Affinity chromatography of yeast α -glucosidase using ligand-mediated chromatography on immobilized phenylboronic acids

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(Received 26 March 1981/Accepted 1 June 1981)

The synthesis of 3-nitro-4-(6-aminohexylamido)phenylboronic acid is described. The properties of two novel forms of immobilized phenylboronate agarose adsorbents [*m*-aminophenylboronic acid–Matrex Gel and 3-nitro-4-(6-aminohexylamido)phenylboronic acid–Sephacrose CL-6B] were investigated. Both gels bind and selectively retard the glycoprotein α -glucosidase from yeast. The retardation is affected by following parameters: (i) pH, (ii) presence of sugar, (iii) concentration of sugar and (iv) buffer species (especially triethanolamine). Five sugars were studied, namely sorbitol, fructose, ribose, glucose and maltose. The concentration of sugar required to produce significant retardation increased in the above order, whereas the ability of sugar to form a complex with boron decreases in the same order. These effects were observed with crude as well as pure enzyme. Since α -glucosidase is a glycoprotein, it is proposed that this protein is mainly bound to these immobilized phenylboronates via sugar (glyco) residues. Displacement of the enzyme from the column is effected by the sugar in the buffer (or in a preincubation mixture). However, the marked pH-dependence (this retardation effect could only be observed at pH 7.4) suggests that these results are not due solely to hydrophobic or ionic mechanisms and are more complex than simple sugar–phenylboronic acid interactions.

Immobilized boronic acid derivatives form reversible complexes with diol-containing compounds such as nucleotides (Rosenberg *et al.*, 1972), nucleosides (Weith *et al.*, 1970), nucleic acids (Pace & Pace, 1980) and carbohydrates (Yurkevich *et al.*, 1975). Use of immobilized phenylboronic acid derivatives to separate diol-containing nucleotides was first suggested by Weith *et al.* (1970).

Hageman & Kuehn (1977) and Dean *et al.* (1979) demonstrated that NAD⁺ and NADP⁺ were able to form complexes with phenylboronic acid–agarose adsorbents. Furthermore, the latter authors showed that NADP⁺-dependent enzymes bound to the NADP⁺ complexes. On the other hand, NAD⁺-dependent enzymes were only slightly retarded by the NAD⁺-immobilized phenylboronic acid complex. In addition, yeast hexokinase was retarded when a phenylboronate–agarose column was pre-saturated with ATP (Bouriotis *et al.*, 1981a).

Another example of the use of presaturation of an immobilized phenylboronate column with a specific biomolecule is in the purification of uridine 5'-diphosphate glucose pyrophosphorylase from the slime mould *Physarum polycephalum*, where the column was saturated with uridine 5'-triphosphate (Maestas *et al.*, 1980).

The ability of boron to form specific complexes with *cis*-diols has also been explored to separate glycoproteins. Recently the measurement of the major glycosylated haemoglobin (haemoglobin A_{1c}) has been improved by using immobilized phenylboronate (Bouriotis *et al.*, 1981b). Furthermore, Williams *et al.* (1981) have fractionated membrane glycoproteins from lymphocytes from patients with chronic lymphocytic leukaemia.

Since α -glucosidase (α -D-glucosidase glycohydrolase, EC 3.2.1.20) is a glycoprotein, we set out to investigate the possibility of using the potential interaction with phenylboronic acids as a means of isolating this protein. However, α -glucosidase also binds diol-containing substrates, which led us to consider the possibility of ligand-(substrate)-mediated chromatography to purify this enzyme. To this end, we have studied the properties of two immobilized phenylboronic acids: *m*-aminophenylboronic acid and 3-nitro-4-(6-aminohexylamido)-phenylboronic acid.

Materials and methods

Materials

m-Aminophenylboronic acid–agarose (Matrex

Gel PBA-60; ligand concn. 75 μ mol of aminophenylboronic acid/ml of gel wet wt.) was supplied by S. Fulton (Amicon Corp., Lexington, MA, U.S.A.). *p*-Nitrophenyl α -D-glucopyranoside, triethanolamine hydrochloride and pure α -glucosidase were kindly donated by Dr. A. Roeder (Boehringer Mannheim, Tutzing, Germany). 3-Aminophenylboronic acid and diphenylcarbazone were obtained from Aldrich Chemical Co., Gillingham, Dorset, U.K. Cyanuric chloride, 4-bromotoluene, magnesium turnings, tributylborate, and hexanediamine were obtained from BDH Chemicals, Poole, Dorset, U.K. Sepharose CL-6B was obtained from Pharmacia Fine Chemicals, London W.5, U.K. All other reagents were of analytical grade.

