

Isomeric glucose recognition using molecularly imprinted polymer hydrogels

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ABSTRACT

The goal of this research is to produce molecular imprinted polymers (MIPs), which selectively bind glucose over other sugars. MIP hydrogels against glucose exhibited binding capacities in excess of 0.6 grams of glucose per gram of dry gel in a 100 % DI H₂O glucose solution, as well as in a 50-50 % glucose-fructose solution mixture. Equilibrium binding capacities of fructose were lower than those observed with respect to glucose, indicating an isomeric preference for the binding of glucose over fructose. Although it is expected that imprinted cavities will be distorted due to the swelling of the hydrogel in water, our experiments show that even the swollen gels exhibit remarkable glucose recognition. This synthetic and characterization methodology for MIPs might thus offer exciting avenues for novel biomimetic recognition and isomeric separation techniques.

INTRODUCTION

In the past three decades many different polymeric hydrogel products have been developed. Hydrophilic polymeric networks are now found in applications including, but not limited to drug delivery, separation, human tissue mimics, liquid absorption, and even medical bandages. Current research attempts to further expand the scope of applications of polymeric hydrogels, through the synthesis, characterization and elucidation of biomimetic sugar recognition in molecularly imprinted polymer (MIP) networks for applications as glucose sensors [1-6].

The clinical relevance of the study presented in this research relates to the development of a pharmaceutical based on a polymer hydrogel MIP, which could aid in the treatment of type II diabetes, a disease which affects over 14 million people in the United States alone. Applying the glucose MIP to a pharmaceutical or a food additive could contribute to the dietary freedom of those who suffer from type II diabetes. By ingesting these materials, the glucose would be absorbed by the hydrogels in the intestine, thereby reducing the amount of sugar actually introduced into the blood stream, and prevent elevation of blood sugar levels after meals. The imprinting strategy of this research involves a polymer gel imprinted against glucose, using a sugar analogue, glucose phosphate monosodium or monobarium salt (GPS) as the template. A novel characteristic of the imprinting technique employed in this research is the choice of the phosphate salt of the glucose, rather than pure unmodified glucose, which results in enhanced affinity and binding of the template to the primary amine groups of the polymer, and thus improved specificity for the sugar.

While methods of template fixation vary among research groups, the large majority of molecular imprinting studies to date have concentrated on synthesizing imprinted polymers from a monomer, rather than crosslinking an existing polymer having the appropriate functional groups. Typically, polymerizable, functional monomers, capable of non-covalent interactions with the template molecule, are mixed in organic solutions with the template and allowed to associate. Our approach employs a more flexible non-covalent imprinting method, starting from a readily available polyamine polymer, and both MIP synthesis and testing are performed in aqueous solutions. The development of general aqueous methods using MIPs capable of specific recognition of biological analytes would have an enormous value in medicine and bioanalytics.

The experimental results presented deal with non-covalent molecular imprinting of poly(allylamine hydrochloride), (PAA•HCl) to produce molecularly imprinted polymer hydrogels (MIP) imprinted against glucose. The chosen polymer matrix, poly(allylamine) has good water solubility and presents a high density of amine groups. In its cross-linked form, this polymer possesses low toxicity and is biocompatible [7,8]. It has also been recently employed as a matrix for the molecular imprinting of glucose [9,10]. The GPS template displaces the previously associated HCl and bonds to the polymeric amine.

EXPERIMENTAL DETAILS

The molecular imprinting technique was used to create specific binding sites for glucose in crosslinked network polymers. Poly (allylamine hydrochloride) (PAA•HCl) was crosslinked in the presence of the Glucose Phosphate Salt (GPS) template molecule. The template was removed by washing the hydrogel with a base, to create cavities of specific shape size and functionality, which are able to rebind the glucose. The randomly crosslinked PAA•HCl networks were prepared by the aqueous reaction of a 25 % w/v solution of linear PAA•HCl chains and epichlorohydrin (EPI), which served as the crosslinker. The PAA•HCl used in the synthesis of the gels had an average molecular weight of 70,000 g/mole. Before imprinting the polymer with GPS, a portion of the HCl groups of PAA•HCl were neutralized with NaOH. This was done not to provide free amine sites for crosslinking. After association of GPS to the primary amine sites on the polymer, epichlorohydrin (EPI) was added to crosslink the neutralized amine sites. To remove the GPS template, the polymers were subjected to a NaOH wash, followed by a deionized water wash to remove excess NaOH. The specific details of the imprinting procedure have been published in [10].

