Hybrid Bioorganic–Inorganic Materials Prepared by Site-Specific Ligation of Peptides to Functionalized Polydisperse Silica Particles

Pascal Joly,[a] Nicolas Ardès-Guisot,[b] Samiran Kar,[b] Michel Granier,[b] Jean-Olivier Durand,*[b] and Oleg Melnyk*[a]

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We describe the synthesis of semicarbazide- or glyoxylyl-functionalized polydisperse silica particles and their use for the preparation of hybrid polypeptide–silica materials. The peptides were attached to the surface of functionalized silicas by site-specific α-oxo hydrazone or semicarbazone ligation. The reaction of semicarbazide silicas with a model glyoxylyl peptide was found to be very efficient under stoichiometric conditions and led to ligation yields of about 90 %. The use of glyoxylyl silicas and of hydrazinoacetyl peptides led to lower yields.

Introduction

Organic–inorganic materials, especially those based on silicas, are widely used for transferring organic, organometallic, or bioorganic molecules to a solid phase.[1] The large number of applications of these materials in fields such as catalysis,[1b] nonlinear optics,[1c] chromatography,[1d] biosensors,[1e] and biological membranes[1f] has stimulated the development of chemical methods for their preparation. In the case of biomolecules such as polypeptides, immobilization was initially performed by exploiting the ability of polypeptides to bind noncovalently to silica supports. Despite the simplicity of the adsorption methods, covalent attachment is often preferred to avoid the leaching of the polypeptide. The covalent bonds with silica supports, which are not hydrolyzed under physiological pH conditions, are made through trialkoxysilyl derivatives.[1] The usual pathways for covalent attachment of biomolecules consist of the functionalization of silica surfaces by epoxides,[2] aldehydes,[1d] or thiol groups.[4] Polypeptides react with supported epoxides through their nucleophilic groups (SH, NH_2, OH). Reaction with aldehydes involves α- or ε-amino groups, but the formed imine is sensitive to hydrolysis and thus is usually reduced.[3] However, polypeptides usually present more than one useful nucleophilic group. Consequently, these methods usually result in a random immobilization[5] through multiple points of attachment.

A better control of molecule orientation at the solid–liquid interface can be achieved by using site-specific ligation methods. Supported amines have been used for the immobilization of oxidized antibodies by reductive amination.[5] This method is limited to glycoproteins and requires the performance of a chemical step on the protein. Thiol chemistry has been used extensively to immobilize polypeptides through disulfide bridges[4] or Michael addition with maleimide groups.[6] However, thiols are sensitive to air oxidation and can be involved in disulfide exchange reactions. Hydrazone ligation between alkyl hydrazides and aldehydes has been used for the preparation of solid supports for high performance affinity chromatography.[7] The preparation of these supports involves the oxidative cleavage of vicinal-diol-bonded silicas into aldehydes, which can be reacted with oxalic or adipic dihydrazide. The poor stability of the formed hydrazone requires a final reduction step with $NaBH_4$. Although these supports have been used successfully for immobilization of oxidized antibodies and t-RNA,[7] their preparation requires a multi-step procedure and leads to a loss of reactive sites due to the formation of cross-linkages between two adjacent aldehyde groups on the surface in the presence of the dihydrazide.

In the course of our studies concerning the preparation of hybrid polypeptide–silica materials, we became interested in the development of simple and alternative procedures for the dense, covalent, and oriented immobilization of polypeptides onto silicas. In this context, immobilization of peptides through site-specific α-oxo hydrazone or semicarbazone chemistry was considered since these bonds can be formed chemoselectively using very mild aqueous experimental conditions and for this reason have often been uti-

[a] UMR 8525 CNRS-Université de Lille 2, Institut de Biologie de Lille,
1 rue du Professeur Calmette, 59021 Lille, France
Fax: +33-32-087-1235
E-mail: oleg.melnyk@ibl.fr
[b] Laboratoire de Chimie Moléculaire et Organization du Solide, UMR 5637, case 007-Université Montpellier II,
Place Eugène Bataillon, 34095 Montpellier Cedex 05, France
Fax: +33-467-143-852
E-mail: durand@univ-montp2.fr

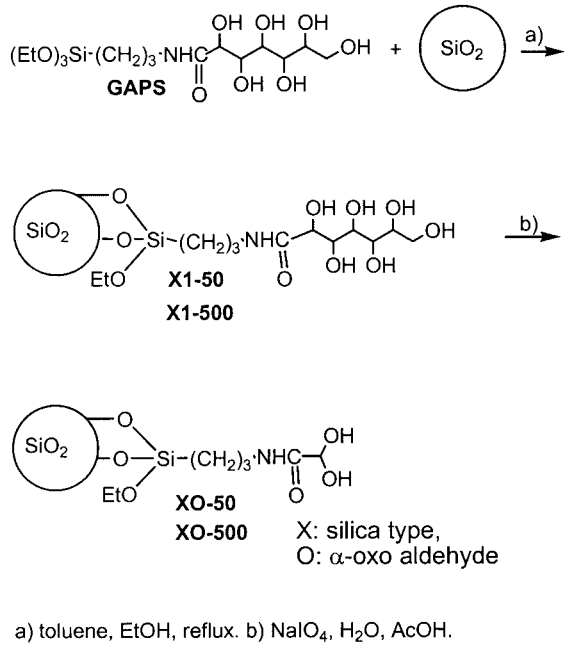
lized for the preparation of conjugates or for the convergent synthesis of large molecular objects.[8] These ligation methods have been scarcely studied in the context of bioorganic–inorganic materials synthesis.[9] We describe here the preparation of α-oxo aldehyde (COCHO) and semicarbazide-functionalized silicas, their reactivity, and selectivity for the covalent immobilization of hydrazinoacetyl- or COCHO-modified polypeptides.

