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Imprinting for the assembly of artificial receptors on a silica surface

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Molecular imprinting of a solid surface provides a means to introduce reactive functionalities with a defined geometry on the surface. A silica surface was imprinted with a tridentate metal complex, $Ru[bipy{-}CH{=}NCH_2CH_2CH_2Si(OEt)_3]_3$. The molecular imprinting process leaves three amino groups per Ru^{II} complex. The imprinted silica was reacted with various aldehydes to assemble artificial receptor sites on the surface. A metal binding site for Fe^{II} was constructed with a bipyridine-modified aldehyde that was selectively adsorbed on the surface in the presence of other two aldehydes and Fe^{II}.

Introduction

The controlled modification and patterning of solid surfaces will continue to be the basis for the advancement of electronic, chemical and biomedical technologies in the future. Such engineered surfaces are a crucial element for the development of modern biochemical analysis and biocompatible materials. Silicon is the predominant material in fabricating microelectronic devices, microelectromechanical systems (MEMS), and microanalytical systems because of the expertise which exists in fabricating functional microstructures from silicon. Because the high surface-to-volume ratio in microscopic systems makes surface interactions more important than in conventional macroscopic systems, control over surface properties often becomes important for these applications. Furthermore, there is an increasing need to develop sensors and microelectronic devices which are specific to particular chemical species.

Some current and quite versatile approaches to the surface organofunctionalization of silicon use organofunctional alkoxy- or chloro-silanes. Chemo-mechanical polishing of silicon wafers followed by oxide stripping and chemical oxidation has been shown to yield very flat SiO_2 surfaces which can be modified with various silane coupling reagents.[1–5] The thickness of this SiO_2 layer has been estimated to lie between 5 and 10 Å.[6] The flatness of of the Si/SiO_2 interface is in large part determined by the initial flatness of the wafer which can be as small as 2–3 Å rms.[7] The outer surface of the oxidized Si is believed to be largely hydroxylated in contact with air and aqueous solutions and is therefore hydrophilic. The development of silane coupling chemistry will, therefore, have a major impact on technology for the production and utilization of microelectronics devices and other nanoscale systems fabricated from silicon.

Various molecules have been attached to a silica surface by a combination of silane coupling reactions and subsequent functionalization of the modified silica surface (Fig. 1).[8–12] Here, we wish to report a novel strategy to introduce a set of functionalities onto a silica surface for the recognition of specific compounds. A silica surface can be modified using the 'molecular imprinting' method[13–19] to create a site with three organized amino groups on the surface. Simple aldehyde-modified ligands are attached to the amino groups through a Schiff base bond to construct a receptor-like structure. Because Schiff base formation is reversible, combinations of certain recognition elements are preferentially enriched on the surface in the presence of a molecule that interacts with the synthetic receptor. Thus, synthetic receptors with a desired specificity will be constructed from a pool of aldehyde-modified ligands by a thermodynamic selection mechanism. We describe here the synthesis of aldehyde-modified bipyridine and amino acid derivatives as recognition elements for metal ions, and the selective enrichment of Fe^{II} binding sites on the surface.

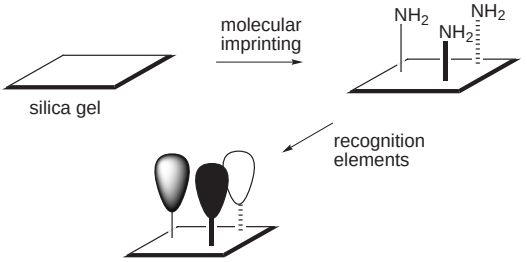

Fig. 1 Assembly of artificial receptors on a solid surface through molecular imprinting process

Experimental

4-(*p*-Formylbenzamidemethy)-4′-methyl-2,2-bipyridine (bipy-CHO)

A solution of 4-carboxyphenyl-1,3-dioxolane[20] (303 mg, 1.58 mmol) and 1-hydroxybenzotriazole (HOBt, 213 mg, 1.58 mmol) in dichloromethane (CH_2Cl_2, 5 ml) and anhydrous *N*,*N*-dimethylformamide (DMF, 3 ml) were mixed with a solution of 4-aminomethyl-4′-methyl-2,2′-bipyridine[21,22] (275 mg, 1.4 mmol). To the mixture was added dicyclohexylcarbodiimide (DCC, 326 mg, 1.58 mmol) dissolved in CH_2Cl_2–DMF (2:1, 3 ml). The reaction mixture was kept stirring overnight at 0 °C under a nitrogen atmosphere. The coupling product was isolated by silica gel column chromatography. The protecting group (1,3-dioxolane) was then removed with trifluoroacetic acid in chloroform to yield the desired aldehyde in 80% yield.

