Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 235–238
Bioorganic & Medicinal Chemistry Letters

Glucose-specific poly(allylamine) hydrogels—A reassessment

Furqan M. Fazal and David E. Hansen*

Department of Chemistry, Amherst College, Amherst, MA 01002, USA

Received 15 August 2006; revised 18 September 2006; accepted 19 September 2006
Available online 10 October 2006

Abstract—Polymer hydrogels synthesized by crosslinking poly(allylamine hydrochloride) with (±)-epichlorohydrin in the presence of D-glucose-6-phosphate monobarium salt do not show imprinting on the molecular level. A series of hydrogels was prepared using the following five templates: D-glucose-6-phosphate monobarium salt, D-glucose, L-glucose, barium hydrogen phosphate ($BaHPO_4$), and D-gluconamide; a hydrogel was also prepared in the absence of a template. For all six hydrogels, batch binding studies were conducted with D-glucose, L-glucose, D-fructose, and D-gluconamide. The extent of analyte sugar binding was determined using ^1H NMR. Each hydrogel shows approximately the same relative binding affinity for the different sugar derivatives, and none displays selectivity for either glucose enantiomer. The results of the binding studies correlate with the octanol–water partition coefficients of the sugars, indicative that differential solubilities in the bulk polymer account for the binding affinities observed. Thus, in contrast to templated hydrogels prepared using methacrylate- or acrylamide-based reagents, true imprinting does not occur in this novel, crosslinked-poly(allylamine hydrochloride) system.

Over the past decade, many new approaches have been reported for the generation and characterization of molecularly imprinted polymers.[1,2] For example, imprints generated from hydrogels, hydrophilic polymer networks that have been exploited in a variety of biological and pharmacological applications,[3] have potential for use as 'intelligent, controlled release' materials.[4] Recent reports indicate that imprinted hydrogels can selectively bind both protein[5,6] and small-molecule[7] templates, including glucose.[8]

We were particularly interested in the novel, carbohydrate-binding system reported by the Kofinas group.[9,10] Unlike the majority of imprinted hydrogels reported to date, this system does not involve the use of methacrylate- or acrylamide-based reagents—rather the hydrogels are synthesized by crosslinking poly(allylamine hydrochloride) (PAA·HCl) with (±)-epichlorohydrin (EPI). When prepared in the presence of 1.5 mol % of D-glucose-6-phosphate monobarium salt (GPS-Ba), these hydrogels show preferential binding of D-glucose relative to D-fructose.[9] As measured by batch studies in deionized water, binding capacities of approximately 600 mg of D-glucose per gram of dry polymer were reported, compared with only slightly more than 100 mg of D-fructose; control hydrogels formed in the absence of template were reported to bind just over 100 mg of both D-glucose and D-fructose per gram of dry polymer. Based upon these results, the Kofinas group had concluded that the GPS-Ba templated hydrogels had 'recognizable cavities in a water-swollen state with an affinity for the imprint's analog, glucose'.[9] To better understand the basis of the binding properties exhibited by this remarkable system, we undertook the additional studies reported here.

Following the general procedure of the Kofinas group,[9,10] we prepared crosslinked hydrogels in the presence of GPS-Ba, D-glucose, L-glucose, $BaHPO_4$, and D-gluconamide;[11,12] a control hydrogel was also prepared in the absence of a template. By using D-glucose and $BaHPO_4$ as templates, we had hoped to dissect the interactions of the GPS-Ba with the PAA·HCl. We included D-gluconamide in our studies because it is a potential substrate for an intramolecular amide cleavage reaction[13] (our ultimate goal is to generate catalytic molecular imprints). Finally, we were especially interested in L-glucose—both as a template and an analyte—as a probe of enantioselective binding.

For all six hydrogels, batch binding studies were conducted in deionized water with the analytes D-glucose, L-glucose, D-fructose, and D-gluconamide.[14] (Although the Kofinas group had also measured the extent of bind-

Keywords: Hydrogels; Molecular imprinting; Poly(allylamine); Glucose; Fructose.
* Corresponding author. Tel.: +1 413 542 2731; fax: +1 413 542 2735; e-mail: dehansen@amherst.edu