Results and Discussion

Synthesis of COCHO Silicas

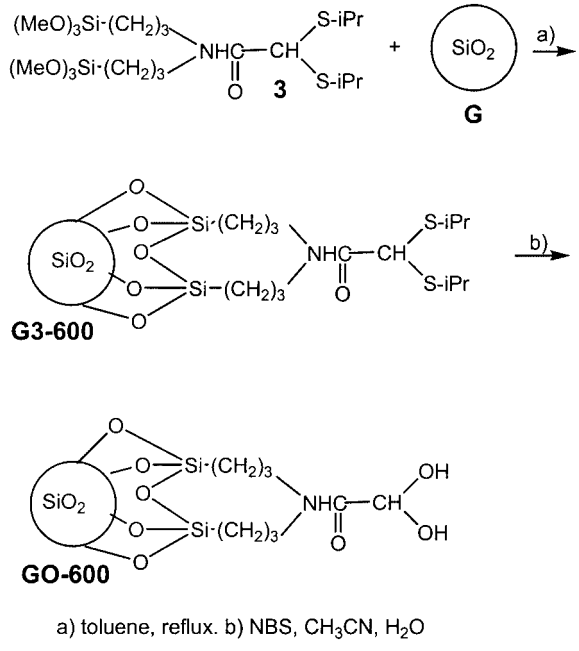
Few methods have been proposed for the modification of surfaces by α-oxo aldehyde moieties.[9] Indeed, this functionality has been found to be attractive for its hydrophilicity and stability towards oxidation compared to aliphatic or aromatic aldehydes. These properties are mainly due to the formation of a hydrate in aqueous media. The introduction of the α-oxo aldehyde group was performed by coupling protected serine to amino-functionalized surfaces followed by periodic oxidation of the β-amino alcohol group,[9a] or by coupling glyoxylic acid dimethoxyacetal followed by deprotection by treatment with strong acids.[9b] Recently, we have described an alternative approach based upon the sol-gel chemistry of triethoxysilylpropylgluconamide (GAPS), whose polyol chain can be converted into an α-oxo aldehyde group by treatment with periodate in aqueous acetic acid.[10] The presence of α-oxo aldehyde groups was demonstrated, in particular, by CP MAS ^{13}C NMR spectroscopy, which shows a signal at δ = 88 ppm typical of the glyoxylyl group in its hydrated form. However, these materials, which show a good reactivity with low-molecular-weight hydroxylamine or hydrazine derivatives, were unable to react with α-hydrazinoacetyl peptides due to poor accessibility.

We envisaged that silanization of the surface of silica particles could give better results than sol-gel chemistry (Scheme 1). Silica particles for flash chromatography **X** (Macherey–Nagel, particle size 43–60 nm, BET surface: 450 $m^2 g^{-1}$) were treated with GAPS. Two types of materials were synthesized with 50 μmol (**X1-50**) or 500 μmol (**X1-500**) of GAPS per gram of silica. IR spectroscopy showed resonances characteristic of the gluconamide chain, in particular the ν(C=O) band appears at 1648 cm^{-1} as in the hybrid organic–inorganic material prepared by sol-gel chemistry. Microanalysis allowed us to quantify the grafting of GAPS at the surface (solid-state NMR spectroscopy was not sensitive enough for this purpose) Treatment of silicas **X1-50** or **X1-500** with an excess of periodate in 10% aqueous acetic acid led to the oxidative cleavage of the polyol chain and to the glyoxylyl-supported silicas (**XO**). IR analysis showed a shift of ν(C=O) to higher wavelength (1682 cm^{-1}) after oxidation, thus confirming the cleavage of the polyol chain.



Scheme 1. Synthesis of COCHO-functionalized silicas by treatment with GAPS reagent and oxidative cleavage with periodate.

Alternatively, silica particles **G** (Carlo–Erba, particle size 35–70 nm, BET surface: 550 $m^2 g^{-1}$; these particles were activated by refluxing in HCl for 3 h) were functionalized with silane **3**[10] bearing an α,α′-diisopropylthioacetic acetyl moiety as a masked glyoxylyl group. This COCHO protection, which was described originally by Badet[11] et al., can be removed by treatment with NBS in acetonitrile/water (Scheme 2).



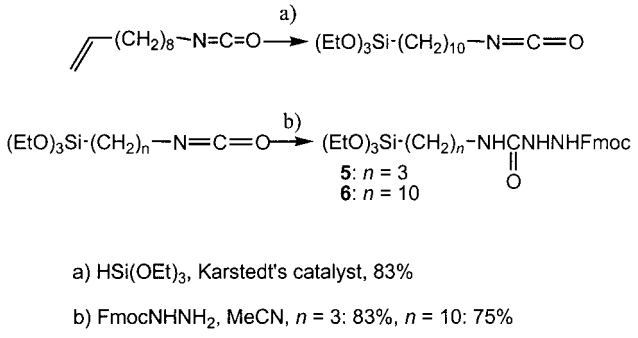
Scheme 2. Synthesis of glyoxylyl silica **GO-600** by using a dithioacetal protective group.

Deprotection of silica **G3-600** with *N*-bromosuccinimide furnished silica **GO-600**, which has a ν(C=O) band at 1638 cm^{-1} for the COCHO group.