^{1}H NMR ($CDCl_3$), δ 2.46 (s, 3H, CH_3), 4.71 (d, 2H, CH_2), 7.21 (dd, 1H, bipy), 7.32 (dd, 1H, bipy), 7.42 (t, 1H, NH), 7.91 (d, 2H, phenyl), 8.02 (d, 2H, phenyl), 8.26 (s, 1H, bipy), 8.46 (s, 1H, bipy), 8.50 (d, 1H, bipy), 8.63 (d, 1H, bipy), 10.08 (s, 1H, CHO); GC–MS: m/z 331 (M^+), 226, 198 (100%), 133, 105, 77; UV–VIS (MeOH): λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1}) 241 (24 400), 274 (18 000).

p-Formylbenzoyl-L-histidine amide (His-CHO)

4-Formylbenzoic acid (600 mg, 4 mmol) and HOBt (540 mg, 6 mmol) were dissolved in a mixture of CH_2Cl_2 (5 ml) and anhydrous DMF (5 ml) with stirring at 0 °C under nitrogen

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atmosphere. L-Histidine amide·2HCl (His-NH_2, 1 g, 4.4 mmol) was neutralized slowly with triethylamine (TEA, 0.89 g, 8.8 mmol) in anhydrous DMF at 0 °C, and the mixture was kept stirring for 30 min. The free base solution of His-NH_2 was added to the solution of 4-carboxybenzaldehyde and HOBt. To the mixture was added DCC (844 mg, 4.1 mmol), and the reaction mixture was stirred at 0 °C for 2 h. After the temperature was increased to room temperature, the mixture was kept stirring overnight. His-CHO was obtained in 60% yield after purification with column chromatography [eluent: CH_2Cl_2–MeOH (7:3)]. ^{1}H NMR [$(CD_3)_2SO$], δ 3.00 (m, 2H, CH_2), 4.61 (m, 1H, CH), 6.82 (s, 1H), 7.07 (s, 1H, $CONH_2$), 7.48 (s, 1H, $CONH_2$), 7.56 (s, 1H), 7.99 (d, 2H), 8.04 (d, 2H), 8.83 (d, 1H, NHCO); MS(FAB): m/z 287 (calc. for $C_{14}H_{15}N_4O_3$ $[M+H]^+$ m/z 287); UV–VIS (MeOH) λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1}) 246 (10 100).

p-Formylbenzoyl-L-alanine amide (Ala-CHO)

Ala-CHO was synthesized from L-alanine amide·HCl using the same procedure as for His-CHO. The yield was 90% after silica gel column chromatography [eluent: CH_2Cl_2–MeOH (8:2)]. ^{1}H NMR [$(CD_3)_2SO$], δ 1.36 (d, 3H, CH_3), 3.04 (m, 2H, CH_2), 4.43 (m, 1H, CH), 7.02 (s, 1H, $CONH_2$), 7.47 (s, 1H, $CONH_2$), 7.99 (d, 2H), 8.09 (d, 2H), 8.70 (d, 1H, NHCO), 10.09 (s, 1H, CHO); UV–VIS (MeOH) λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1}) 246 (10 800).

Binding of aldehyde-modified amino acids and bipyridine to silica surfaces

(**A**) **Stock solutions.** Bipy-CHO (4.5×10^{-5} mol), Ala-CHO (4.5×10^{-5} mol) and His-CHO (4.5×10^{-5} mol) were dissolved in 0.1 ml of $(CH_3)_2SO$, and each solution was diluted with 15 ml of methanol. Aliquots (1.5 ml) of each of the three aldehyde solutions was mixed with each other in a vial (Stock 1). To the stock solution was added methanolic buffer solution [0.45 ml; MeOH : buffer = 1 : 1; buffer; 0.01 M HEPES (pH = 5.9) with 0.05 M KCl] (Stock 2). Iron(II) ammonium sulfate (1.2×10^{-5} mol) was dissolved in 1.5 ml of methanolic buffer solution [0.45 ml; MeOH : buffer = 1 : 1; buffer; 0.01 M HEPES (pH = 5.9) with 0.05 M KCl]. (Fe^{II} stock).

(**B**) **Binding of aldehydes to silica surfaces.** *Without* Fe^{II}. Stock 2 [0.45 ml, Bipy-CHO (3×10^{-7} mol), Ala-CHO (3×10^{-7} mol) and His-CHO (3×10^{-7} mol)] and SiO_2-NH_2 (20 mg, 1.2×10^{-6} mol NH_2 groups) were placed in a small vial and stirred for 20 h at room temperature. The suspension was centrifuged to separate silica, and the supernatant was analyzed by HPLC.