General methods

α -Glucosidase activity was assayed routinely at 37°C in semimicro cuvettes in a total volume of 1.01 ml. The reaction mixture contained *p*-nitrophenyl α -D-glucopyranoside (1.65 mM) in 0.1 M-KH₂PO₄/K₂HPO₄ buffer, pH 7.2. The reaction was initiated by the addition of enzyme solution (10 μ l) and was monitored by measuring the rate of *p*-nitrophenol formation at 405 nm with a Gilford 250 spectrophotometer. Activity was calculated as follows:

$$\text{'Volume activity' (units/ml)} = \frac{1.01 \text{ ml}}{18.5 \times 0.01} \times \Delta A_{405} / \text{min}$$

Protein was determined from the absorbance at 280 nm by assuming $A_{280}^{1\%, 1\text{cm}} = 10$. Specific activity is expressed as units/mg of protein. In these instances protein was measured by the tannin micro method (Katzenellenbogen & Dobryszcka, 1959).

T.l.c. was performed on Kieselgel CF 254-coated plates (Merck). The spots of boron-containing compounds were made visible by spraying with a 0.2% (w/v) solution of diphenylcarbazone in methanol (Neu, 1960). A 0.2% (w/v) ninhydrin solution in acetone was used for detecting primary amino groups. N.m.r. spectra were recorded at 220 MHz on a Perkin-Elmer R-34 instrument.

3-Nitro-4-(6-aminohexylamido)phenylboronic acid was coupled to Sepharose CL-6B by using the method described by Biagioni *et al.* (1978), with minor modifications described by Dean *et al.* (1979).

Synthesis of 3-nitro-4-(6-aminohexylamido)phenylboronic acid

(a) *Synthesis of tol-4-ylboronic acid.* The Grignard synthesis was performed as described by Bean & Johnson (1932) with minor modifications; the Grignard reagent (tol-4-ylmagnesium bromide) was prepared from 4-bromotoluene (0.19 mol), magnesium (0.22 mol) and diethyl ether (250 ml).

The reagent was filtered through glass wool into a solution of tributylborate (0.17 mol) in diethyl ether (100 ml) at -78°C with stirring. The addition was performed over 1 h. Stirring was continued for 1 h at -78°C and then over 1 h while the mixture warmed to 0°C. The mixture was then added to cold 10% (v/v) H₂SO₄ (300 ml). The aqueous phase was extracted with diethyl ether (3 $\times$ 250 ml). The ether extracts were combined, dried (over anhydrous MgSO₄) and the ether removed *in vacuo*. Tol-4-ylboronic acid was recrystallized (from water) as colourless needles (yield 10.2 g, 41%). T.l.c. showed one spot [*R_F*, 0.51 (ethyl acetate); 0.61 (toluene/acetone, 1:1, v/v)]. N.m.r. ([²H₆]dimethyl sulphoxide): δ , 2.32 (s, 3H = CH₃); 7.15, 7.19, 7.71, 7.75 (9 = 4H = aromatic hydrogen atoms). No clear boron hydroxy groups were observed except a very broad multiplet (7.5–8.2), which disappeared on 'D₂O (= ²H₂O) shake'. λ_{max} (methanol) = 256, 263, 268, 274 (shoulder) nm.

(b) *Synthesis of 3-nitro-4-methylphenylboronic acid.* Fuming HNO₃ (15 ml) in a cylindrical Pyrex tube was cooled to -40°C in a bath of methanol. Dried *p*-tolylboronic acid (15 mmol) was then added slowly with stirring. The reaction mixture was maintained at -40°C throughout the addition and

Fig. 1. Structures of 3-nitro-4-methylphenylboronic acid (I), 3-nitro-4-carboxyphenylboronic acid (II), 3-nitro-4-(6-aminohexylamido)phenylboronic acid (III) and *m*-aminophenylboronic acid (IV)

then poured on to 30 g of ice. The suspension was stirred for $\frac{1}{2}$ h. 3-Nitro-4-methylphenylboronic acid was precipitated and filtered off under suction and washed with small portions of ice-cold water and recrystallized (from water) to give needles (yield 1.6 g, 58%). T.l.c. showed one spot [R_F , 0.47 (ethyl acetate); 0.55 (toluene/acetone, 1:1, v/v)]. N.m.r. ($^{12}\text{H}_6$]dimethyl sulphoxide) = δ , 2.52 (s, 3H = CH₃): 7.52, 7.48 (d, H = aromatic hydrogen, a, structure I, Fig. 1): 8.0, 8.21 (d, H = aromatic hydrogen = b, structure I, Fig. 1): 8.38 (s = H = aromatic hydrogen = c, structure I, Fig. 1): 8.42 (broad complex multiplet which disappeared on D₂O shake = boron hydroxy groups).

