A systematic approach to forming micro-contact imprints of creatine kinase

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A systematic approach has been used to form molecular imprints of creatine kinase (CK) using micro-contact imprinting. Using thermocalorimetry data, we selected poly(ethylene glycol) 400 dimethacrylate (PEG400DMA) as our crosslinker, on the basis that it would be expected to have minimal specific recognition when incorporated into the imprinted polymer. The functional monomer used, methacrylic acid (MAA), was chosen from a panel of six candidates on the basis of it giving the highest differential affinity with respect to a non-imprinted polymer. A polymer formed with 5% MAA and 95% PEG400DMA showed excellent imprint recognition, with CK binding to the imprinted material being $2.05 \pm 0.07 \times 10^{-10}$ mol cm^{-2} compared to $9.1 \pm 4.5 \times 10^{-12}$ mol cm^{-2} control binding. The imprinted polymers (approximate thickness 22.6 μm as measured by Alpha-step) showed clear two-phase binding with maximum absorption achieved after approximately 2 hours. Data extracted from Scatchard plots showed the K_d for the high affinity binding site population to be 2.56×10^{-10} M and the binding site population to be 1.97×10^{-10} mol cm^{-2}, corresponding data for low affinity binding sites shows the $K_d = 3.27 \times 10^{-9}$ M and the binding site population to be 2.32×10^{-10} mol cm^{-2}. Re-binding the molecularly imprinted polymers (MIPs) with non-template proteins, namely myoglobin, human serum albumin (HSA) and immunoglobulin G (Ig G), showed these proteins to have comparatively little affinity for the CK imprinted films. The percentage re-binding figures, relative to CK binding, were: 18.7, 3.5, and 3.5 for myoglobin, HSA, and Ig G respectively. This pattern of binding was maintained in competitive binding protocols with two proteins in solution at equal concentrations, where the percentage re-binding figures, relative to CK binding ($4.5 \pm 0.06 \times 10^{-10}$ mol cm^{-2}), were 17.2, 4.5, and 2.9 for myoglobin, HSA, and Ig G respectively. The presence of multiple competing analytes in undiluted human serum did not significantly decrease template protein recognition. Finally, we used circular dichroism to monitor protein denaturation, and showed that the denatured template protein loses a significant proportion (76.8%) of its MIP affinity after being heated at 80 °C for 10 minutes.

Introduction

Creatine kinase (CK), also known as creatine phophokinase, normally functions as an intracellular enzyme that has a pivotal role in energy production by catalysing the formation of adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and creatine phosphate.

As a result of being a dimeric molecule [with subunits termed M (muscle) and B (brain)], creatine kinase is able to exist as 3 distinct isoenzymes (CK-MM, CK-MB and CK-BB). The expression pattern of the CK isoenzymes is tissue dependent. Not surprisingly, skeletal muscle expresses predominantly CK-MM (98%) and correspondingly low levels of CK-MB and CK-BB (both at approximately 1%). Cardiac muscle however expresses a significantly higher percentage of CK-MB (~25%). Healthy individuals have serum CK in which the dominant isoenzyme is CK-MM. However, in individuals suffering from cardiac trauma, with associated tissue disruption, there will be a reduction in the CK-MM/CK-MB ratio measured in circulating CK.[1–6]

Although CK isoenzyme ratios have been used extensively as indicators of myocardial damage in heart attacks, producing standard reference values to express the 'normal' concentration of circulating CK is problematic, due to the diversity of the assay methods currently in use. Among the methods commonly employed to determine the amount of CK in circulation and its isoenzyme composition are agarose gel/cellulose acetate electrophoresis followed by fluorometric/spectrophotometric based quantification.[3] Other methods in use for CK determination include column chromatography, radioimmunoassay and NMR spectroscopy.[7–9]

Molecular imprinting is a rapidly maturing branch of synthetic recognition chemistry that employs artificial recognition structures formed either on the surface, or within cavities, of synthetic polymers. The recognition structures formed are ideally complimentary in both form and function to either a given 'template' or to a smaller part, or epitope representative of the template. Non-covalent molecular imprinting is a means to create such recognition structures using relatively simple chemistry in which the shape of the recognition structure together with the placement of specific molecules (functional monomers), able to mediate recognition, is directed by the template itself prior to the polymerisation process.[10]