Synthesis of Semicarbazide-Functionalized Silicas

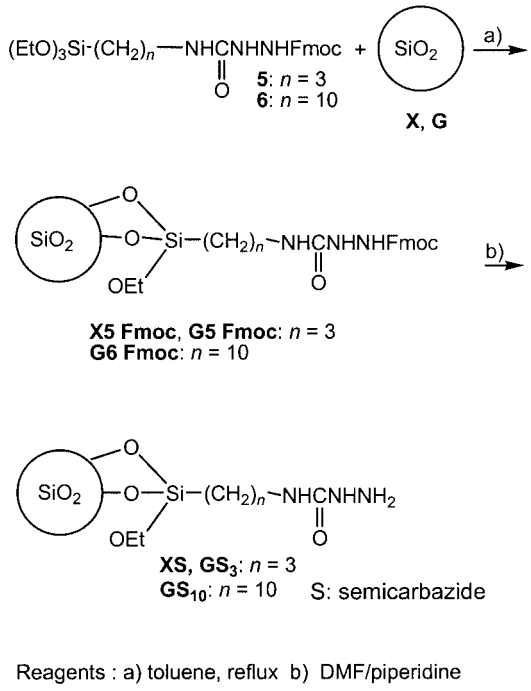
Silicas modified by hydrazine derivatives have been described by a few authors. These materials were prepared by multi-step procedures involving the reaction of dihydrazides with supported electrophiles such as aliphatic aldehydes or epoxides.[7] Surprisingly, semicarbazide silica supports[12] have been scarcely studied despite the interest of the semicarbazide group for site-specific ligations in solution, as discussed before.

The preparation of semicarbazide silicas needed the synthesis of compounds **5** or **6** (Scheme 3). Reagent **5**[12e] was synthesized by treating commercially available triethoxysilylpropyl isocyanate with $FmocNHNH_2$[13] (96% yield). Triethoxysilyldecyl isocyanate (a product which has not been described yet) was easily obtained by hydrosilylation of 9-*n*-decenyl isocyanate[14] with triethoxysilane in the presence of Karstedt's catalyst. Speier's catalyst (H_2PtCl_6) was less efficient in this reaction. Condensation with $FmocNHNH_2$ furnished protected semicarbazide **6** in 75% yield.



Scheme 3. Synthesis of silanes **5** and **6**.

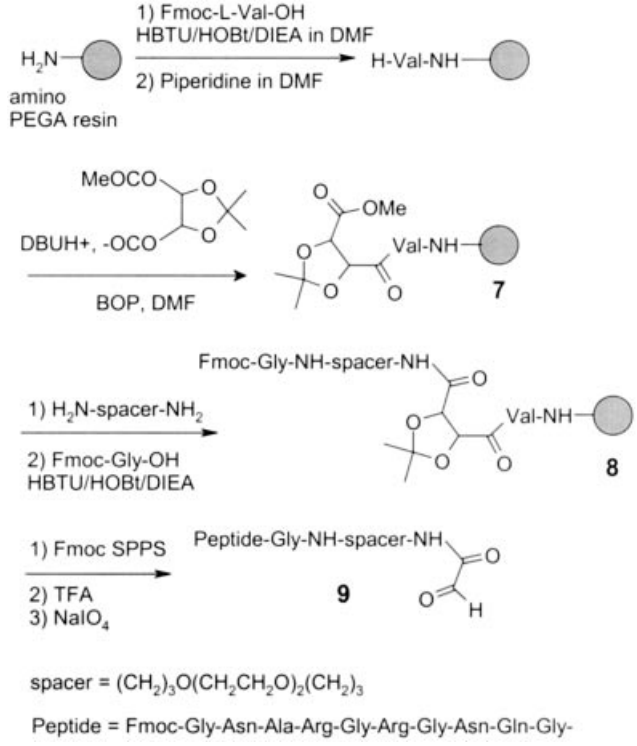
Then, solid supports **XS**, $\mathbf{GS_3}$, and $\mathbf{GS_{10}}$ were prepared in two steps by treating silanes **5** or **6** with silica particles **X** or **G**: 50 μmol g^{-1} of **5** (**XS-50**) or 500 μmol g^{-1} of **5** (**XS-500**) were used with silica **X**, whereas 2 mmol g^{-1} of **5** ($\mathbf{GS_3}$-**500**) or **6** ($\mathbf{GS_{10}}$-**500**) were used with silica **G** (Scheme 4). All the prepared semicarbazide silicas show characteristic ν(C=O) resonances for the carbonyl groups at about 1730 and 1654 cm^{-1}. One of the benefits of the Fmoc protecting group is the possibility to determine the loading of the solid supports by treatment with piperidine followed by spectrophotometric UV analysis of the dibenzofulvene-piperidine adduct.[15] Loadings of 36, 320, 513, and 500 μmol g^{-1} were obtained for solid supports **XS-50**, **XS-500**, $\mathbf{GS_3}$-**500**, and $\mathbf{GS_{10}}$-**500** respectively. The higher loading of 500 μmol obtained for silica $\mathbf{GS_3}$-**500** compared to silica **XS-500** shows the importance of the activation procedure.