(c) *Synthesis of 3-nitro-4-carboxyphenylboronic acid.* 3-Nitro-4-methylphenylboronic acid (2.7 mmol) was dissolved in 0.26 M-NaOH. The mixture was heated to 50°C and a saturated solution of KMnO₄ (1 g) was added in portions over 5 days. After filtration, the filtrate was acidified with HCl (testing with litmus paper) and evaporated to dryness. The residue was extracted with cold ethanol (3 $\times$ 15 ml). The extracts were combined, evaporated, and the oily mass that was produced crystallized on the addition of a small amount of water (yield 0.41 g, 70%). T.l.c. showed one spot [R_F , 0.41 (ethanol)]. N.m.r. ($^{12}\text{H}_6$]dimethyl sulphoxide): δ , 7.79, 7.92 (d, H = aromatic hydrogen, a, structure II, Fig. 1): 8.15, 8.28 (d, H = aromatic hydrogen, = b, structure II, Fig. 1): 8.33 (s = H = aromatic hydrogen, c, structure II, Fig. 1): 9.98 (broad s = CO₂H, disappears on D₂O shake). λ_{max} . (10 mM-sodium phosphate buffer, pH 7.0) = 220, 268 (shoulder) nm.

(d) *Synthesis of 3-nitro-4-(6-aminohexyl-amido)phenylboronic acid.* 3-Nitro-4-carboxylphenylboronic acid (1.8 mmol) was made to react with thionyl chloride (0.5 ml). The mixture was heated and refluxed for 90 min, cooled and the excess of thionyl chloride removed *in vacuo*. The solid acid chloride that was formed was added in portions to an aqueous solution of hexanediamine (20 mmol in 6 ml of water) at 0°C. The solution was then acidified to precipitate 3-nitro-4-(6-aminohexyl-amido)phenylboronic acid, which was filtered off and washed with small portions of ice-cold water (yield 0.34 g, 61%). T.l.c. showed one spot [R_F , 0.24 (methanol)]. N.m.r. ($^{12}\text{H}_6$]dimethyl sulphoxide + $^2\text{H}_2\text{O}$): δ , 1.21, 1.37, 1.57 (broad, t, 8H = 4 $\times$ CH₂ = B + C + D + E, structure III, Fig. 1): 2.81 (broad t, 2H = CH₂ = A, structure III, Fig. 1): 3.25 (broad t, 2H = CH₂ = F, structure III, Fig. 1): 7.57, 7.60 (d, H = aromatic hydrogen = a, structure III, Fig. 1): 8.12, 8.15 (d, H = aromatic hydrogen = b, structure III, Fig. 1): 8.33 (s, H = aromatic hydrogen = c, structure III, Fig. 1). λ_{max} . (0.1 M-sodium bicarbonate, pH 9.0) = 275 nm. Mass spectra were recorded for all the above compounds and were in agreement with the relevant structures.

Column chromatography with pure α -glucosidase

Matrex Gel PBA-60 (structure IV, Fig. 1) (2 ml) was packed in polycarbonate columns (0.7 cm $\times$ 3 cm) and the columns were equilibrated with buffer (20 ml) either 50 mM-triethanolamine/HCl, or 50 mM-KH₂PO₄/K₂HPO₄, pH 7.4 (phosphate) plus or minus sugar.

Between each experiment, the columns were washed with 0.1 M-sorbitol (20 ml) in the buffer followed by 0.1 M-sodium acetate, pH 5.0 (20 ml). α -Glucosidase (16 units, 200 μ l) was dialysed against the relevant buffer, applied to phenylboronate columns and eluted with equilibration buffer (30 ml). Tightly bound α -glucosidase activity was eluted by including 0.1 M-sorbitol in the buffer. The temperature of the columns was 4°C throughout. Flow rates were maintained at 2.0 ml/h by using a peristaltic pump (LKB Varioperpex). Fractions (1 ml) were collected and assayed for α -glucosidase activity.