Until recently ideal imprinting targets were restricted to relatively small, but often highly functionalised molecules; however,

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recently several groups have made significant in-roads into the imprinting of bio-macromolecules, especially proteins. Several excellent reviews have recently been published relating to the state-of-the-art with respect to protein imprinting.[11,12]

Among the advantages of molecularly imprinted polymers (MIPs) are: their ease of fabrication, robustness, simplicity of use, and the cost effectiveness of their synthesis. Having become established as viable materials in separation techniques such as HPLC and solid phase chromatography, MIPs are now being shown to a practical means to achieve bio-macromolecular recognition.[13]

Given the advantages of MIPs noted above, a sensor incorporating such materials and able to detect and quantify CK would potentially offer a simple and cost-effective means of determining a subject's CK status. An interesting and up-to-date review focused on the use of MIPs in sensing applications is given by McClusky *et al.*[14]

Materials and methods

Materials

Methacrylic acid (MAA), creatine kinase (MM, mol wt. ~81,000) from rabbit, 2-hydroxyethyl methacrylate, human serum albumin (96–99%), myoglobin (mol wt. 17,600; 90%), immunoglobulin G and Tween 20 were all obtained from Sigma. Poly(ethylene glycol) dimethacrylate (mol. wt. 550), poly(ethylene glycol) dimethacrylate (mol. wt. 875), trimethylolpropane trimethacrylate, glycerol dimethacrylate (GDMA), pentaerythritol tetraacrylate (PTTA), 1,3 butanediol dimethacrylate and glycerol 1,3-diglycerolate diacrylate (GDGDA) were obtained from Aldrich. Styrene and sodium hydroxide were obtained from Showa, Japan. Acrylamide and sodium dodecyl sulfate were purchased from J.T. Baker. Acrylic acid (AA), ethylene glycol dimethacrylate (EGDMA), divinyl benzene (DVB) and 1,6 hexanediol dimethacrylate (HEMA) were obtained from Fluka. Methyl methacrylate (MMA) was purchased from the Kanto Chemical Co. Inc., Tokyo, Japan. Potassium chloride, sodium phosphate dibasic dehydrate, sodium chloride and hydrochloric acid were purchased from Riedel-de-Haën. Goat polyclonal antibody to creatine kinase (MM) was obtained from Abcam (UK) while Enhanced Chemiluminescence (ECL) reagents were supplied by Amersham Biosciences. All other chemicals/solvents were obtained as either Analar or HPLC reagent-grade materials from normal commercial sources.

Microcalorimetry measurements were made using a Thermometric 2277 thermal activity monitor supplied by Thermometric AB Sweden. Atomic force microscopy images were made using a scanning probe microscope from Digital Instruments Inc., Santa Barbara, USA. Ultra violet/visible spectra/absorbances were recorded using a Shimadzu UV-160A spectrophotometer. Film thickness determinations were made using an Alpha-step 500 supplied by Tencor. Photochemical reactors manufactured by the Panchum Scientific Corp., Taiwan, were used to form the polymeric thin-films.

Methods

Preparation of materials. As in our previous studies,[15–18] a micro-contact approach was used to form the imprinted sites for the target protein. In this study a glass support was used to 'carry' the functional monomer and the crosslinker; while a cover glass, treated so as to increase protein adhesion, was used to introduce the CK into the polymer mixture prior to polymerisation. Prior to use the supporting slide and the cover glass were prepared in a manner broadly similar to our previous studies.

In brief, the supporting substrate glasses (1.3 cm × 1.3 cm) were sequentially washed (10 minutes each stage in a sonicator bath) in: sodium hydroxide solution (1 M); deionised water; hydrochloric acid (1 M) and finally again deionised water. Finally, prior to use the glasses were modified by immersion in a homogenous solution made as glacial acetic acid (69 μl), additionally containing 0.4% methacrylic acid 3-triethoxysilylpropyl ester (769 μl) at 80 °C for 4 hours before being dried in a nitrogen stream.