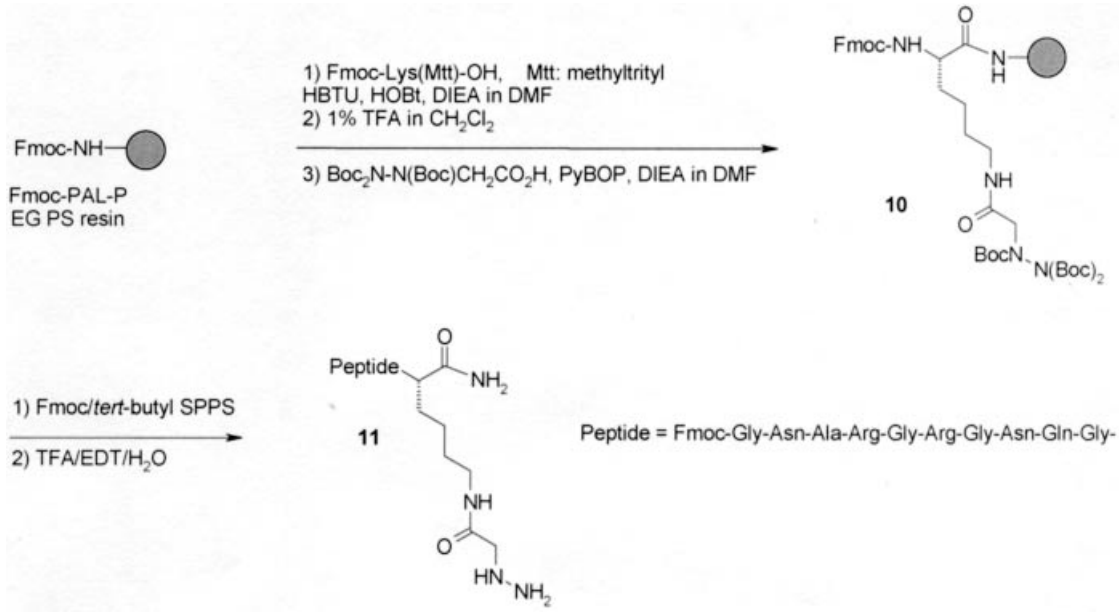
Scheme 4. Synthesis of semicarbazide silicas **XS**, $\mathbf{GS_3}$, and $\mathbf{GS_{10}}$.

Peptide Synthesis

Peptides **9** and **11** were designed to determine the chemoselectivity of the ligation of glyoxylyl or α-hydrazinoacetyl peptides with the prepared semicarbazide or glyoxylyl silicas. The functional groups were placed at the C-terminus, whereas the N-terminus was functionalized with an Fmoc



Scheme 5. Synthesis of glyoxylyl peptide **9**.



Scheme 6. Synthesis of α-hydrazinoacetyl peptide **11**.

group to allow the titration of the peptides immobilized covalently or not at the surface.

Glyoxylyl peptide **9** (Scheme 5) was synthesized on the solid phase using an isopropylidene tartrate linker, which was assembled on an amino PEGA resin as described elsewhere.[16] The peptide was elaborated using standard Fmoc/*tert*-butyl chemistry.[15] Resin deprotection of both the peptide side-chains and the vicinal diol moiety was followed by an oxidative cleavage of the diol with periodate, which permitted the simultaneous formation of the glyoxylyl group and the separation of the product from the resin. Peptide **7** was isolated with a 37% yield following purification by RP-HPLC.

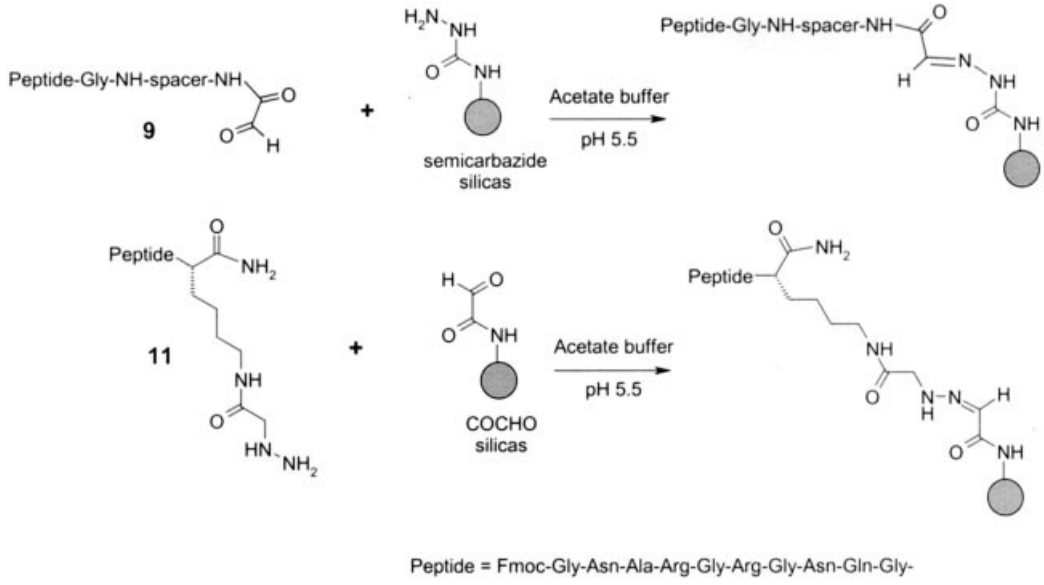
α-Hydrazinoacetyl peptide **11** was assembled on an Fmoc-L-Lys[$COCH_2$NBocN$(Boc)_2$]-PAL-PEG PS resin, which was obtained as described elsewhere[17] (Scheme 6). The peptide was elaborated on the solid phase using standard Fmoc/*tert*-butyl protocols and then deprotected and cleaved from the resin with concentrated TFA in the presence of the appropriate scavengers. Hydrazinoacetyl peptide **11** was isolated with a 45% overall yield following RP-HPLC purification.

Ligation Experiments

The glyoxylyl peptide **9** was used to estimate the level of physisorption on COCHO silicas and the yield of site-specific ligation on semicarbazide silicas. Analogously, α-hydrazinoacetyl peptide **11** was utilized to estimate the level of physisorption on semicarbazide silicas and the yield of site-specific ligation with COCHO silicas (Scheme 7).

Ligations and control experiments were performed in pH 5.5 sodium acetate buffer at a fixed peptide concentration of about 1 mM; 36 μmol of peptide **9** or **11** per gram of silica were used with XO-50, XS-50 and 50 μmol of peptide **9** or **11** per gram of silica were used with XS-500, GS3-500, GS10-600, XO-500. Thus, the stoichiometry of the functionalized silica ranged from 1 to about 10 equivalents depending on the loading of the silica particles. After the incubations, the solid supports were washed with an acetate buffer containing 0.5% of Tween® 20, and then with water. The results are presented in Figure 1. The yields are expressed relative to the peptide quantity engaged in the experiments.

