Talanta 76 (2008) 1119–1123

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Analysis of recognition of fructose by imprinted polymers

Rajagopal Rajkumar[a,b], Axel Warsinke[a], Helmuth Möhwald[c], Frieder W. Scheller[a,b], Martin Katterle[b,*]

[a] Analytical Biochemistry, Potsdam University, D-14476 Potsdam-Golm, Germany
[b] Fraunhofer Institute for Biomedical Engineering (IBMT), D-14476 Potsdam-Golm, Germany
[c] Max Planck Institute of Colloids and Interfaces, D-14476 Potsdam-Golm, Germany

ARTICLE INFO

Article history:
Received 10 January 2008
Received in revised form 12 May 2008
Accepted 14 May 2008
Available online 23 May 2008

Keywords:
MIP sensor
Thermistor
Covalent imprinting
Fructose
Cross-reactivity
TRIM

ABSTRACT

Binding of fructose to the fructose imprinted polymer (MIP(Frc)) and pinacol imprinted polymer (control) were studied both in batch and a flow through mode. The influence of the cross-linkers ethylene glycol dimethacrylate (EDMA) and trimethylolpropane trimethacrylate (TRIM) on the binding characteristics was analysed. TRIM cross-linked MIPs showed a lower (unspecific) binding for the control polymer (pinacol imprinted) and higher binding of fructose as compared with the EDMA-MIPs. Furthermore interactions of a TRIM cross-linked molecularly imprinted polymer against fructose and its corresponding template were studied using a thermistor. Label-free detection of fructose was realised in the range of 0.5–10 mM. The difference in enthalpy changes between specific binding of fructose to boronic acid moieties of the MIP and non-specific binding to the matrix leads to an 18-fold higher apparent imprinting factor than batch binding studies. Cross-reactivity studies using MIP sensor indicate that the interaction of fructose to MIP generates higher signal than disaccharides. The studies described in this paper demonstrate the potential of direct characterisation of molecular binding events.

1. Introduction

Molecular imprinting is a method for preparing polymers possessing microcavities having a predetermined selectivity for an analyte of interest. Based on template–monomer interactions MIPs have been synthesised using covalent, non-covalent and semi-covalent approaches [1–5].

MIPs towards carbohydrates and their derivatives were synthesised making use of hydrogen or covalent bonds between functional monomers and templates. These MIPs were found to be suitable for chromatography and sensing. Aromatic boronic acids have the unique property of forming reversible complexes with *cis*-diols, e.g., in sugars. The resulting cyclic diesters can be prepared either in organic solvents or in aqueous alkaline media. Apart from aryl boronic acids, metal complexes and non-covalent interactions were successfully used for preparing MIPs targeting carbohydrates and their derivatives [6,7].

In this paper the covalent approach was employed to prepare a MIP against fructose. Since typically 80–90% of the MIP matrix consists of cross-linker and the template–monomer content is usually between 5 and 10% the cross-linking agent will have an influence on the binding properties of the polymers [8–10]. For this reason we compared MIPs prepared by using two different cross-linkers.

As MIPs are robust and can be re-used, their application in sensing is promising and they are used as recognition elements with transducers like Quartz crystal microbalance, field effect transistors, surface plasmon resonance, and calorimeters [11–37]. MIP based fructose sensors use fluorescence or electrochemical methods for signal generation [38,39]. In this paper, we investigate the MIP–analyte interactions by using a flow calorimeter whereby label-free detection of fructose is demonstrated. Further the cross-reactivity was evaluated using the MIP thermistor.

2. Experimental

2.1. Chemicals and reagents

D-Fructose was purchased from Merck, Germany. D-Sucrose, D-maltose was obtained from Serva Feinbiochemica, Germany. Nitrobenzene, trimethylolpropane trimethacrylate (TRIM) and 4-vinylphenyl boronic acid were purchased from Aldrich, Germany. Anthrone, 2,2′-azobisisobutyronitrile (AIBN), dioxane and ethylene glycol dimethacrylate (EDMA) were obtained from Fluka, HPLC grade methanol (MeOH) was purchased from Carl Roth, Germany.

* Corresponding author. Tel.: +49 331 58187 503; fax: +49 331 58187 299.
E-mail address: Martin.Katterle@ibmt.fraunhofer.de (M. Katterle).

0039-9140/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2008.05.022

2.2. MIP synthesis

MIPs were synthesised according to Wulff's covalent approach described earlier [40]. To prepare the MIP(Frc) 1 g of β-D-fructopyranose 2,3;4,5-bis-*O*-((4-vinylphenyl)boronate), 1 ml of toluene:acetonitrile (1:1), 26.5 mmol TRIM or 45.3 mmol of EDMA and 3.98 mmol of AIBN were added, mixed well and purged with nitrogen for 10 min. Free radical polymerisation was initiated and carried out at 65 °C for 48 h. Temperature was increased to 95 °C and kept constant for the next 24 h for the final curing of polymer. In analogy the control polymer (pinacol as template) and the MIP(Fru-Val) were prepared by applying 1 g of the respective 4-vinylphenyl boronate esters. Therefore the control polymer contains almost 70% more and the MIP(Fru-Val) has 20% less binding sites as fructose imprinted polymer. The polymer monoliths obtained were crushed, ground in a ball mill (Retsch type S 100, Germany) for 10 min at 400 rpm and wet sieved (mesh 25 μm) using acetone to remove the fines. For template removal polymer particles were washed with 500 ml of water/methanol (1:1, v/v) and the final washing was done with 100 ml methanol.