The cover glasses were sequentially treated (30 minutes each stage in a sonicator bath) in a solution of Sodosil® RM02 (10 ml in 30 ml deionised water) at 55 °C prior to being washed in deionised water and then in isopropanol and again in water. This washing procedure was finally repeated with ethanol, after which the cover glasses were blown dry in a nitrogen stream.

Imprint formation and template protein extraction. The cover glasses, treated as above, were immersed in a solution of CK [made with CK-MM as 2.25 mg in 100 ml phosphate buffer (adjusted to pH 7.4)] at 37 °C for 2 hours prior to drying in a nitrogen stream. The crosslinkers and functional monomers examined in this study were mixed and added to the substrate glass together with the initiator 2,2-dimethoxy-2-phenylacetophenone [DMPAP (2 wt.%)]. Imprinted films (having an approximate thickness of 22.6 μm as measured by Alpha Step) were formed by bringing together, *i.e.* contacting, the support and cover glasses prior to UV irradiation for 1 hour. Non-imprinted polymers made as controls were formed in the same way except that no protein was adhered to the cover glass prior to contacting.

Protein was removed from the films in a two-stage process. Initially the films were treated in trypsin/phosphate buffer at 37 °C for 3 hours prior to being washed (4 washes each of 10 minutes) in phosphate buffer. The films were subsequently washed in 1% SDS, containing 0.4 wt.% NaOH, at 80 °C for 30 minutes and then again washed (4 washes each of 10 minutes) in phosphate buffer.

Protein re-binding. All proteins were re-bound at ambient temperature in phosphate buffer (pH 7.4) using protocols similar to those described in our previous papers.[15,18] In brief, the films – imprinted or controls – were incubated for 2 hours with protein made in phosphate buffer to a final concentration (for each individual protein present in solution) of 2.7×10^{-7} M. After incubation, but prior to protein quantification, the films were immersed in fresh phosphate buffer and agitated (50 rpm for 10 minutes using an orbital shaker) to remove non-adherent protein.

Protein detection. CK in solution was able to be directly determined spectrophotometrically while adherent protein on the surface of the thin-films was quantified by ELISA. Briefly, to quantify adherent CK, the films were immersed in a blocking solution comprising reconstituted dried milk powder (5 wt.% in PB), additionally containing BSA (2 wt.%), and then left overnight in a refrigerator, after which they were washed (4 times, 10 ml/10 min each wash) in phosphate buffered saline also containing Tween20 (0.1 wt.%), adjusted to pH 7.4 (PBST). A

primary CK antibody (2 ml for each glass substrate made as 1 µl/ml in PBS, pH 7.4) was added to the films. These films were then incubated (3 h at 37 °C) before washing in PBST as described above, after which a secondary antibody, goat anti-rabbit IgG, carrying the peroxidase conjugate to the anti-CK antibody, was introduced (2 ml for each glass substrate made as 2 µl/ml in PBS, pH 7.4) to allow colour development. Incubation was allowed to proceed for 3 h at room temperature. ECL reagents were introduced to the incubation wells in accordance with the manufacturer's instructions and incubation was allowed to proceed for 3 min prior to plate reading. Data points reproduced in this paper show error bars as SEM values with $n \geq 3$.

Thermocalorimetry measurements. Heat change measurements were made in a manner broadly similar to those described in our previous paper.[19] Briefly, duplicate glass slides (2.6 cm × 2.6 cm), each carrying either the NIP or the MIP thin-film (after template protein extraction), were placed in the titration and reference cells together with 3 mL phosphate buffer (pH 7.4). Protein solutions (100 µL), made as 3 mg/10 mL phosphate buffer, were injected into the reaction cell after base-line stabilisation. Data collection was completed after the re-establishment of the baseline (maximum 4 hours). All area integrations were performed using dedicated software from the microcalorimeter's manufacturer.

Results and discussion

Imprinting strategy – selection of crosslinker and functional monomer

We initially took a panel of six crosslinkers as shown in Fig. 1, with which we formed non-imprinted films in the absence of a functional monomer.