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.09.054

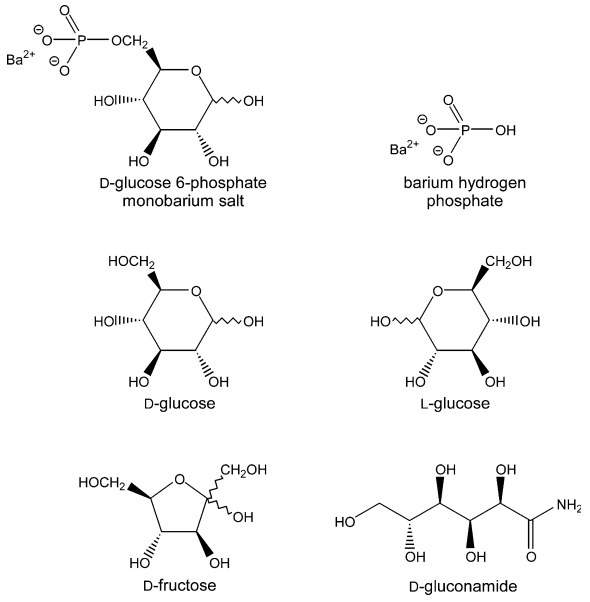

Figure 1. The structures of the derivatives employed in this study.

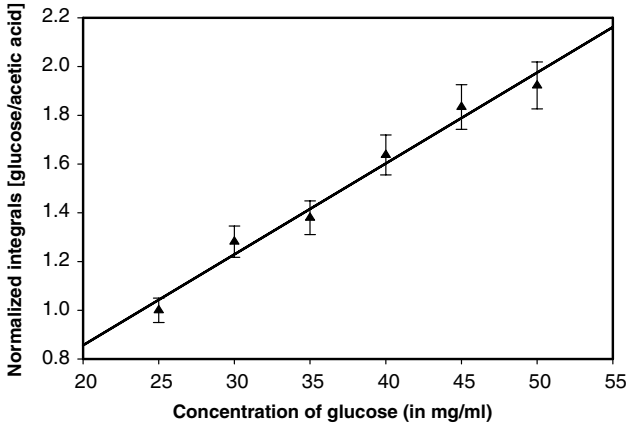

Figure 2. Calibration curve for D-glucose ($n = 6$, $R^2 = 0.981$) generated from the ^{1}H NMR spectra of six solutions of known concentration. The ratio of the integral of 25 mg/mL D-glucose relative to that of the acetic acid standard is normalized to 1.0. The error bars indicate the ±5% range routinely observed in the measurement of the integrals.

ing in pH 7 buffer, greater discrimination for glucose over fructose had been observed in deionized water,[9] and we thus did not use buffer in our experiments.) The structures of all the templates and analytes are shown in Figure 1.

While the Kofinas group had determined the extent of binding colorimetrically,[9,10] we employed an ^{1}H NMR assay that entails integrating signals for the sugar relative to an internal acetic acid standard; the amount of sugar remaining in solution is then determined by reference to a standard curve. The calibration curve for D-glucose is shown in Figure 2. (This assay does not require reagents specific to the sugar of interest and thus is general for any analyte.)

The raw data from our binding studies are shown in Table 1; the sugar-binding capacities of the hydrogels (in mg of sugar bound per gram of dry hydrogel) calculated from these data are shown in Table 2. Our results indicate that all of the hydrogels, irrespective of template, preferentially bind D-glucose over D-fructose. The values for the separation factors α—defined as [(binding capacity for glucose)/(binding capacity for fructose)]— shown in Table 3 indicate that this enhanced binding of glucose is essentially independent of template. Moreover, in contrast to the results reported by the Kofinas group, we observed that the hydrogel formed in the absence of template has a higher affinity both for glucose and for fructose as compared with the hydrogel formed in the presence of GPS-Ba. The α value for D-glucose verses D-fructose binding by the untemplated hydrogel is lower than that for all five of the templated hydrogels, but the difference is not large.

To further probe the specificity of binding, we measured the affinity of all six hydrogels for L-glucose—enantioselective binding is a hallmark of organic polymers imprinted with optically pure chiral templates.[15] (The hydrogels prepared with achiral $BaHPO_4$ and in the absence of template serve as controls.) All of the hydrogels, however, bound both enantiomers of glucose with essentially equal affinity (Tables 1 and 2).