With Fe^{II}. Stock 1 [0.3 ml, Bipy-CHO (3×10^{-7} mol), Ala-CHO (3×10^{-7} mol) and His-CHO (3×10^{-7} mol)] was placed in a small vial. Fe^{II} stock (0.15 ml, 1.2×10^{-6} mol) was added with stirring. After stirring for 30 min, SiO_2-NH_2 (20 mg, 1.2×10^{-6} mol NH_2 groups) was added and stirred for 20 h at room temperature. The suspension was centrifuged to separate the silica, and the supernatant was analyzed by HPLC.

(**C**) **HPLC assay of bound aldehydes.** After the above binding experiment, the silica gel was collected by centrifugation. The silica gel was washed with methanol (0.5 ml × 3) to remove any physically adsorbed aldehydes. After washing, the silica gels were treated with acidic aqueous methanol (0.4 ml, 0.25 M HCl, 20% H_2O), and the mixture was stirred for 10 min with sonication. This hydrolyzes the Schiff base formed between aldehydes and surface amino groups. The silica gel was separated by centrifugation, and washed twice more with acidic aqueous methanol. The supernatant and washing solutions were collected for HPLC analysis. Only negligible amounts of ligands were released after the second washing.

Results and Discussion

A silica surface was imprinted with a tridentate metal complex, $Ru[bipy{-}CH{=}NCH_2CH_2CH_2Si(OEt)_3]_3$ **1**.[23] The modified silica gel was treated with a catalytic amount of HCl in aqueous methanol to hydrolyze the Schiff base and to release the Ru^{II}(bipy-CHO)$_3$ complex. Molecular imprinting with **1** should leave three amino groups per Ru^{II} complex released if the surface is covered with a monolayer of **1**. The ratio of amino groups on the surface and the amount of Ru^{II}(bipy-CHO)$_3$ complex released remained aproximately 3 : 1 up to a substitution level of 3.3×10^{-5} mol g^{-1} of **1** on the surface, suggesting successful imprinting at low substitution levels. The substitution level used in this work is 2.1×10^{-5} mol g^{-1} of **1** (6.2×10^{-5} mol NH_2 groups g^{-1}) on the surface.

p-Formyl benzoic acid was coupled to His-amide and Ala-amide to prepare aldehyde-modified amino acids [His-CHO and Ala-CHO, respectively (Fig. 2)]. The His side chain is involved in coordination to heavy metal ions in the active sites of many metalloproteins. Aldehyde-modified bipyridine (bipy-CHO) was also synthesized as a Fe^{II}-specific recognition element. The formyl group of *p*-formylbenzoic acid was protected as a cyclic acetal to avoid Schiff base formation during the coupling reaction. The DCC-mediated coupling reaction gave the desired bipyridine and amino acid derivatives in protected form, and the final deprotection of cyclic acetal with acid afforded the desired aldehyde-modified recognition elements.

Aldehyde-modified recognition elements can be *reversibly* attached to imprinted silica by Schiff base formation. When a mixture of Ala-CHO, His-CHO and bipy-CHO is reacted with the imprinted silica, various metal binding sites should be formed by combinations of these three aldehydes. Metal binding to a site with the right combination of recognition elements would crosslink the recognition elements. The crosslink should decrease the rate of Schiff base hydrolysis. This entropic