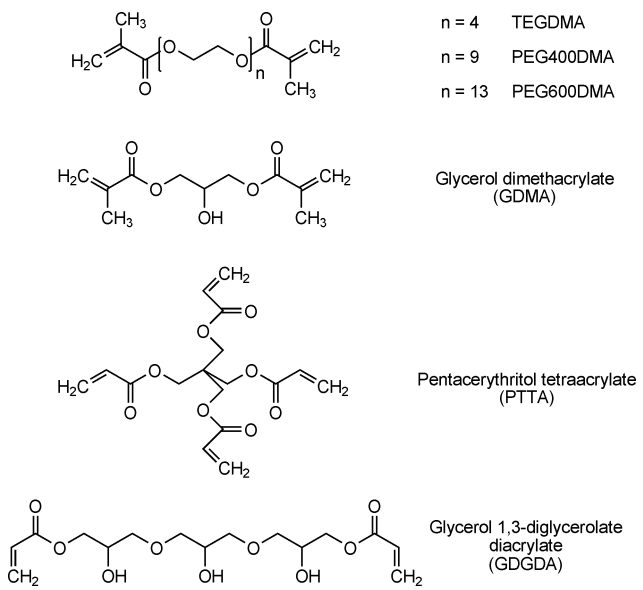

Fig. 1 The crosslinkers that were investigated in this study.

Each of these films was subjected to isothermal calorimetry, in which we measured the enthalpy change in response a single injection (100 µl) of CK solution made as 3 mg/10 ml in phosphate buffer adjusted to pH = 7.4. We reasoned that a significant exothermic change would be indicative of strong polymer-protein interaction: a situation we wished to avoid as our initial objective was to minimise non-specific interactions in our non-imprinted control polymers and thereby hopefully increase the selectivity of the final CK imprinted material.

Table 1 Measured heat changes ΔQ (mJ) shown in comparison with the measured amount of CK re-bound to the non-imprinted films. Conditions for film formation are described in the text. CK re-binding concentration (2.7×10^{-7} M in phosphate buffer), with detection of re-bound protein by ELISA. Re-bound amounts are the results of triplicate determinations

Functional monomer	Measured heat change ΔQ (J/mol)	Amount of CK re-bound to film ($\times 10^{-11}$ mol)
TEGDMA	-5.8×10^{7}	12.91 ± 0.25
PEG400DMA	-3.3×10^{8}	4.43 ± 0.71
PEG600DMA	-1.1×10^{8}	5.64 ± 0.50
GDMA	-1.0×10^{7}	26.7 ± 1.74
PTTA	-7×10^{6}	27.78 ± 2.65
GDGDA	-2.3×10^{7}	33.44 ± 0.61

All of the crosslinkers examined above showed enthalpy changes that were in varying magnitudes endothermic with respect to CK binding to the non-imprinted films, formed without functional monomer. Following the thermometric determinations we repeated the formation of non-imprinted films and measured the amount of adherent protein. Interestingly, we found a very good correlation between the amount of bound CK and the extent of the endothermic change observed for the films. These results are given in Table 1.

As PEG400DMA showed the lowest re-bound amount of CK when challenged with the protein we pursued our imprinting investigation using this as our crosslinker of choice. Using an initial ratio of crosslinker to functional monomer of 9:1 we examined the binding of CK to separate films made with a variety of functional monomers as shown in Fig. 2.

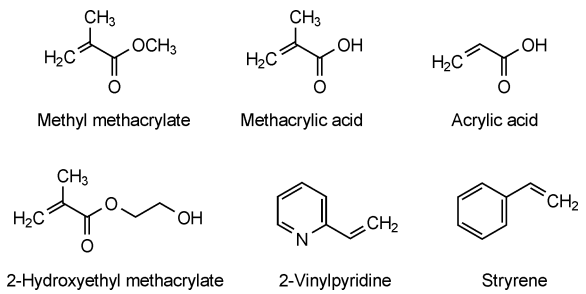

Fig. 2 The functional monomers used in this study.

The data generated from the comparison of films made with varying functional monomers enabled us to calculate, in a simple manner an imprinting factor α (eqn (1)), which takes into account non-specific binding by the control polymer and therefore provides us with a measure that indicates the specificity of binding by the imprinted material (all terms are in protein concentration units).

$$\alpha = \frac{\mathrm{MIP(after\ rebinding)} - \mathrm{MIP(after\ wash)}}{\mathrm{NIP(after\ rebinding)} - \mathrm{NIP(after\ wash)}} \quad (1)$$

The imprinting factors generated by this equation for the methacrylate functional monomers (MAA, HEMA and MMA)

Table 2 Imprinting factors for polymers formed with methacrylate-functional monomers

	MAA	HEMA	MMA
Imprinting factor (α)	49.8	8.4	2.3

are given in Table 2, while results obtained for styrene, acrylic acid and 2-vinylpyridine are given separately below.