2.3. Batch experiments

To 10 mg of polymer 1 ml of fructose (0.1–5 mM) in 100 mM sodium carbonate buffer containing 10% MeOH (pH 11.4) was added and incubated at 26 °C. After 12 h the MIP suspension was centrifuged at 13,000 rpm for 10 min and the supernatant was used to determine the concentration of unbound fructose. Two hundred microliters of supernatant and 1800 μl of anthrone tryptophan reagent was added, mixed well and incubated for 1 h at 65 °C. The absorbance was measured at 540 nm with a Shimadzu 160-A UV–vis spectrophotometer. The fructose concentrations in the supernatants were calculated using a fructose calibration curve [41]. All measurements were performed as triplicates and the error bars indicate the derived standard deviation.

2.4. Thermometric measurements

Thermometric measurements were carried out in an enzyme thermistor developed by Mosbach and Danielsson [42]. Hundred milligrams of the imprinted or control polymer was packed in a 500 μl plastic column. The MIP column was placed in an insulated thermistor block. Analyte solution was pumped into the thermistor block using a peristaltic pump for 10 min followed by neat buffer (100 mM sodium carbonate pH 11.4, 10% MeOH) for the next 10 min. A constant flow rate of 1 ml/min was used in all experiments. All measurements were performed as triplicates and the error bars indicate the derived standard deviation. Thermometric experiments with control polymer was done in the same way as described for imprinted polymer. All analytes (fructose and disaccharides) were dissolved in 100 mM carbonate buffer, pH 11.4, 10% MeOH before the start of the experiments and used immediately.

3. Results and discussion

3.1. Batch binding studies

Batch binding studies were carried out for different time intervals by incubating the MIP suspension with 0.1 mM fructose in 100 mM carbonate buffer/pH 11.4 containing 10% methanol to determine the time to reach the equilibrium. Fig. 1 shows that the equilibrium was reached after 2 h. For further binding studies an incubation period of 2 h was therefore considered as sufficient.

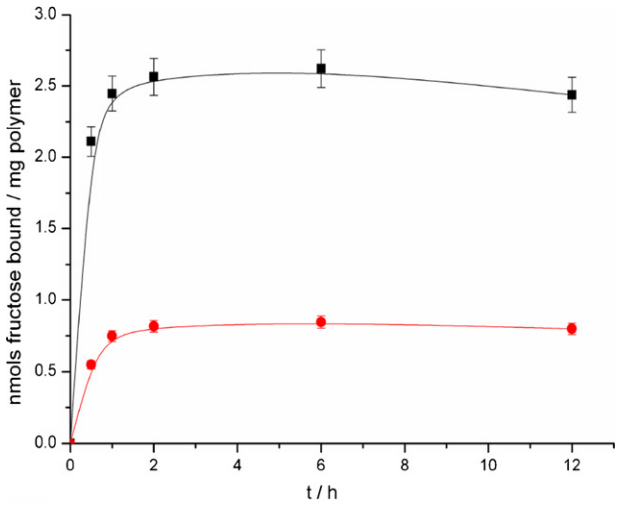

Fig. 1. Time dependence for fructose binding to fructose imprinted (squares) and pinacol imprinted (circles) polymers. Ten milligrams of TRIM cross-linked polymer + 1 ml of 0.1 mM fructose in 100 mM sodium carbonate buffer containing 10% methanol, pH 11.4; 0–12 h; 26 °C.

3.2. Influence of cross-linker on molecular recognition

Polymers prepared in parallel with EDMA and TRIM were compared to study the influence of the cross-linker.

Since the selectivity of MIPs might not only be determined by the print molecule but also by the nature of the cross-linker used for polymerisation, binding of fructose to MIPs prepared by EDMA and TRIM, respectively, against fructose MIP(Frc), fructosyl valine MIP(Fru-Val) and pinacol (control polymer) were compared in batch studies (Table 1).

At equilibrium TRIM cross-linked MIP(Frc) binds 3.6-fold more fructose than control polymer and 1.4-fold more fructose than MIP(Fru-Val). On the other hand, the EDMA cross-linked MIP(Frc) binds only 2.5-fold more fructose than the control polymer, and almost the same amount is bound to the MIP(Fru-Val). In general the TRIM cross-linked polymers showed lower unspecific binding compared with EDMA cross-linked polymers. These results indicate that better complementarity to the template is obtained with TRIM cross-linked materials than using EDMA, making TRIM a better cross-linker for fructose imprinting.

TRIM is a trimethacryl ester and it has been shown to be an ideal cross-linker in comparison with DVB and EDMA for imprinting penicillin [43], R-phenylbutyric acid [44] and protected dipeptides [8]. Wulff has shown that the enantiomeric selectivity of covalent MIPs is strongly dependent on the nature and amount of cross-linker [9]. When divinylbenzene was used as a cross-linker the stiffness and hyrdophobicities of the polymer increased severely thereby reducing the accessibility of cavities as shown by smaller percentage of templates that can be removed. However when using

Table 1
Influence of cross-linkers TRIM and EDMA on fructose binding to MIP and control polymer

	TRIM as cross-linker (nmol fructose/mg)	EDMA as cross-linker (nmol fructose/mg)
MIP(Frc)	2.5	2.3
MIP(Fru-Val)	1.7	2.2
Control	0.7	0.9