The yields of immobilization of peptides **9** and **11** on glyoxylyl silica **XO-50** are similar (about 20%), thus indicating that little or no site-specific ligation occurs on this solid support. Alternately, support COCHO-silica **XO-500** led to about 60% of immobilization with hydrazinoacetyl peptide **11**. The Fmoc loading obtained in the control experiment with peptide **9** was below 10%, thus showing that site-specific ligation occurs between hydrazinoacetyl peptide **11** and COCHO-silica **XO-500**. For comparison, COCHO-silica **GO-600**, obtained as described before by using the α,α′-dithioisopropylacetic acid derivative as a masked glyoxylyl equivalent, led to an immobilization yield of 77% yield with peptide **11** under the same experimental conditions. Thus, silica **XO-500** appears to be an interesting solid support for the preparation of hybrid materials owing to its ease of preparation from the cheap, commercially available reagent GAPS. The preparation of glyoxylyl support **GO-600** has to be preferred if higher yields of immobilization are desirable, even though a multi-step procedure is necessary to install the glyoxylyl functionality. The approaches presented



Scheme 7. Reaction of peptides **9** and **11** with semicarbazide (S) or α-oxo aldehyde (O) silicas (**X**,**G**) in sodium acetate buffer (pH 5.5).

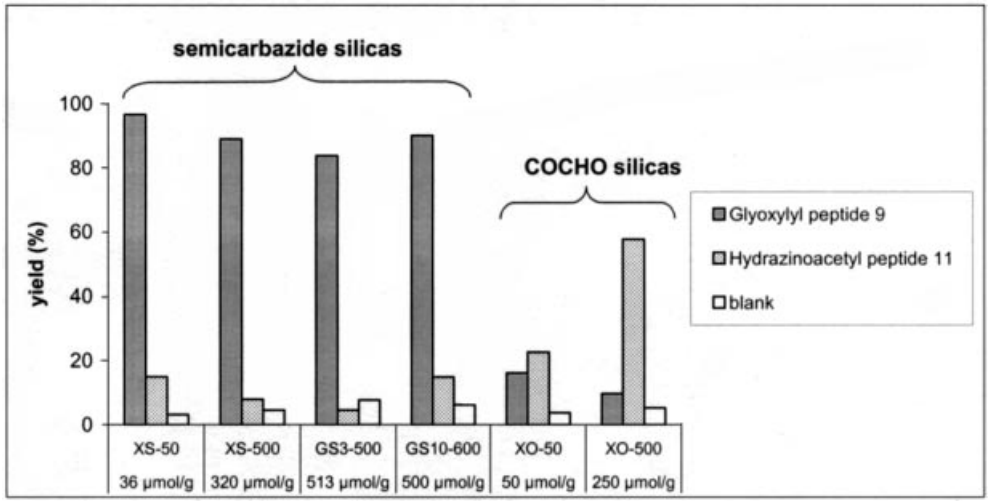


Figure 1. Immobilization yield of peptides **7** and **8** on semicarbazide (S) or α-oxoaldehyde (O) silicas.

here are interesting alternatives to the methods described in the literature.[9] In particular, Lam et al. have described the preparation of glyoxylyl-functionalized glass slides by periodic oxidation of a surface-linked serine, oxidation of acrylic acid residues with $NaIO_4/OsO_4$, or by acid-catalyzed hydrolysis of dimethylacetal-protected glyoxylic acid residues.[9] These surfaces were treated with hydroxylamine derivatives. However, the ligation yields and the chemoselectivity of the immobilization were not quantified.

If we consider now the semicarbazide substrates, reaction of glyoxylyl peptide **9** with a stoichiometric amount of solid support **XS-50** led to a high yield (97%) of immobilization, with only 15% of noncovalent immobilization in the control experiment with hydrazinoacetyl peptide **11**. Utilization of higher loadings (support **XS-500** or **GS_3-500**) or of a longer spacer (**GS_{10}-500**) led to similar results in term of ligation yield (84–90%) and noncovalent adsorption (5–15%). The solid supports **XS** and **GS_{10}-500** seemed to give the highest ratios of covalent/noncovalent immobilization.

The behavior of the functionalized particles described in this study differs significantly from the sol-gel materials described recently.[10] Indeed, hybrid organic–inorganic semicarbazide[12b] or glyoxylyl[10] sol-gel matrices were found to be unable to react with the corresponding functionalized peptides, although reaction with small chemical reagents was observed, showing that the absence of reaction with peptides is mainly due to the inability of the polypeptide chain to diffuse into the sol-gel matrix.

The good reactivity of semicarbazide silicas toward COCHO peptides and the low level of noncovalent adsorption observed in this study can be compared to recent results obtained in the peptide microarray field.[12d–12f] Peptide α-oxo semicarbazone microarrays were prepared by printing glyoxylyl peptide probes onto semicarbazide-functionalized

microscope glass-slides. The chemoselectivity of the immobilization was demonstrated with Rho-Lys-Arg-NH$(CH_2)_3$-NHCOCHO and Rho-Lys-Arg-NH_2 peptides, where Rho is (5)-6-carboxytetramethylrhodamine, and fluorescence detection. In this experiment, the functionalized glass slides were immersed in the peptide solutions and then washed to remove the excess of reagent. Typically, the ratios of covalent to noncovalent immobilization were close to 10, i.e. the ratios obtained in the present study. In another experiment, biotinylated peptides functionalized or not by a COCHO group were dissolved in a pH 5.5 acetate buffer and printed on the semicarbazide glass surface.[12e,12f] The microarrays were incubated with tetramethylrhodamine-labeled goat antibiotin antibodies in the presence of 5% BSA. The signal displayed by the glyoxylyl biotinylated peptide printed at 10^{-4} M was about sevenfold higher than those given by the control peptide. Thus, about the same ratios of covalent to noncovalent immobilization were obtained with peptides reacted with different semicarbazide surfaces, as determined by the detection of the Rho label attached to the peptide or to an antibody specifically captured by the peptide probe.