The remaining, *i.e.* non-methacrylate monomers, styrene, acrylic acid and 2-vinylpyridine had imprinting factors of 38.9, 1.3 and 2.4 respectively. The mechanistic reason why the acrylates should give such significantly lower imprinting factors is not entirely clear, although it may be that differences in reactivity lead to phase separation. For the remainder of this study MAA (clearly having the largest imprinting factor when used together with PEG400DMA) was used to form the polymeric materials for imprinting and for the controls.

Effect of crosslinker/functional monomer ratio

Having initially used a CL to FM ratio of 9:1 we examined the imprinted and control re-binding profiles of polymers formed with varying ratios of cross-linker to functional monomer. We determined the binding to materials formed when the functional monomer was present in amounts varying between 5 and 25 vol.% of the final film. The re-bound amounts of CK-MM determined for these materials are shown in Fig. 3.

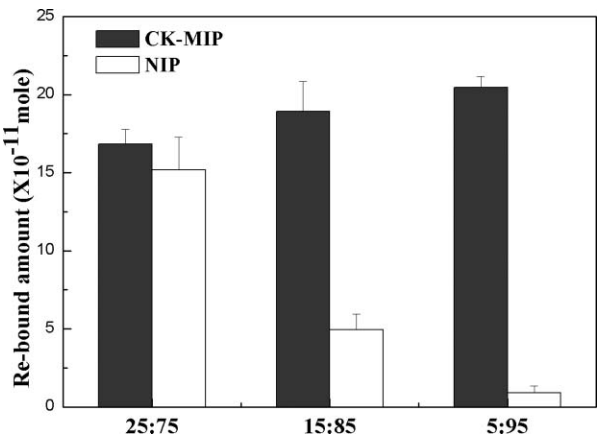

Fig. 3 Resulting binding determined after forming CK-MIPs and NIPs with PEG400DMA and MAA. The x-axis ratio is the functional monomer to cross-linker volume ratio.

The degree to which the functional monomer is present in the polymer is critical for generating recognition in the imprinted materials, in comparison to the non-imprinted controls, when the template is re-bound.[20,21] From the figure, binding to the NIP is seen to decrease rapidly in the absence of the functional monomer, indicating that at higher functional monomer concentrations recognition becomes less specific.

Effect of trypsin concentration on CK removal

We investigated varying the amount of trypsin in the initial stage of protein template extraction from the films. Amounts ranging from 0.01 g to 0.4 g trypsin in 100 mL phosphate buffer were found to be almost uniformly effective in removing the template protein, leaving residual bound amounts of CK typically quantified at $<2.5 \times 10^{-11}$ mol. Similarly, experiments to examine the effect of temperature on the second stage of the extraction process, *i.e.* the base wash with 1% SDS, additionally containing 0.4 wt.% NaOH, showed no appreciable difference in CK extraction (residual CK per film $<5 \times 10^{-11}$ mol in each case) over temperatures ranging from 22 °C to 80 °C.

Effect of re-binding time on CK adherence to the imprinted films

A series of imprinted films were made and incubated as above. At various time intervals, some were withdrawn from the CK-phosphate buffer incubation media to allow quantification of surface adherent protein. The amount of protein bound with respect to time is shown in Fig 4. As little or no change was observed after 120 minutes, we used this period of time for subsequent re-binding studies.