Our results indicate that molecular imprinting is not responsible for the different binding properties of these hydrogels. Rather, the binding data correlate to the octanol–water partition coefficients (P_{oct}) of the analyte sugars. As shown in Figure 3 for the hydrogel generated in the absence of a template, a plot of log [binding capacity] vs. $\log P_{oct}$ yields a straight line. (Similar plots are obtained from the data for each of the templated

Table 1. The results of the ^{1}H NMR binding assay[a]

Template	Analyte sugar (mg/mL remaining in solution after incubation of a 50 mg/mL solution with hydrogel)			
	D-Fructose	D-Glucose	L-Glucose	D-Gluconamide
GPS-Ba	45.7 ± 2.3	38.6 ± 2.2	37.7 ± 2.4	31.2 ± 1.7
$BaHPO_4$	43.5 ± 1.0	34.3 ± 0.1	34.2 ± 2.3	32.6 ± 1.8
D-Glucose	43.6 ± 0.7	35.3 ± 1.1	36.1 ± 1.3	30.3 ± 1.4
L-Glucose	45.2 ± 0.4	35.7 ± 0.8	36.2 ± 2.6	31.0 ± 1.6
D-Gluconamide	46.3 ± 0.4	36.2 ± 0.0	38.2 ± 0.5	31.4 ± 1.1
None	41.8 ± 0.5	32.7 ± 1.0	35.4 ± 1.2	28.8 ± 0.7

[a] The final concentration of analyte sugar (in mg/mL) remaining in solution after incubation of a 50 mg/mL solution of sugar in deionized water with the hydrogels. A 0.40:15 w/v ratio of the dry polymer hydrogels to the analyte sugar solution was used. Each value shown is the average of two independent measurements with the range indicated as '±'.

Table 2. Sugar-binding capacity (mg of sugar bound/g of dry polymer hydrogel)[a]

Template	Analyte sugar			
	D-Fructose	D-Glucose	L-Glucose	D-Gluconamide
GPS-Ba	161 ± 86	428 ± 83	461 ± 90	705 ± 64
$BaHPO_4$	244 ± 38	589 ± 04	593 ± 86	653 ± 68
D-Glucose	240 ± 26	551 ± 41	521 ± 49	739 ± 53
D-Glucose	180 ± 15	536 ± 30	518 ± 98	713 ± 60
D-Gluconamide	139 ± 15	518 ± 00	443 ± 19	698 ± 41
None	308 ± 19	649 ± 38	548 ± 45	795 ± 26

[a] The values shown are calculated from the raw data in Table 1: Sugar-binding capacity = [(50 mg/mL initial analyte sugar concentration − analyte sugar concentration remaining in solution)/0.02667 g polymer per mL analyte sugar solution]. The larger errors than in Table 1 are due to the small amount of sugar bound relative to that remaining in solution.

Table 3. Separation factors α for D-glucose vs. D-fructose binding for each hydrogel[a]

Template	Range	Average
GPS-Ba	1.39–6.80	2.65
$BaHPO_4$	2.08–2.87	2.42
D-Glucose	1.92–2.77	2.30
L-Glucose	2.60–3.43	2.98
D-Gluconamide	3.37–4.18	3.73
None	1.87–2.38	2.11

[a] The α value = [(binding capacity for D-glucose)/(binding capacity for D-fructose)]. The range in each α value reflects the experimental error in Tables 1 and 2; the average α values are calculated from the average binding values in Table 2.

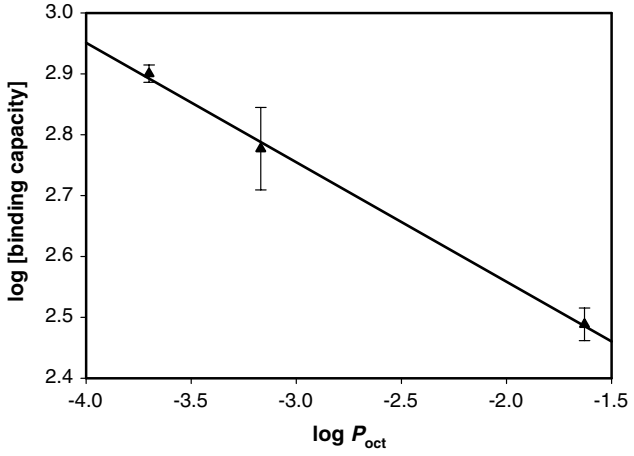

Figure 3. Log [binding capacity untemplated hydrogel] vs. log P_{oct} for the analyte sugars. The binding capacity for glucose was taken to be the weighted average of the binding capacity for D-glucose and L-glucose. The log P_{oct} values at 25 °C for glucose (both enantiomers will have the identical value), D-fructose, and D-gluconamide have been calculated to be −3.169 ± 0.858, −1.629 ± 0.870, and −3.700 ± 0.870, respectively.[17] The log P_{oct} value for glucose has also been experimentally determined as −3.24 by Sangster[18] and −2.82 ± 0.04 by Mazzobre et al.,[19] numbers that agree, within experimental error, with the calculated value.

hydrogels.) As log P_{oct} becomes more negative, indicative of an increased sugar polarity, adsorption into the hydrogel also increases. Thus, the differential solubilities of each sugar in the bulk polymer account for the binding data obtained. (An earlier study[16] on the partitioning of a series of drugs into the hydrogel poly-2-hydroxyethyl methacrylate also revealed a linear dependence on analyte polarity, but with more hydrophobic analytes preferentially binding in the hydrogel.)