Conclusions

We have described in this article the preparation of hybrid peptide–silica-based materials by site-specific ligation of glyoxylyl or hydrazinoacetyl peptides to semicarbazide- or COCHO-functionalized silica particles, respectively. The selectivity of α-oxo hydrazone or semicarbazone formation was demonstrated by using control peptides lacking the reactive group for ligation. The best results in term of ligation yields and low levels of adsorption were obtained with the semicarbazide-functionalized particles and the glyoxylyl peptide. The size of the spacer separating the semicarbazide group from the silica surface has little influence. Interestingly, the yields of site-specific immobilization were good even under stoichiometric conditions. Thus, the cost associated with the peptide part of the hybrid material is minimal. For glyoxylyl silicas, the yields of immobilization were lower and only highly loaded supports were efficient for ligation. However, the low cost of the GAPS precursor and simplicity of preparation make these supports attractive for peptide immobilization. These results bring additional insight into the utility of semicarbazide-functionalized silicas for the site-specific immobilization of polypeptides under mild experimental conditions.

Experimental Section

Manipulations of air-sensitive compounds were carried out under N_2. FTIR spectra (KBr pellets) were recorded on a Nicolet instrument. Microanalyses were performed at the central service of microanalyses (CNRS at Vernaison). High resolution mass spectra (FAB^+) were performed on a JEOL DL-100 spectrometer, with a nitrobenzyl alcohol (NBA) or a glycerol thioglycerol (GT) matrix. NMR spectra were recorded on 200 MHz and 300 MHz Bruker Advance DRX spectrometers.

Synthesis of (10-Isocyanatodecyl)triethoxysilane: Karstedt's catalyst (0.2 mol-% Pt) was added to a solution of 10-decenyl isocyanate (5.07 g, 28 mmol) and triethoxysilane (7.44 g, 45.37 mmol). An exothermic reaction occurred. The reaction was stirred for 5 h and the excess of triethoxysilane was then evaporated under vacuum. Distillation under vacuum (0.3 Torr, b.p. 133 °C) afforded 8.04 g of a colorless liquid (83%). IR (NaCl pellets): $\tilde{\nu}$ = 2974, 2926, 2856, 2274, 1460, 1389, 1167, 1104, 1080, 958 cm^{-1}. ^1H NMR ($CDCl_3$): δ = 0.60 (m, 2 H, CH_2Si), 1.27 [m, 23 H, CH_3(ethoxy) and CH_2(alkyl)], 1.63 (m, 2 H, CH_2), 3.32 (t, ^3J = 6.67, 2 H, CH_2NCO), 3.86 (q, ^3J = 6.99, 6 H, CH_2O) ppm. ^{13}C NMR ($CDCl_3$): δ = 10.72 (CH_2Si), 18.60 (CH_3), 23.10 (CH_2), 26.87 (CH_2), 29.29 (CH_2), 29.54 (CH_2), 29.74 (CH_2), 29.81 (CH_2), 31.67 (CH_2), 33.46 (CH_2), 43.29 (CH_2N), 58.57 (CH_2O), 122.44 (N=C=O) ppm. ^{29}Si NMR ($CDCl_3$): δ = –44.3 (s, CH_2Si) ppm.

9-Fluorenylmethyl 1-[(Triethoxysilyldecyl)aminocarbonyl]-2-hydrazinecarboxylate (6): 9-Fluorenylmethylcarbazate (1.09 g, 4.29 mmol) and (10-isocyanatodecyl)triethoxysilane (1.52 g, 4.40 mmol) were refluxed for 1 h in MeCN (60 mL). The hot reaction mixture was filtered, and the filtrate was crystallized at 0 °C. The solid was filtered and was washed with cold EtOH to give 1.94 g of a white powder (m.p. 130 °C; 75% yield). IR (KBr): $\tilde{\nu}$ = 3312, 2964, 2920, 2855, 1705, 1652, 1564, 1450, 1411, 1339, 1105, 1080, 956, 759, 739 cm^{-1}. ^1H NMR ($CDCl_3$): δ = 0.66 (m, 2 H, CH_2Si), 1.26 [m, 23 H, CH_3(ethoxy) and CH_2(alkyl)], 1.45 (m, 2 H, CH_2), 3.22 (q, ^3J = 5.98 Hz, 2 H, CH_2N), 3.85 [q, ^3J = 7.00 Hz, 6 H, CH_2O (ethoxy)]), 4.24 (t, ^3J = 6.63 Hz, 1 H, CH), 4.48 (d, ^3J = 7.03 Hz, 2 H, CH_2O), 5.38 (s, 1 H, NH), 6.78 (s, 1 H, NH), 6.95 (s, 1 H, NH), 7.29–7.46 (m, 4 H, CH arom.), 7.60 (d, ^3J = 7.11 Hz, 2 H, CH arom.), 7.78 (d, ^3J = 7.41 Hz, 2 H, CH arom.) ppm. ^{13}C NMR ($CDCl_3$): δ = 10.79 (CH_2Si), 18.71 (CH_3), 23.15 (CH_2), 27.23 (CH_2), 29.63 (CH_2), 29.71 (CH_2), 29.86 (CH_2), 29.96 (CH_2), 30.45 (CH_2), 33.59 (CH_2), 40.65 (CH), 47.34 (CH_2N), 58.51 [CH_2O-(ethoxy)], 68.43 (CH_2O), 120.46 (CH arom.), 125.45 (CH arom.), 127.55 (CH arom.), 128.27 (CH arom.), 141.72 (CH arom.), 143.76 (CH arom.), 157.41 (C=O), 158.66 (C=O) ppm. ^{29}Si NMR ($CDCl_3$): δ = –44.1 (s, CH_2Si). MS (FAB^+): *m/z* = 622 [M + Na^+], 600 [MH^+]. HRMS: MH^+ calculated: 600.3469; found 600.3472.