Column chromatography with crude yeast protein

Method A. Matrex Gel PBA-60 column (15 ml, 1.6 cm $\times$ 9 cm) was equilibrated with 50 mM-triethanolamine/HCl, pH 7.4, which contained 100 mM-maltose. Crude yeast protein mixture [containing 36 units (23 mg) of α -glucosidase] was dialysed against buffer and then applied to phenylboronate column. The column was eluted with the equilibration buffer (120 ml). The flow rate was 10 ml/h. Fractions (3.3 ml) were collected and assayed for α -glucosidase activity and for protein (A_{280}). Retarded fractions were pooled, concentrated by ultrafiltration (Amicon, model 202 or 12) and assayed for α -glucosidase activity and for protein by the tannin micro method (see above).

Method B. Columns (5 ml, 5.3 cm $\times$ 0.35 cm) of Matrex Gel PBA-60 and 3-nitro-4-(6-aminohexyl-amido)phenylboronic acid-Sepharose CL-6B (ligand concn. 35 μ mol/ml wet wt.) were equilibrated with 50 mM-triethanolamine/HCl, pH 7.4. Crude yeast protein mixture (2 ml, dialysed against buffer) was preincubated with various concentrations of maltose at 0° for 2.5 h and then applied to phenylboronate columns. Columns were eluted with the equilibration buffer (100 ml). Flow rates were 9 ml/h. Fractions (3 ml) were collected and assayed for α -glucosidase activity and for protein (A_{280}). Specific activities of pooled fractions were measured as in Method A.

Results and discussion

Experiments with pure enzyme

Pure α -glucosidase bound tightly to columns of Matrex Gel PBA-60 in 50 mM (I = 36.6 mM)-triethanolamine/HCl or 50 mM (I = 127.9 mM)-phosphate buffers, pH 7.4. However, when the two buffer

Fig. 2. Chromatography of pure α -glucosidase on *m*-aminophenylboronic acid-Matrex Gel in the presence of maltose

Columns of immobilized aminophenylboronic acid (Matrex Gel-PBA-60) (2 ml, 4.7 cm $\times$ 0.5 cm) were equilibrated with 50 mM-triethanolamine/HCl, pH 7.4 (20 ml), containing various amounts of maltose. Pure α -glucosidase (16 units, 200 μ l) was applied and the column eluted with the same buffer (30–40 ml). The flow rate was 2 ml/h. Fractions (1 ml) were collected and assayed for α -glucosidase activity. ●, 50 mM-maltose; ○, 100 mM-maltose.

ions were combined, the enzyme was merely retarded. This was shown not to be an effect related to salt concentration, since replacement of K_3PO_4 by KCl resulted in the enzyme activity being tightly bound. However, the enzyme was also retarded (rather than tightly bound) when various sugars were present in the buffer. Furthermore, the elution volume (and profile) of emergent α -glucosidase activity depended on the concentration and the nature of the sugar in the buffer (Table 1, Fig. 2).

Five different sugars were studied, namely D-maltose, D-glucose, D-fructose, D-ribose and sorbitol. Maltose and glucose are substrates (or products) of α -glucosidase, whereas ribose, fructose and sorbitol have no measurable effect on the enzyme activity (Halvorson & Ellias, 1958). Higher concentrations of maltose and glucose (50 mM) were needed to produce the same retardation as 5 mM-fructose, 5 mM-sorbitol or 20 mM-ribose. The sugar-dependent elution volumes agree well with (i) the complex-formation constants for these sugars with phenylboronic acid (Lorand & Edwards, 1959; Torssell *et al.*, 1958) (thus fructose has a complex-formation constant with phenylboronic acid that is 40 times higher than with glucose) and (ii) published retention volumes of these sugars on immobilized phenylboronic acid columns, which increase in the order: glucose, ribose, fructose and sorbitol (Weith *et al.*, 1970). We conclude from these data that, since α -glucosidase is a glycoprotein, the enzyme is probably bound mainly via its covalently attached sugar (glyco) residue(s) to the phenylboronate

Table 1. Retardation volumes of pure α -glucosidase on *m*-aminophenylboronic acid-Matrex Gel in the presence of different sugars

Retardation volumes were measured as in Fig. 2. Retardation volume is defined as the elution volume of the peak of emergent activity minus the column volume.

