Cooperative Binding of Polyols by Phenylboronic Acids

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The complexation of phenylboronic acid with glucose and galactose to form a 1 : 2 (polyol–phenylboronic acid) complex has been shown to involve positive cooperativity, contrary to previous assumptions.

The formation of complexes between phenylboronic acid and polyols is the basis of chromatographic separations,[1] asymmetric syntheses,[2] an enzyme immobilization technique[3] and the preparation of polymers capable of molecular recognition.[4] Although several acid–polyol complexes have been isolated[5] the complexation equilibria to form these complexes is not well understood. Complexation is envisaged to involve the formation of anions either prior to complexation[6] or following complexation.[7] The anionic complexes are stabilized at alkaline pH.

The equilibrium constants for complexes formed between phenylboronic acid and polyols have been determined by potentiometric titration,[8] pH depression,[6] polarimetry[9] and spectrometry.[10] Complex formation has been described by equilibria involving 1 : 1 and 1 : 2 complexes (Fig. 1) in which it is assumed that there is no cooperativity in binding. As a consequence of this assumption, deviations of the formation constant from ideal behaviour have been found frequently and attributed to several causes including the inaccuracy of the measurement of pH depression.[6]

To obtain a better understanding of the equilibrium between phenylboronic acid and polyols, we have designed a differential pH meter to simplify the task of undertaking accurate measurements of complexing constants by the method of pH depression. We report here results which indicate that there is cooperativity in the binding of polyols by phenylboronic acid. The instrument used was constructed using operational amplifiers and was designed to display the difference in pH between the working and reference electrodes. After calibration at pH 7 and 9, measurements were made by placing the reference electrode in a phenylboronic

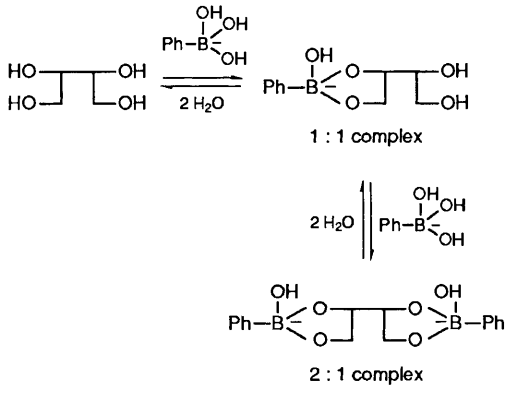
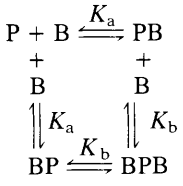


Fig. 1 Equilibria between phenylboronic acid and a polyol function at pH > 8.5

Table 1 Dissociation constants for the complexation of polyols with phenyl boronic acids

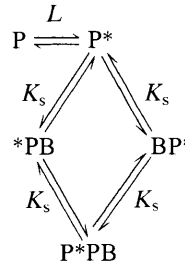
	Sequential		Concerted		
Substitutent	K_a	K_b	L	K_s	Polyol
H	2.42×10^{-2}	2.76×10^{-3}	7.76	2.77×10^{-3}	Glucose
3-NH_2[a]	1.14×10^{-2}	3.58×10^{-3}	2.19	3.58×10^{-3}	Glucose
H	2.48×10^{-2}	6.87×10^{-3}	2.6	6.87×10^{-3}	Galactose

[a] In this experiment the pH was 8.9.



$$1 - 10^{\Delta pH} = \frac{\frac{[P]}{K_a} + \frac{[P]^2}{K_a K_b}}{1 + \frac{2[P]}{K_a} + \frac{[P]^2}{K_a K_b}}$$

Scheme 1



$$1 - 10^{\Delta pH} = \frac{\frac{[P]}{K_s} + \left(1 + \frac{[P]}{K_s}\right)^2}{L + \left(1 + \frac{[P]}{K_s}\right)^2}$$

Scheme 2

acid buffer solution (pH 8.86) and the working electrode in the same buffer solutions to which was added varying concentrations of polyol.[6] The data were analysed using eqn. (1) where K_c is the pseudo-association constant,[6] ΔpH the difference in pH and $[P_f]$ the amount of uncomplexed polyol.

$$K_c = 10^{-\Delta pH} - 1/[P_f] \quad (1)$$

In the case of complexes formed between one polyol and two boronic acid molecules, K_c is related to K_1 and K_{12}, the formation constants for a 1 : 1 and 1 : 2 complex, by eqn. (2) where $[B_f^-]$ is the concentration of free boronic acid.[6] For a 1 : 1 complex $K_c = K_1$

$$K_c = K_1 + 2K_{12}[B_f^-] \quad (2)$$

For the interaction of glycerol with the anion of phenylboronic acid a plot of $10^{-\Delta pH}$ *vs.* [glycerol] was linear according to eqn. (1), over the range 0.01–0.3 mol dm^{-3} and a value of 20.2 calculated for the association constant, which agreed with the value of 19.9 reported by Lorand and Edwards.[6] However, the corresponding plots for the interaction of phenylboronic acid with glucose and galactose over the range 0.001–0.1 mol dm^{-3} were non-linear and inconsistent with the analysis reported[6] for compounds which form a 1 : 2 complex.

