Journal of Chromatography, 176 (1979) 239–241
© Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 11,942

Note

Phenyl(dihydroboryl) polyacrylamide beads for chromatography of ribonucleosides in tissue extracts

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(Received April 24th, 1979)

Recent reports illustrate the usefulness of ligand exchange chromatography employing the succinamidophenyl(dihydroboryl) derivative of polyacrylamide beads in the analysis of ribonucleosides in urine[1,2]. These beads are synthesized by succinylating the hydrazide derivative of polyacrylamide followed by coupling of *m*-aminophenylboronic acid by means of a water-soluble carbodiimide (Fig. 1).

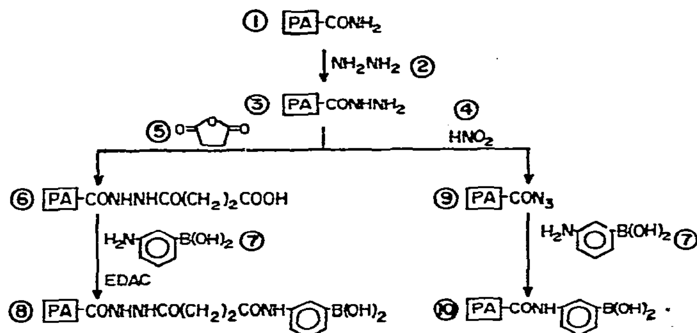

Fig. 1. Synthesis of phenyl (dihydroboryl) derivatives of polyacrylamide beads by the method of Gehrke *et al.*[2] and by the acrylazide procedure described here. Both methods employ polyacrylamide hydrazide (3). In the method of Gehrke *et al.*[2], the hydrazide is succinylated with succinic anhydride (5), and *m*-aminophenylboronic acid (7) is coupled to the succinylated gel (6) by means of a water-soluble carbodiimide, 1-ethyl-3(3-dimethylaminopropyl)carbodiimide (EDAC). In the present method the polyacrylamide hydrazide is diazotized with nitrous acid (4), and the polyacrylamide azide (9) is then reacted directly with *m*-aminophenylboronic acid. See text for details. In this diagram the rectangle labelled PA represents the polyacrylamide backbone.

This report concerns the phenyl(dihydroboryl) derivative of polyacrylamide beads prepared by coupling this acid directly via the general acyl azide procedure[3]. This preparative route is simpler, cheaper and yields beads which perform as well as the succinamidophenyl(dihydroboryl) resin. We have successfully applied this technique to the preliminary purification of samples of blood, cardiac muscle and pericardial fluid for the assay of adenosine by a competitive radioligand binding assay[4].

Bio-Gel P-2 hydrazide beads (200–400 mesh) were purchased from Bio-Rad Labs. (Richmond, Calif., U.S.A.), anhydrous hydrazine from Pierce (Rockford, Ill.,

U.S.A.); 1-ethyl-3(3-dimethylaminopropyl)carbodiimide (EDAC) and *m*-aminophenylboronic acid hemisulfate were from Sigma (St. Louis, Mo., U.S.A.). Other chemicals, all of reagent grade, were from Fisher Scientific (Pittsburgh, Pa., U.S.A.). Hydrazinolysis of the polyacrylamide beads for 5 h and conversion to the acyl azide followed the description of Inman and Dintzis[3] exactly. After thoroughly washing with water, the pale yellow azide resin was suspended in a solution containing, per g xerogel, 186 mg (1 mmole) *m*-aminophenylboronic acid hemisulfate in 5 ml water, pH being adjusted to 8.6 with NaOH. This mixture was stirred magnetically for 24 h at 4°, warmed to room temperature, and diluted with an equal volume of 0.2 *N* NH_4OH. After 30 min the gel was washed in a büchner funnel with 1 l of 0.2 *N* NH_4OH and then with water until the effluent was neutral. The gel was stored in 0.1 *M* NaCl containing 0.02% Na_3N. The degree of substitution estimated from the adsorbance of uncoupled phenylboronate remaining in the washings ($\lambda_{max.} = 293$ nm, $\varepsilon = 1610$ mole^{-1} at pH 7) was 1.0 mequiv./g xerogel. The succinamidophenyl-(dihydroboryl) derivative of Bio-Gel P-2 hydrazide prepared exactly as described by Gehrke *et al.*[2] contained 1.39 mequiv. phenylboronate/g xerogel.

Table I compares the performance of the two gels. Adenosine, inosine and 5′-AMP were retained essentially quantitatively by both resins whereas adenine, 2′-deoxyadenosine and cyclic 3′:5′-AMP, which do not contain the *cis*-glycol moiety required for borate complex formation, were recovered quantitatively in the column washings. Recovery of the three purine ribosides in the formic acid eluate was quantitative. These results indicate that the phenyl(dihydroboryl) resin performs as well as the succinamidophenyl(dihydroboryl) resin. Both of the gels described here were prepared from 200–400 mesh beads, which tend to shrink on exposure to ammonium acetate and have very low flow-rates after 4 or 5 samples. This problem is reduced by using 100–200 mesh beads.

Preparing the phenyl(dihydroboryl) resin is quicker and cheaper than preparing the succinamidophenyl(dihydroboryl) resin (1.5 days *vs.* 3 days and US$ 3.18 *vs.*

TABLE I

RETENTION OF PURINES ON SUCCINAMIDOPHENYL(DIHYDROBORYL) AND PHENYL(DIHYDROBORYL) DERIVATIVES OF POLYACRYLAMIDE

^{3}H-labelled purines were added to aliquots of an $HClO_4$ extract of dog blood which had been neutralized with KOH and centrifuged to separate $KClO_4$. This solution was diluted with an equal volume of 0.4 *M* ammonium acetate, pH 8.8 and 0.500 ml aliquots were either counted as standards or applied to 0.5 × 5 cm columns of the two types of gel previously equilibrated with 0.2 *M* ammonium acetate pH 8.8. After the sample had soaked in, the columns were washed with 5 ml equilibrating buffer and eluted with 5 ml 0.1 *N* formic acid. The washings and eluates were collected separately and counted. Results are expressed as percentage of counts recovered, which averaged 99.6 ± 3.4 (S.E.M.) %.

Purine	*Phenyl (dihydroboryl)*		*Succinamidophenyl (dihydroboryl)*	
	CH_3COONH_4	*HCOOH*	*CH_3COONH_4*	*HCOOH*
Adenosine	0.17	99.9	0.20	99.8
Adenine	98.3	1.7	98.9	1.6
2′-Deoxyadenosine	97.2	2.8	99.2	0.8
Inosine	1.6	98.4	3.3	95.7
Cyclic 3′:5′-AMP	99.4	0.6	99.7	0.3
5′-AMP	0.8	99.2	1.5	98.5

US$ 6.98/g xerogel, at current catalog prices, respectively). Much of the cost difference is due to the fact that commercial polyacrylamide hydrazide is 10 times more expensive than the underivatized gel. If one prepares the hydrazide by the simple and reproducible method of Inman and Dintzis[3] the coat of the succinamidophenyl(dihydroboryl) polyacrylamide gel is only 25% greater than that of the gel described here.

ACKNOWLEDGEMENTS

This work was supported by a Grant-In-Aid from the Suncoast Chapter, Florida Affiliate, American Heart Association, Fla., U.S.A.

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