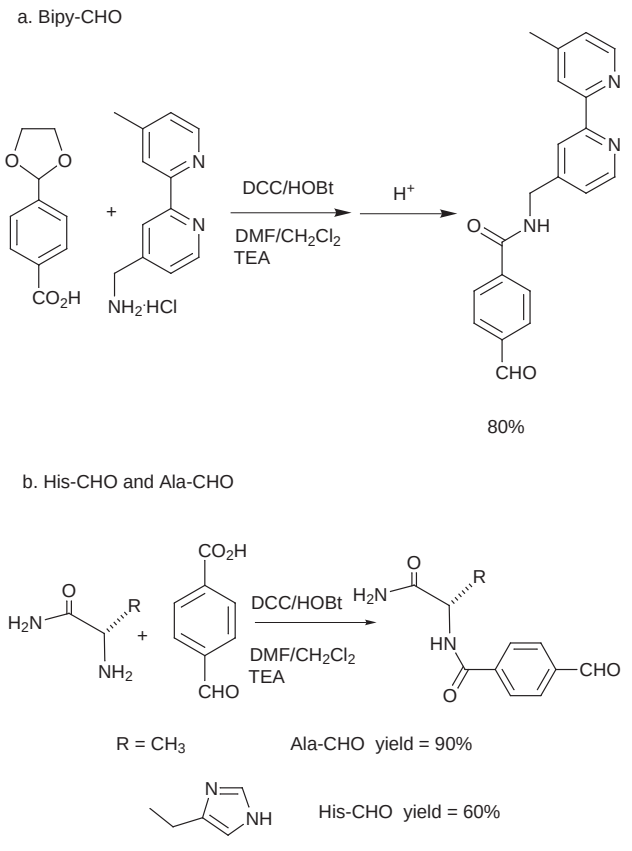

Fig. 2 (a) Synthesis of bipy-CHO. (b) Synthesis of His-CHO and Ala-CHO

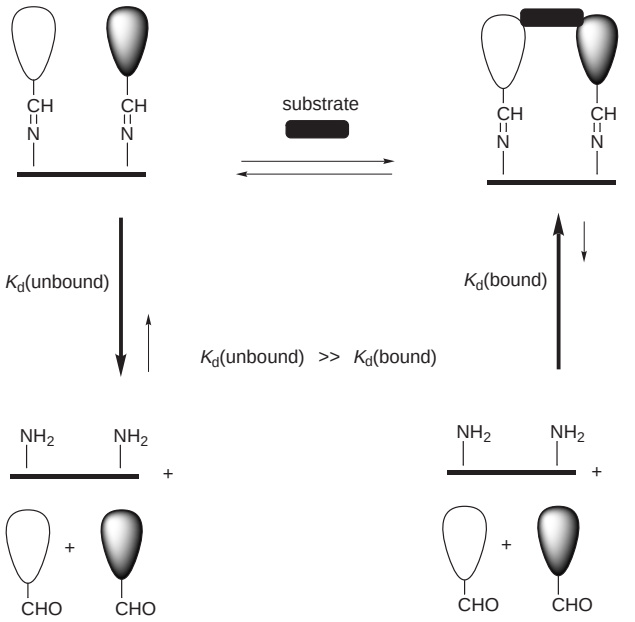

Fig. 3 A pair of surface Schiff bases can be entropically stabilized against hydrolysis by substrate crosslinking

stabilization of Schiff's base bond can be used as a selection mechanism to enrich the binding site for a particular metal ion on a silica surface (Fig. 3) The modified surface could be treated with $NaBH_4$ to 'fix' the selected combinations of recognition elements by reductive amination.

A mixture of Ala-CHO, His-CHO and bipy-CHO was reacted with the imprinted silica in methanol with and without Fe^{II}. After 20 h, the supernatant was analyzed by HPLC, showing selective adsorption of bipy-CHO in the presence of Fe^{II} [Fig. 4(a), (b)]. The silica was then isolated, washed with methanol to remove any non-specifically adsorbed aldehydes, and treated with aqueous acid to release any chemically adsorbed aldehydes through a Schiff base bond. HPLC analysis of the released aldehydes showed only bipy-CHO, and no His-CHO and Ala-CHO were detected above noise level [Fig. 4(c)]. Bipyridine is known to form a very stable iron(II) complex,[24] consistent with the observed selective adsorption of bipy-CHO. Ala-CHO and His-CHO do not appreciably interact with Fe^{II} under the experimental conditions used in this work, as determined by UV spectroscopy. Bipy-CHO shows drastic UV changes in the presence of Fe^{II}, characteristic of the formation of its iron(II) complex. The absorption maxima of the iron(II) complex of Bipy-CHO, 286, 296, 350 and 527 nm, are similar to that in $[Fe(bipy)_3]^{2+}$.[24] On the other hand, selective binding of His-CHO was observed in the absence of Fe^{II} (Fig. 5). This may be due to a favorable interaction between the basic imidazole group of His-CHO and acidic Si—OH groups on the silica surface.

In conclusion, we have demonstrated that a silica surface can be imprinted with a tridentate silane coupling reagent to create a site with three organized amino groups that are subsequently modified with various recognition elements. Reversible Schiff base formation can be used as a selection mechanism for the assembly of artificial receptors on the surface of silicon- and silica-based materials. Also, molecular imprinting provides a series of silica surfaces with designed metal binding properties that may become important tools for studies of biomineralization *in vitro*. Surface induced biomineralization has been demonstrated using a self-assembled monolayer (SAM) on a TiO_2 surface.[25] The SAM has a negatively charged surface that interacts with Ca^{2+} to initiate the formation of a thin hydroxyapatite film. Such surface modification with hydroxyapatite is crucial for the development of biocom-