The assumption that cooperativity was absent in the binding of polyols by phenylboronic acid was tested for by plotting log $([B_0] - [B_f^-]/[B_f^-])$ *vs.* log [polyol] (Hill plot),[11] where $[B_0]$ is the initial concentration of boronic acid and $[B_f^-] = 10^{\Delta pH}[B_0]$ (see ref. 6). The plots for glucose and galactose were linear, as shown in Fig. 2 over the range 10–90% with a value for the Hill coefficient (n) of 1.47 and 1.62 respectively. For $n = 1$, the binding sites are independent and there is no cooperativity. However a value of $n > 1$ but <2 implies that there is weak positive cooperativity, in the binding of both glucose and galactose with phenylboronic acid to form a 1 : 2 complex.

Two models of cooperativity were evaluated based on the sequential[12] and concerted[13] binding schemes shown in Scheme 1 and 2 respectively and expressions derived relating

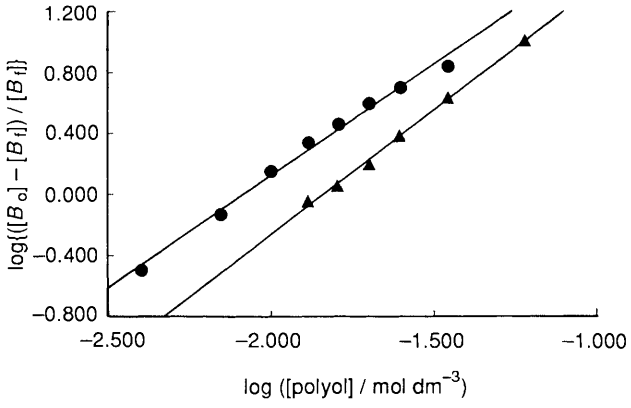


Fig. 2 A Hill plot of data for the complexation of glucose (●) and galactose (▲) with phenylboronic acid (pH 8.86)

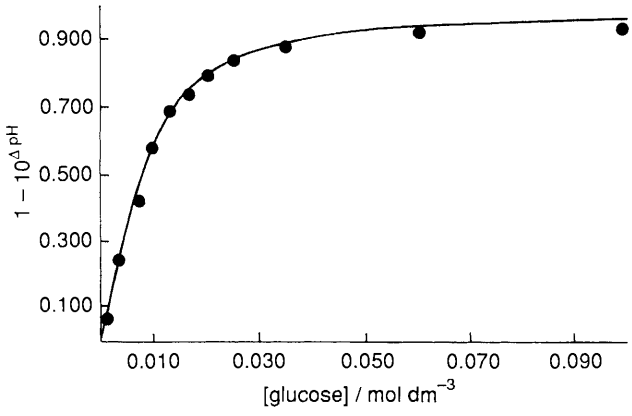


Fig. 3 A plot of the pH depression function ($1 - 10^{\Delta pH}$) *vs.* glucose concentration for the complexation of glucose with phenylboronic acid (●). Also shown is the best theoretical fit to Schemes 1 and 2. Both Schemes yield the same dependence on [P].

ΔpH to the amount of polyol added [P]. The sequential model (Scheme 1) assumes that the binding of the first boronate 'activates' the molecule to further binding of a second. In this model K_a and K_b are the dissociation constants for the 1 : 1 and 1 : 2 complexes respectively. The concerted model, however, assumes the polyol to exist in two different mutually interconvertible forms [P] and [P*] (anomers or in pseudo equilibrium)[14] and that complexation occurs exclusively with the latter form. In this scheme L is the equilibrium constant for the interconversion of the two forms and K_s the dissociation constant for the formation of a complex.

Fig. 3 shows typical results obtained for the interaction of phenylboronic acid with glucose fitted to the equations given in Schemes 1 and 2. Table 1 gives the corresponding dissociation constants K_a, K_b, L and K_s calculated using these models for glucose and galactose with phenylboronic acid and 3-aminophenylboronic acid. Both models yield identical curves with glucose and galactose so preventing differentiation between the two models. However, the fact that $K_a > K_b$ and $L > K_s$ is consistent with a model of positive cooperativity.

The values of K_a, K_b and K_s reported here are slightly larger than values previously reported[6] but no direct comparison is possible as the earlier reported treatment for the determination of dissociation constants of polyols did not assume cooperativity. The treatment described here yields data both on complexation constants and on the nature of the complexes formed. Measurements on fructose yielded pH depression data giving a value of $n = 3.89$ from the Hill plot, indicating the possibility of a 1:4 polyol–phenylboronic acid complex rather than a 1:2 complex as previously assumed. The accommodation of four phenylboronic acid residues around fructose would require the formation of two diphenylcyclodiboronic acid ester complexes. This type of complex is common with *trans*-related hydroxy groups,[15,16] of which fructose contains two pairs.

Although cooperativity is a property normally associated with binding processes involving large biomolecules, this study has shown it is also possible in reactions involving small biomolecules. Analysis of pH depression data can afford valuable insights into the type and strength of complexes formed.

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