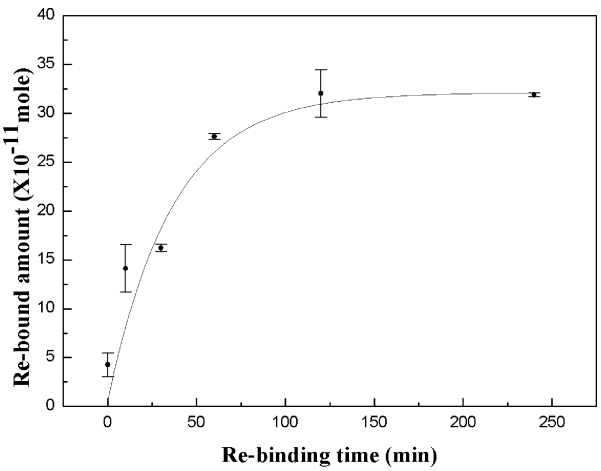

Fig. 4 The effect of changing the binding time on the re-bound amount of CK found on the CK-MIP.

Saturation curve and Scatchard plot

In order to establish an optimal protein concentration for subsequent protein re-binding experiments and also to examine the relative populations of high-affinity, *i.e.* well-imprinted sites, we performed re-binding experiments to generate a saturation curve (Fig. 5) and also a Scatchard Plot (Fig. 6).

Interestingly, our saturation curve shows little indication of protein-protein interaction, but rather is indicative of well-ordered binding to the point of saturation (approximately 10×10^{-8} M) and thereafter ceases to increase, as would be the case if additional protein in solution was binding to protein already immobilised on the polymer's surface.

While proteins are often considered 'difficult' templates to imprint in comparison with small, semi-rigid well functionalised molecules, we were nevertheless able to generate a distinct two-phase Scatchard plot for CK; see Fig 6 for the plot and Table 3 for extracted data.

Table 3 The equilibrium constant and binding site populations extracted from the Scatchard plot[a]

	Slope (M^{-1})	K_d (M)	Intercept	n^b (mol cm^{-2})
High affinity binding	−38.975	2.56×10^{-10}	129.84	1.97×10^{-10}
Low affinity binding	−30.56	3.27×10^{-9}	12.049	2.32×10^{-10}

[a] Surface area: 1.3 × 1.3 cm, re-binding volume: 10 mL. [b] n is the binding site population per square centimeter of CK-MIP surface.

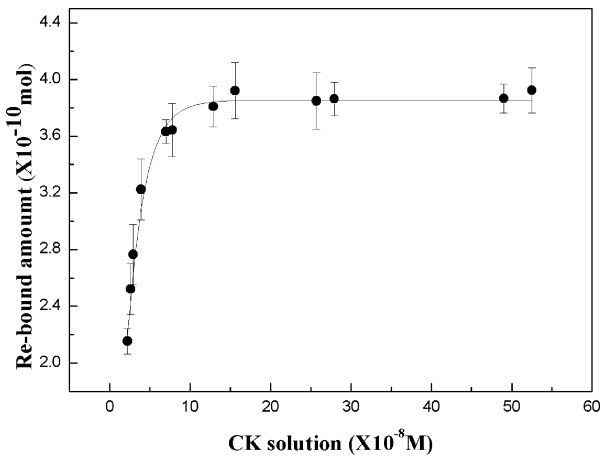

Fig. 5 Saturation curve of CK imprinted polymer re-bound with different concentrations (x-axis) of creatine kinase solution.

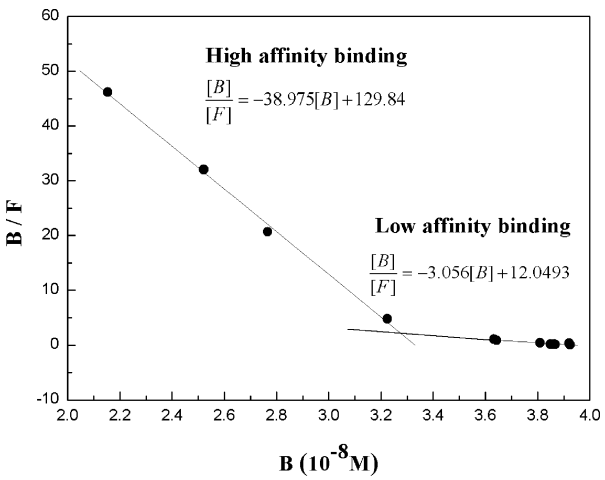

Fig. 6 Scatchard plot for CK re-binding by imprinted polymer.