Finally, we would note that two factors likely account for the discrepancies in the values reported here, as compared with those reported earlier by the Kofinas group,[9] for the glucose and fructose-binding affinities of the hydrogels prepared with GPS-Ba and prepared in the absence of template: one, the binding assays are run in unbuffered, deionized water (for the reasons discussed earlier), and thus small differences in pH are inevitable; and two, binding equilibrium is not reached in the 'standard testing time' of 4 h,[10] and thus kinetic factors will influence the data obtained.

In summary, polymer hydrogels prepared by crosslinking poly(allylamine hydrochloride) with epichlorohydrin in the presence of sugar templates do not exhibit imprinting on the molecular level. Instead, differences in the solubilities of the analyte sugars in the polymer hydrogel bulk account for the observed data, a conclusion consistent with the fact that far more sugar is bound by the hydrogels than the 1.5 mol % of template used in their generation.

Acknowledgments

We are grateful to the National Institutes of Health (R15 GM63776) and to the Amherst College Faculty Research Award Program, as funded by the H. Axel Schupf '57 Fund for Intellectual Life, for financial support. We are also pleased to thank the Howard Hughes Medical Institute for support of F.M.F. (through an Undergraduate Biological Sciences Education Program award to Amherst College).

References and notes

1. *Molecularly Imprinted Materials: Science and Technology*; Yan, M., Ramström, O., Eds.; Marcel Dekker: New York, 2005.
2. van Nostrum, C. F. *Drug Discov. Today: Technol.* **2005**, *2*, 119.
3. Kashyap, N.; Kumar, N.; Ravi Kumar, M. N. V. *Crit. Rev. Ther. Drug Carrier Sys.* **2005**, *22*, 107.

