitrophenyl β-D-galactopyranoside were performed as described[17]. For the calculation of K_i, the classical methods[18,19] were used. For at least ten concentrations of substrate, the initial velocity (v_i) was determined, and the maximal rate V and K_{app} were calculated by the method of Wilkinson[20]. The process was then repeated with the same concentrations of substrate, but with the addition of a constant concentration of inhibitor. The inhibition constant K_i was then calculated from $K_{app} = K'_{app}[1 + (I)K_i]$[18,19], with K'_{app} the value with, and K_{app} the one without, inhibitor. The process was then repeated with different concentrations of substrate and inhibitor. Each value of K_i is the arithmetical mean of at least five determinations. All determinations were carried out in sodium phosphate buffer (pH 7.5; 0.1M, mM $MgCl_2$). The standard enthalpy of binding ($\Delta H°$) was calculated from the K_i values at five temperatures (Tables II and III) by the method of least-squares (log K_i *versus* 1/T). $\Delta S°$ was then calculated from $\Delta G° = \Delta H° - T\Delta S°$.

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