CK imprinted polymer binding and selectivity

We examined the imprinted polymer's binding in both simple *i.e.* non-competitive situations with only one protein in solution together with the films, and in competitive binding experiments with CK and a competing protein present in solution at the same concentration. We then took this approach one stage further and replaced the phosphate buffer previously used for the re-binding protocols with undiluted human serum and again examined the re-binding to the films. As a final step we took our template protein and denatured it at 80 °C, a process which we were able to follow using ellipsometry, and were able to demonstrate a marked reduction in affinity of the denatured protein for its imprinted films compared to the native form of the protein.

In non-competitive environments

Imprinted and non-imprinted polymers were initially incubated with a series of proteins to assess the selectivity of the MIPs in a single protein environment. Fig. 7 shows the amounts of myoglobin, human serum albumin and immunoglobulin G bound to the imprinted and control films after incubation from 2.7×10^{-7} M protein solutions.

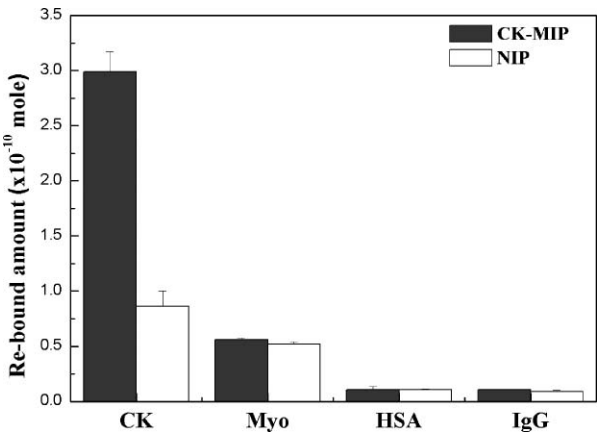

Fig. 7 Selectivity of CK-MIPs in single protein, *i.e.* non-competitive environments.

Interestingly, the CK imprinted material showed significantly greater affinity for its template protein with respect to the control, while the non-template proteins showed little or no difference in MIP vs. control binding. Put simply, none of the non-template proteins showed any marked degree of differential affinity for the CK-imprinted polymer or its control (i.e. MIP and control binding were nearly equal in each case), thereby supporting the notion that the CK imprinted sites are genuinely selective for CK.

In a two-protein competitive environment

Competitive binding experiments with two proteins (each present at 2.7×10^{-7} M) in solution were undertaken to establish if the materials made could still 'recognise' the original template in the presence of a competing protein-template and protein-protein interactions. Solutions were prepared with CK plus either myoglobin, HSA or IgG. In each case the CK imprinted material showed a clear and distinct recognition, as measured by binding, for the native template protein. Shown in Table 4 are the three protein combinations investigated together with the amount of each protein re-bound to the CK-imprinted and control films. In each case the films show little affinity for the competitor, and virtually no difference in affinity is noted between imprinted films and controls for the competitor proteins.

Re-binding in human serum

An additional experiment was undertaken to examine CK-imprinted film binding to its native template in undiluted human serum. Human serum is a solution comprising a complex natural matrix containing a variety of proteins and other significant

Table 4 CK binding to CK-MIPs and to non-imprinted controls in competitive environments: using myoglobin, human serum albumin and immunoglobulin G as the competing proteins

Protein combinations used for competitive binding		Protein re-bound ($\times 10^{-11}$ mol) CK-MIP	NIP
CK:Myo (1:1)	CK	45.3 ± 0.6	8.65 ± 1.5
	Myo	7.88 ± 0.2	6.5 ± 0.8
CK:HSA (1:1)	CK	38.0 ± 2.3	6.28 ± 1.0
	HSA	1.41 ± 0.2	1.19 ± 0.2
CK:IgG (1:1)	CK	40.7 ± 1.7	5.88 ± 1.4
	IgG	1.19 ± 0.1	1.18 ± 0.1

biomolecules. For any imprinted recognition material to find application in a practical sensor it must be able to maintain its recognition properties in such complex solutions.

Imprinted and control polymers were incubated in undiluted serum (9.9 mL) spiked with CK (0.1 mL) made as 1 mg CK/10 mL phosphate buffer. Remarkably, it was shown that the amount of CK rebound by the imprinted polymer was $1.2 \pm 0.03 \times 10^{-10}$ mol while only 20.8% of this amount (*i.e.* $2.51 \pm 0.26 \times 10^{-11}$ mol) was re-bound to the non-imprinted films under the same conditions. This result shows that the imprinted materials can maintain their recognition abilities in complex natural matrices.