4. Byrne, M. E.; Kinam, P.; Peppas, N. A. *Adv. Drug Delivery Rev.* **2002**, *54*, 149.
5. Hawkins, D. M.; Stevenson, D.; Reddy, S. M. *Anal. Chim. Acta* **2005**, *542*, 61.
6. Xia, Y-q.; Guo, T-y.; Song, M-d.; Zhang, B-h.; Zhang, B-l. *Biomacromolecules* **2005**, *6*, 2601.
7. Hiratani, H.; Alvarez-Lorenzo, C. *Biomaterials* **2004**, *25*, 1105.
8. Oral, E.; Peppas, N. A. *J. Biomed. Mat. Res., Part A* **2006**, *78A*, 205.
9. Parmpi, P.; Kofinas, P. *Biomaterials* **2004**, *25*, 1969.
10. Wizeman, W. J.; Kofinas, P. *Biomaterials* **2001**, *22*, 1485.
11. In a typical procedure, a 25% w/v aqueous solution of poly(allylamine hydrochloride) (average molecular weight 15,000) [1.0 g PAA·HCl (10.7 mmol allylamine·HCl monomer) in 4.0 deionized water] was allowed to stir with 0.16 mol of the template (GPS-Ba, D-glucose, L-glucose, $BaHPO_4$, or D-gluconamide) for 2 h. 0.534 mL of a 10 M NaOH solution (5.34 mmol) was then added and the resultant solution was allowed to stir for 20 minutes, followed by the addition of 0.109 mL of (±)-epichlorohydrin (1.39 mmol). As indicated by the numbers above, the molar ratio of monomer/template/NaOH/crosslinker was 200:3:100:26, the values employed in the earlier reports.[9,10] Upon addition of the EPI, gelation occurred in 10–15 min, and the hydrogel was then allowed to sit undisturbed overnight. The next day, the hydrogel was cut into approximately 4 mm cubes with a razor blade and was then washed with gentle shaking in 4 M NaOH solution for 24 h to remove the template and any unreacted reagents. The NaOH solution was decanted off the hydrogel cubes, which were then repeatedly washed by gently shaking in deionized water over a period of 5 days. Each day, the hydrogels were washed 3–4 times for 1–2 hours, and after every wash the solution was decanted, and the pH measured with pH paper. After the 5 day period, overnight incubation of the hydrogels prepared with D-glucose, L-glucose, and D-fructose, as well as the one prepared in the absence of a template molecule, yielded a wash solution that was no longer basic (pH ≈ 6.5). However, even after the 5 days of washing, the pH of the wash solution for the GPS-Ba and $BaHPO_4$ hydrogels was still slightly basic (pH ≈ 8). These hydrogels were thus washed repeatedly for an additional 3–4 days, but the pH did not drop further. All the polymers were then dried, open to the air, for 18–24 h in an oven at 50 °C. Between 0.6 and 0.7 g of washed and dried hydrogel was routinely obtained. Assuming that all of epichlorohydrin had fully reacted (with loss of HCl) with the polyallylamine and that all salts and template had been fully washed away, 0.69 g of dried hydrogel would correspond to a 100% yield.
12. D-Gluconamide was synthesized using the method of Wolfrom et al.[13] 5.0 g of δ-gluconolactone (28.0 mmol) was dissolved in 18.3 mL of concentrated ammonium hydroxide (28–30%, ~0.3 mol), and the solution was allowed to sit for 24 h at room temperature. The solvent was removed by rotary evaporation, and the resultant solid was dried under vacuum for 2–3 days. The product obtained was used without further purification [mp 147.2–155.1 °C; ^{13}C NMR (DMSO-d_6): δ 175.7, 73.0, 72.6, 71.9, 71.0 and 63.9 ppm].
13. Wolfrom, M. L.; Bennett, R. B.; Crum, J. D. *J. Am. Chem. Soc.* **1958**, *80*, 944.
14. For the batch binding studies, a 0.40:15 w/v ratio of the dry polymer hydrogels to a 50 mg/ml solution of the sugar analyte (i.e., D-glucose, L-glucose, D-gluconamide or D-fructose) in deionized water (prepared from house distilled water using a Corning MP-3A still) was used. Typically the binding studies were performed on a scale of 50 mg of dried hydrogel in 1.875 mL of sugar solution. The mixture was gently agitated for 4 h,[9,10] and the amount of sugar still in solution (and hence not bound to hydrogel) was then immediately measured using ^1H NMR (recorded at 400 MHz on a JEOL GSX spectrometer). An aliquot of the solution was removed (typically 0.5 mL) and added to an equal volume of a standard acetic acid solution in D_2O [5:1000 (v/v) glacial acetic acid in D_2O]. An ^1H NMR spectrum was recorded and integrated (though the final solution contained 50% H_2O, the signals for sugar and the acetic acid methyl group were well resolved from the large water peak). The amount of sugar still present in solution was determined by reference to a calibration curve (see below), and the binding capacity of the hydrogels (mg of sugar bound per gram of dry hydrogel) then calculated. All binding studies were performed in duplicate, and the average of the two measurements was used to calculate the binding capacities. Calibration curves were constructed for D-glucose, L-glucose, D-gluconamide, and D-fructose by employing solutions of known concentration (25, 30, 35, 40, 45, and 50 mg/mL). As above, an aliquot of each of these solutions (usually 0.5 mL) was added to the same volume of the 5:1000 glacial acetic acid solution in D_2O, and an ^1H NMR spectrum recorded. The integration for signals of the analyte compound and the acetic acid methyl group was measured, and the ratio for the 25 mg/mL solution of analyte normalized to 1.0. A calibration curve was then constructed for each of the four analytes ($n = 6$; $R^2 = 0.991$ for D-fructose, 0.996 for D-gluconamide, 0.981 for D-glucose, and 0.994 for L-glucose).
15. Ramström, O.; Yan, M. In *Molecularly Imprinted Materials: Science and Technology*; Yan, M., Ramström, O., Eds.; Marcel Dekker: New York, 2005; p 1.
16. Pitt, C. G.; Bao, Y. T.; Andrady, A. L.; Samuel, P. N. K. *Int. J. Pharm.* **1988**, *45*, 1.
17. Data obtained from SciFinder® Scholar [calculated using Advanced Chemistry Development (ACD/Labs) Software V8.14 for Solaris, © 1994–2006 ACD/Labs].
18. Sangster, J. *J. Phys. Chem. Ref. Data* **1989**, *18*, 1111.
19. Mazzobre, M. F.; Román, M. V.; Mourelle, A. F.; Corti, H. R. *Carbohydr. Res.* **2005**, *340*, 1207.