Synthesis of Silicas X1-50 and X1-500: Silica **X** (1 g) was suspended in EtOH (10 mL). Triethoxysilylpropylgluconamide [44 μL (50 μmol) for **X1-50** and 440 μL (500 μmol)] for **X1-500** was added and the mixture was refluxed for 2 h. After cooling, the solution was filtered and the functionalized silica washed with EtOH and H_2O, and dried by lyophilization.

Data for X1–50: Microanalysis: C 0.82, N 0.1, Si 42.90, which corresponds to a loading of 60 μmol g^{-1}.

Data for X1–500: Microanalysis: C 3.63, N 0.36, Si 41.3, which corresponds to a loading of 257 μmol g^{-1}. IR (KBr): $\tilde{\nu}$ = 3450, 2932, 1648, 1545, 1075, 956 cm^{-1}.

Synthesis of Silicas XO-50 and XO-500: A suspension of **X1-500** or **X1-50** in 10% AcOH in water (3 mL) was treated with $NaIO_4$ (740 mg). After 20 min, the suspension was filtered, washed with a solution of 10% AcOH in water, water, and EtOH, four times each. The particles were dried under vacuum.

Data for XO-500: Microanalysis: C 3.37, N 0.35, Si 41.2, which corresponds to a loading of 250 μmol g^{-1}. IR (KBr): $\tilde{\nu}$ = 3420, 2932, 1682, 1558, 1059, 945 cm^{-1}.

Synthesis of Silica G3-600: A solution of **3** (400 mg, 1.08 mmol) and silica **G** (500 mg, activated by refluxing in 6 N HCl for 2 h then filtration and drying) in toluene (40 mL) was refluxed with stirring for 4 h. The cooled, functionalized silica gel was filtered off and washed with diethyl ether and dichloromethane. The functionalized

silica gel was dried under vacuum at 120 °C for 2 h. **Data for G3-600:** IR (KBr): $\tilde{\nu}$ = 3444.2, 2964.7, 2921.2, 1632.4, 1463.5 cm^{-1}. Micronalysis: C 10.40, H 2.21, N 0.95, S 2.82, Si 35.99, which corresponds to a loading of 670 $\mu mol\,g^{-1}$.

Synthesis of GO-600: A suspension of **G3-600** (500 mg) in MeCN (20 mL) and water (5 mL), was treated with *N*-bromosuccinimide (476 mg, 2.7 mmol) whilst stirring. After 4 h, the suspension was filtered, and washed with water and MeCN. The powder was dried under vacuum for 2 h at 100 °C. IR (KBr): $\tilde{\nu}$ = 3466, 2953.8, 1637.8, 956.7, 798.7 cm^{-1}.

Synthesis of Silicas XS-50 and XS-500: Silica **X** (400 mg) was treated with silane **5** [10 mg (19.8 μmol) for **X5–50**; 100 mg (0.198 mmol) for **X5–500**] in toluene (10 mL) for 2 h. The suspension was filtered, washed with toluene, EtOH, and water, and then dried. Titration of the Fmoc group (Fmoc cleavage and determination of the piperidine adduct) gave a loading of 36 $\mu mol\,g^{-1}$ for **X5–50** and 320 $\mu mol\,g^{-1}$ for **X5–500**.

Synthesis of Silicas GS_3-500 and GS_{10}-500: Silica **G** (1 g) was treated with silane **5** (1 g, 2 mmol) or silane **6** (1.2 g, 2 mmol) in refluxing toluene (20 mL) for 12 h. The suspension was then filtered, washed with EtOH and Et_2O, and dried under vacuum at 80 °C. Titration of the Fmoc group gave a loading of 513 $\mu mol\,g^{-1}$ for **GS_3-500** and 500 $\mu mol\,g^{-1}$ for **GS_{10}-500**.

Peptide Synthesis: General. Eluent A: water containing 0.05% TFA by volume; eluent B: 2-propanol/water (2:3 by volume) containing 0.05% TFA by volume; Eluent C: acetonitrile/water (4:1 by volume).

Synthesis of Fmoc-GNARGRGNQG-$NH(CH_2)_3O(CH_2CH_2O)_2(CH_2)_3NHCOCHO$ (peptide 9)

Preparation of Solid Support 7 (1 mmol Scale): Water (72 μL, 4 mmol) was added to (+)-dimethyl-2,3-*O*-isopropylidene-D-tartrate (7.59 mL, 40 mmol) at room temp. DBU (598 μL, 4 mmol) was then added and the mixture was stirred for 1 h. H-Val-NovaSyn TG® [obtained from 3.57 g of NovaSyn TG® resin (0.28 mmol/g, Novabiochem); 1.0 mmol] was washed with DIEA (5% in CH_2Cl_2) and DMF. The above mixture, DMAP (48.9 mg, 0.4 mmol), and PyBOP reagent (2.08 g, 4 mmol dissolved in 2 mL of DMF) were added successively to the resin swelled in the minimum volume of DMF. The resin was shaken for 1 h, then washed with DMF and CH_2Cl_2 (4 × 2 mL), and dried in vacuo.