Examination of denatured CK binding to imprinted and control films

Proteins, being heat labile, are easily denatured by moderate heating. The extent of degradation, especially of the protein's secondary structure, can be monitored using circular dichroism to show a decline in the measured ellipticity of polararization (θ) over a period of time (Fig. 8).

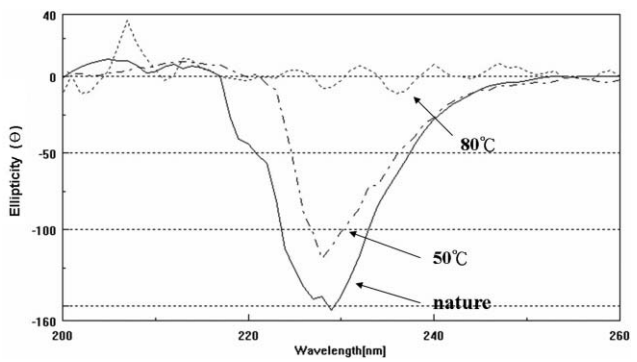

Fig. 8 Circular dichroism spectra for CK at ambient temperature and after heating at 50 °C and 80 °C. Time of heating at 80 °C was 10 minutes, CK concentration 3 mg in 10 mL PB.

The degradation of optical activity is seen in the figure as the temperature is raised towards 80 °C. At the level of the structure of CK this represents a loss of integrity, especially in the α-helix structures, of the molecule. This loss of structural information on heating is reflected in a markedly reduced binding affinity for the denatured protein by its imprinted film (Fig. 9). Although a degree of imprint control/selectivity was still apparent, the level of rebinding to the imprinted films was 2.05×10^{-10} mol for the native protein, compared to 4.75×10^{-11} mol in the case of the denatured CK.

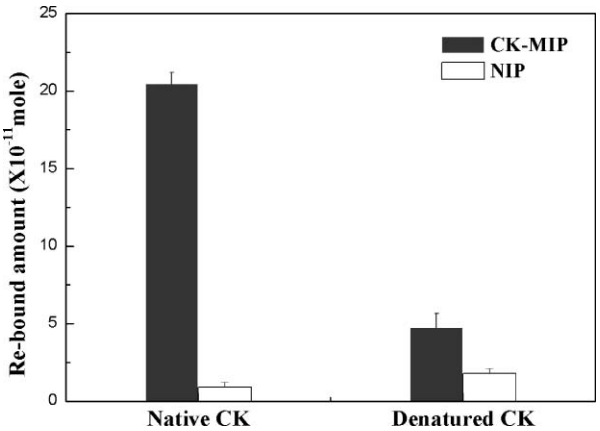

Fig. 9 Comparison of re-binding of native un-denatured CK and CK subjected to denaturation by heat treatment with imprinted and control films.

Conclusions

The micro-contact imprinting method has been successfully used to fabricate molecularly imprinted materials able to recognise CK. Interestingly, both styrene and MAA when used as functional monomers, with PEG400DMA as the cross-linker, produced materials with good template recognition abilities. We would assume that the two functional monomers mediate recognition, possibly to different sites on the template molecule, by different mechanisms, given their contrasting hydrophilic and hydrophobic natures. Given the similarity in the pI values of CK (6.8) and myoglobin (7.2) in the pH 7.4 rebinding buffer; in which they will both be negatively charged, it was noteworthy that such a good level of discrimination was able to be maintained. This is especially interesting considering that myoglobin (mol wt. 17,600) is considerably smaller than CK (mol wt. ~81,000) and is therefore easily able to occupy any recesses in the polymer's surface made by the template, implying that the recognition mechanism is, at least in part, dependent upon shape complementarity and not size-exclusion, as we assume to be the case with HSA.

We are confident, from the competitive binding experiments in human serum and the evidence that denaturing CK causes an obvious loss of template/polymer interaction, that the materials made are genuinely imprinted and may have the potential to be developed into smart-recognition materials for use in sensing devices.

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