(a) 50 mM-Triethanolamine/HCl buffer, pH 7.4

Sugar	Concn. (mM)	Retardation volume (ml)
Sorbitol	2	23
	5	12
	20	6
Fructose	5	11
	20	6
Ribose	5	24
	20	16
Maltose	50	13
	100	7
Glucose	10	24
	50	14

(b) 50 mM-Phosphate buffer, pH 7.4

Sorbitol	2	12
Sorbitol	5	6

column and that the sugar in the buffer displaces the enzyme from phenylboronic acid by competing for this ligand. The mechanism of binding to the boronate appears to be complicated, but could involve (i) diol complexation or (ii) ionic and hydrophobic interactions or (iii) a combination of all three. It is possible to explain the data for glucose and maltose in terms of substrate-mediated chromatography, but, in the light of the result with other sugars, this seems unlikely.

The enzyme retardation observed in the present work is a general feature of (affinity-) chromatography systems that is frequently found in the literature (Lowe & Dean, 1974). Furthermore, such (biospecific) retardation can be a useful aid in protein purification, since, as can be seen from the above experiments, rather subtle changes in mediating ligand concentration can be used to manipulate the emergent elution profile of an enzyme.

Experiments with crude preparations of α -glucosidase

The effects (described above with pure enzyme) were confirmed and extended by using crude yeast protein. A mixture of crude yeast protein was applied to a phenylboronate column in the presence of 100 mM-maltose in 50 mM-triethanolamine/HCl buffer, pH 7.4 (Fig. 3). α -Glucosidase was selectively retarded with 29-fold purification (a yield of 44% was recorded). In the presence of 5 mM-sorbitol under the same conditions, α -glucosidase was selectively retarded with 14-fold purification (a yield of 37% was recorded) (results not shown).

Fig. 3. Chromatographic retardation of yeast α -glucosidase on *m*-aminophenylboronic acid-Matrex Gel in the presence of maltose

The column (15 ml, 1.6 cm $\times$ 9 cm) of *m*-aminophenylboronate-Matrex Gel PBA-60 was equilibrated with 50 ml of 50 mM-triethanolamine/HCl, pH 7.4, which contained 100 mM-maltose. A 2 ml portion of yeast protein mixture (36 units of glucosidase, 23 mg) was applied and eluted with equilibration buffer (120 ml). Fractions (2 ml) were collected and assayed for α -glucosidase activity (○) and for protein (A_{280} , ●). The flow rate was 10 ml/h. Retarded fractions (55–110 ml) were pooled and the specific activity was measured (see the Materials and methods section).

Preincubation of the enzyme preparation with sugars

The above retardation effect was also observed when yeast protein mixture was preincubated at 0°C for 2.5 h with various sugars (Figs. 4a and 4b). The elution volume of α -glucosidase changed to an extent depending on the amount of sugar in the incubation mixture. The observed elution volumes are entirely in agreement with the previous data (Fig. 2) providing that the sugar concentration is calculated as occupying the entire column volume plus the sample volume. We do not understand the mechanism of this retardation. This effect was only observed in triethanolamine/HCl buffer at pH 7.4, which suggests that retardation is affected by the nature of the buffer, since with phosphate and *N*-ethylmorpholine/HCl buffers the enzyme activity was tightly bound (but could be eluted with 0.1 M-sorbitol). These results could be explained by the specific effect of triethanolamine on borate complexes (Brown & Fletcher, 1951).

In order to study the effect of different boronates as ligands we compared two phenylboronic acid derivatives, namely *m*-aminophenylboronic acid and 3-nitro-4-(6-aminoethylamido)phenylboronic acid. α -Glucosidase from crude yeast protein mixture was tightly bound to the latter ligand i.e. immobilized 3-nitrophenylboronic acid. However, the enzyme could not be eluted with sorbitol (0.1 M), maltose (0.1 M) or KCl (1 M). Preincubation in maltose (240 μ M) weakened the binding of α -glucosidase to

Fig. 4. Chromatographic retardation of α -glucosidase preincubated with maltose on *m*-aminophenylboronic acid-Matrex Gel and 3-nitro-4-(6-aminoethylamido)-phenylboronic acid-Sepharose CL-6B