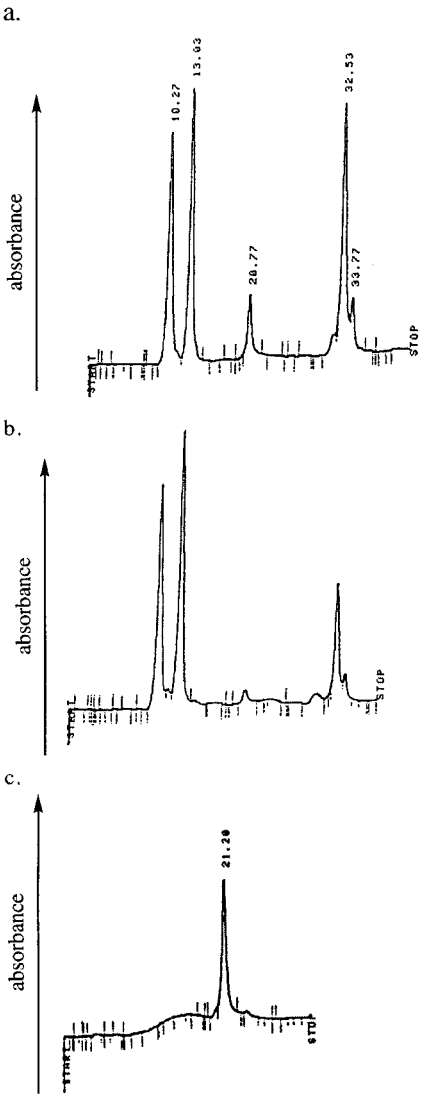

Fig. 4 HPLC analysis of a thermodynamic selection process with Ala-CHO, His-CHO and bipy-CHO on imprinted silica in the presence of Fe^{II}. Retention times; His-CHO, 10.27 min; Ala-CHO, 13.03 min; bipy-CHO, 20.77 min; $Fe^{II}(bipy\text{-}CHO)_3$, 32.53 min. (a) Before addition of imprinted silica. All three aldehydes are seen in a roughly equimolar ratio based on peak area. (b) Supernatant, 20 h after addition of imprinted silica to the mixture of the three aldehydes. Bipy-CHO appears to be selectively removed from the solution. (c) Silica bound aldehydes after an acid-catalyzed hydrolysis of the Schiff's base bond. Only bipy-CHO binds to the imprinted surface in the presence of Fe^{II}.

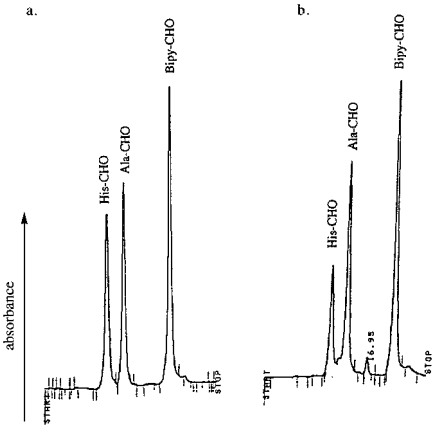

Fig. 5 HPLC analysis of aldehyde binding to imprinted silica in the absence of Fe^{II}. (a) supernatant, $t=0$; (b) supernatant, $t=20$ h.

patible Ti implants. Further mechanistic studies of the selection step with other metal ions and other recognition elements are now in progress.

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22 4-(Aminomethyl)-4′-methyl-2,2′-bipyridine·HCl was synthesized from 4-(bromomethyl)-4′-methyl-2,2′-bipyridine[21] and potassium phthalimide followed by hydrazinolysis in 90% yield. General procedures of Gabriel synthesis are described in M. S. Gibson and R. W. Bradshaw, *Angew. Chem., Int. Ed. Engl*., 1968, **7**, 919. ^{1}H NMR [$(CD_3)_2SO$], δ 2.66 (s, 3H, CH_3), 4.28 (t, 2H, CH_2), 7.87 (d, 1H), 7.90 (d, 1H), 8.68 (s, 1H), 8.77 (d, 1H), 8.88 (s, 1H), 8.90 (d, 1H), 9.08 (br t, 2H, NH_2). The free base form of the amino bipyridine was obtained by a treatment of the HCl salt with an aqueous NaOH solution, followed by extraction with CH_2Cl_2. ^{1}H NMR ($CDCl_3$), δ 2.43 (s, 3H), 3.98 (s, 2H), 7.13 (d, 1H), 7.28 (d, 1H), 8.23 (s, 1H), 8.33 (s, 1H), 8.53 (d, 1H), 8.61 (d, 1H).
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