Glucose and fructose binding capacities of the hydrogels were determined via batch reactor studies. The dried polymers were added to a 50 mg/mL aqueous solution of either glucose or fructose or a mixture of the two sugars. The 50 mg/mL concentration was intended to mimic the sugar concentration likely to be found in the stomach and duodenum after the consumption of a soft drink or sugary snack. This assumes a stomach volume of 1 liter and a consumption of 50 grams of sugar. The test solution and the MIP or non-imprinted polymer (NIP) being tested was then stirred slowly for 4 hours, whereupon filtered aliquots of the solution were taken to determine the remaining concentration of sugar in the test solution. In addition to the 4 hour glucose binding tests, an equilibrium binding experiment was performed, using a different gel batch, to determine the time necessary for complete equilibration and the maximum glucose

binding capacity of the MIP. In this experiment the test solution and the MIP were allowed to equilibrate under slow stirring for 36 hours.

RESULTS AND DISCUSSION

The MIPs synthesized during the preliminary studies using PAA•HCl and GPS have shown considerable affinity and specificity for the binding of glucose versus fructose. The glucose and fructose binding capacities of these MIPs were determined using equilibration tests in aqueous environments and are presented in Table I.

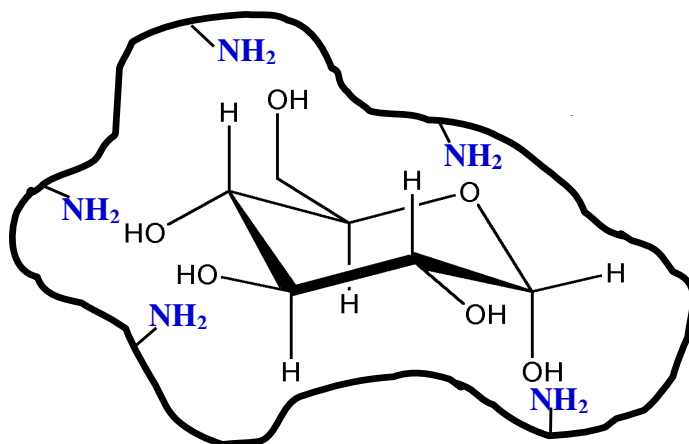


Figure 1. Schematic of glucose imprinting within a PAA•HCl cavity

MIP gels imprinted with GPS were tested in sugar solutions. Aqueous solution mixtures consisting of 50 % glucose and 50 % fructose were also tested with MIPs and non-imprinted control samples to determine whether the imprinted polymers can discriminate one sugar from the other in competitive binding experiments conducted in mixed sugar media. Table I shows the binding capacities for the barium glucose imprinted hydrogels. A non-imprinted hydrogel, NIP, was used as a control specimen to determine the effectiveness of the imprinting procedure. Imprinted hydrogel polymers showed higher affinity for glucose over fructose.

Glucose binding for the GPS-Ba MIPs was shown as high as ~ 600 mg of glucose per g of dry polymer hydrogel in a DI water glucose solution and in 50-50 % mixed sugar media. Fructose binding was considerably lower. The glucose imprinted gel bound only 110 mg of fructose per g of dry polymer hydrogel in a DI water fructose solution, and 84 mg of fructose per g of dry polymer hydrogel in 50-50 % mixed sugar media. The non-imprinted hydrogels showed considerably lower binding capacities compared to their imprinted analogues. The separation factors (shown at the end of each row in Table I indicates that there is 5- to 7- fold specificity for glucose over fructose in the imprinted gels compared to the non-imprinted control polymers. The separation factors, (shown at the bottom of each column in Table I) indicate that the imprinting procedure produces a 3- to 4- fold enhancement in glucose binding for the imprinted polymer, compared to a non-imprinted control sample with no significant increase in fructose binding. There is therefore significant specificity for glucose in the imprinted polymer gel. The

data also indicate that the MIPs are also able to separate glucose out of a 50-50 glucose-fructose mixture, thus demonstrating their ability to function in competitive environments.