Yeast protein mixture containing α -glucosidase [52 units, 50 mg (a) and 52 units, 60 mg (b)] was applied to a column (5 ml, 5.3 cm $\times$ 0.95 cm) of *m*-aminophenylboronate-Matrex Gel PBA-60 (a) and 3-nitro-4-(6-aminoethylamido)phenylboronic acid-Sepharose CL-6B (b) after preincubation at 0°C for 2.5 h with 240 μ M (a) and 640 μ M (b) of maltose. Columns were equilibrated and eluted with 50 mM-triethanolamine/HCl, pH 7.4, containing no maltose. The flow rate was 9 ml/h. Fractions (3 ml) were collected and assayed for α -glucosidase activity (○) and for protein (A_{280} , ●). Retarded fractions [18–90 ml (a) and 24–90 ml (b)] were pooled and the specific activity was measured (see the Materials and methods section).

immobilized 3-nitrophenylboronic acid such that the enzyme could be eluted with 0.1 M-sorbitol. Nitrophenylboronate columns required higher sugar concentrations (640 μ M of maltose per 5 ml column) to produce the same retardation as immobilized *m*-aminophenylboronic acid (240 μ M per 5 ml column). In the maltose-preincubation experiments, yields of α -glucosidase in the retarded fractions of 50.7 and 57.5% were recorded, with 13-fold purification in each case.

Formation of boron complexes with neutral sugars is dependent on a number of variables including pH. Alkaline pH favors complex-formation (Foster, 1957). Nitrophenylboronate forms complexes in free solution with fructose and glucose even at pH 4. On the other hand the lowest pH value for complexation with *m*-aminophenyl-

boronic acid is pH 5 (Barker *et al.*, 1973). We attempted to repeat these experiments as described in Fig. 4(a) at pH values of 6.8 and 8.5. However, at pH 6.8 the enzyme was bound but could be eluted with 0.1 M-sorbitol, whereas at pH 8.4 the enzyme did not bind at all. The binding at pH 6.8 is explained in part by hydrophobic interactions, which diminish at the higher pH values. Elution by sorbitol is explained by the ability of glycols to elute proteins from hydrophobic matrices (Lowe & Mosbach, 1975). On the other hand, at pH 8.5, the triethanolamine buffer may well exclude other competing diols because of its affinity for boronates at this pH value. The principal difference between these two boronate derivatives lies in the pK value of the 3-nitrophenylboronic acid, which is lower (7.0) than that of *m*-aminophenylboronic acid (8.8), owing to the electron-withdrawing effect of the nitro group.

Our explanation of the above data is that the mechanism of binding at pH 7.4 centres on the sugar residues of the glycoprotein and that substrate-sugar (maltose)-mediated phenylboronate-enzyme interactions are probably of minor importance. We base these conclusions on: (1) the observed concentration-dependence of the retardation volumes; (2) the dependence of the retention volumes on the diols' ability to complex to phenylboronic acid rather than the known substrate specificity of the diol for the enzyme. Thus sorbitol and fructose, which bind well to phenylboronate and do not interact with the enzyme, have the same effect on retardation at a concentration one-tenth that of maltose; (3) the marked pH-dependence of these effects suggests that these results are not due solely to hydrophobic or ionic mechanisms and further, must be more complex than simple diol (sugar)-phenylboronic acid interactions. We suggest that triethanolamine participates in the complex-formation either by sharing oxygen or nitrogen co-ordination (or both). Nevertheless, the use of presaturating ligands enables the effective manipulation of group-specific ligands such as phenylboronates to separate glycoproteins and sugar-dependent enzymes. The success of these separations is determined by: (i) use of the appropriate buffer (in this case, triethanolamine); and (ii) choice of the optimum concentration of the mediating ligand (a sugar, in this case, maltose).

This technique is in contrast with the more commonly used method of gradient (or pulse) affinity elution. Thus we have described (Williams *et al.*, 1981) the use of 0.1 M-sorbitol to desorb glycoproteins from immobilized phenylboronic acid. The present work suggests that the addition of potential eluate species to the pre-equilibration buffer of a column is a method to improve the performance of affinity ligands such as phenylboronates.

In conclusion, the behaviour of α -glucosidase on

immobilized phenylboronic acid columns is affected by a number of parameters: pH, presence of sugar, concentration of sugar and buffer species.

Manipulation of these parameters can result in useful purifications of this enzyme by adjusting its elution relative to other emergent proteins.

We are grateful to Dr. A. Roeder for helpful discussions and Boehringer Mannheim G.m.b.H., Tutzing, Germany, and The Amicon Corporation for financial support. We also wish to thank Mr. Colin Longstaff for repeating the synthesis of 3-nitro-4-(6-aminoethyl-amido)phenylboronic acid and providing part of the n.m.r. data.

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