	100 % glucose	100 % fructose	α
Ba imprint	593 \pm 3	110 \pm 19	5.39
No imprint	139 \pm 15	105 \pm 3	1.32
α	4.27	1.05	

	50-50% glucose-fructose		α
	glucose	fructose	
Ba imprint	601 \pm 32	84 \pm 2	7.15
No imprint	132 \pm 21	79 \pm 11	1.67
α	4.55	1.06	

Table I Glucose binding in pure and mixed sugar solutions

The results of the 36 hour equilibration test are given in Figure 2. Glucose binding capacities were calculated at 1, 2, 4, 6, 10, 16, 24, and 36 hours. It is clear that, initially the glucose binding capacity increases exponentially with positive curvature. However, the curvature of the trend becomes negative somewhere between 4 and 10 hours, leading to the final equilibrium binding capacity conditions seen at 24 and 36 hours. Consecutive equivalent binding capacities at 24 and 36 hours were taken to be sufficient evidence of equilibrium. It is interesting to note that at 4 hours, our chosen standard testing time, the binding capacity is approximately half of the observed maximum at 24 and 36 hours. However, the application of the MIP hydrogels as a pharmaceutical most likely would not be able to take advantage of the increased binding capacities observed at longer times due to the relatively rapid rate of glucose absorption in the human body.

A key issue in MIPs is the conformational relaxation of the binding pocket that is left exposed when the template is dissociated from the matrix polymer. Although it is expected that the imprinted cavities will be distorted due to the swelling of the hydrogel in water, the experimental results show that even the swollen gels show remarkable affinity to glucose.

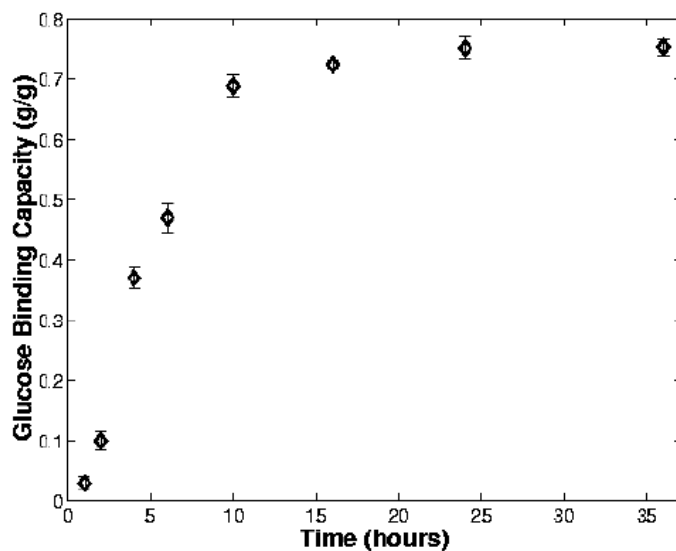


Figure 2. Equilibrium glucose binding experiment

CONCLUSIONS

The technique presented here not only employs the more flexible non-covalent approach to imprinting, but also begins with a polymer having an appropriate functionality, instead of functional monomer, and is performed in aqueous solution under air. Our experiments have shown that the proposed MIP gels have overcome the main problem of recognition in a water-swollen state, i.e. the influence of flexibility of the polymer sequence between two crosslinking points. It is worthy to note that the prevalence of compounds capable of ionic interactions and hydrogen bonding with amines may allow an immense number of compounds to be imprinted using the poly(allyl amine) polymer system.

The experimental results presented in this paper demonstrated that the molecular imprinting procedure produced recognizable cavities in a water-swollen state with an affinity for the glucose imprint. Success has been demonstrated in discriminating between glucose and fructose. The work presented here showed that the binding capacities of glucose in the molecularly imprinted hydrogels using GPS-Ba as a template were as high as 600 mg of sugar bound per grams of dry polymer. The binding capacities in the non-imprinted control polymers were significantly lower in all cases. In addition, significant specificity for glucose was demonstrated by the MIPs using GPS-Ba and this was demonstrated by 7-fold separation factors. The glucose and fructose binding capacities in mixed sugar media indicated that the glucose imprinted gels exhibited affinity and specificity for glucose over fructose in competitive binding environments.

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