Preparation of Solid Support 8 (0.2 mmol Scale): 4,7,10-Trioxa-1,13-tridecanediamine (TTD; 3.38 mL, 15.4 mmol) was mixed with DMF (624 μL; final concentration 3.85 M) and the mixture was added to the resin swelled in the minimum volume of DMF. After 45 min, the resin was washed with DMF (5 × 2 mL) and acylated immediately with Fmoc-Gly-OH (595 mg, 2.00 mmol) activated with HBTU/HOBt/DIEA [759 mg (2.00 mmol)/270 mg (2.00 mmol)/1.05 mL (6.00 mmol)] for 45 min in DMF at room temp.. The completion of the reaction was verified by the TNBS test.[19]

Peptide Elongation, Deprotection, and Cleavage (0.1 mmol Scale); Synthesis of Peptide 9: The rest of the synthesis was performed using a Pioneer Perseptive Biosystems syntheziser as described above. The dry peptidyl resin was deprotected by treatment with 10 mL of TFA/anisole/thioanisole/ethanedithiol/H_2O (90:2.5:2.5:2.5:2.5 by volume) for 2 h at room temp. The resin was then washed with CH_2Cl_2 (twice, 2 mL), MeOH (twice, 2 mL), and 33% aqueous acetic acid (twice, 2 mL). Sodium periodate (128.4 mg, 0.6 mmol) was dissolved in 10 mL of 5% aqueous acetic acid and added to the peptidyl resin swollen in the minimum volume of 33% aqueous acetic acid. The resin was shaken for 10 min, filtered, and washed three times with 3 mL of 5% acetic acid. The combined filtrates were purified by preparative RP-HPLC on a C18 Hyperprep 15 × 500 mm column using buffers A and C (0–33% C in 7 min, 33–42% in 33 min, 42–100% in 5 min, 3 $mL\,min^{-1}$, detection at 215 nm). Following a freeze-drying step, peptide **9** was obtained as a white powder (62.5 mg, 37% based on the starting NovaSyn TG® resin). MALDI-TOF $[M + H]^+$: calcd. 1466.71; found 1466.72. RP-HPLC purity (215 nm): one peak >95%.

Synthesis of Fmoc-GNARGRGNQGK($COCH_2NHNH_2$)-NH_2 (11)

Preparation of Solid Support Fmoc-L-Lys($COCH_2NBocN(Boc)_2$]-PAL-PEG-PS (10): Fmoc-Pal-PEG-PS resin (0.16 $mmol\,g^{-1}$, Applied Biosystems, Foster City, CA; 3 mmol) was deprotected with 20% piperidine in DMF (twice, 2 min and 15 min). Fmoc-L-Lys(Mtt)-OH (7.5 g, 12 mmol) was coupled using HBTU (4.55 g, 12 mmol)/HOBt (1.84 g, 12 mmol)/DIEA (3.38 mL, 48 mmol) activation. The Mtt protecting group was removed by treatment with 1% TFA in CH_2Cl_2 and the deprotection was monitored by RP-HPLC as reported previously.[16] Following washing with CH_2Cl_2 and DMF, $(Boc)_2N$-N(Boc)CH_2COOH (1.41 g, 3.6 mmol) was coupled using PyBop (1.87 g, 3.6 mmol) and DIEA (1.25 mL, 7.2 mmol) activation (30 min at room temp.). The completion of the reaction was monitored with the TNBS test. The resin was then successively rinsed with DMF, CH_2Cl_2, and diethyl ether, and dried under vacuum.

The loading was determined by UV quantification of the dibenzofulvene-piperidine adduct following deprotection with 20% piperidine in DMF. Loading found: 0.153 $mmol\,g^{-1}$.

Peptide Elongation; Synthesis of Peptide 11: The resin (0.1 mmol) was loaded into a reactor vessel for SPPS in a Pioneer Perseptive Biosystems syntheziser using an Fmoc/*tert*-butyl strategy[18] Each coupling step was followed by treatment with Ac_2O/DIEA/DMF (3:0.3:96.7 by volume). Cleavage and deprotection steps were performed with 10 mL of TFA/anisole/thioanisole/ethanedithiol/H_2O (90:2.5:2.5:2.5:2.5 by volume) for 2 h at room temp. The crude peptide was precipitated with cold diethyl ether/heptane (1:1 by volume) and centrifuged. The crude peptide was washed/centrifuged three times with diethyl ether/heptane, (1:1 by volume), redissolved in 20 mL of water/acetic acid (10:1 by volume), and lyophilized. Purification was performed on a C18 Hyperprep 15 × 500 mm column using buffers A and B (0–18% B in 15 min, 18–26% in 30 min, 26–100% in 15 min, 3 $mL\,min^{-1}$, detection at 215 nm). Following a freeze-drying step, peptide **11** was obtained as a white powder (78.7 mg, 45%). MALDI-TOF $[M + H]^+$: calcd. monoisotopic 1407.70; found 1407.72. RP-HPLC purity (215 nm): one peak >95%.

Typical Ligation Experiment. Ligation of Peptide 9 with Silica Support X5-50: Peptide **9** (6.12 mg, 3.61 μmol) was dissolved in 745 μL of 100 mM sodium acetate buffer (pH 5.5). Silica **X5-50** (40.4 mg, 1.45 μmol based on the Fmoc loading of 36 $\mu mol\,g^{-1}$) was deprotected with 20% piperidine in DMF (2 × 4 mL, 5 min and 20 min), washed with 20% piperidine in DMF, DMF (3 × 2 mL), and water (4 × 2 mL). The peptide solution (300 μL) and 700 μL of 100 mM sodium acetate buffer (pH 5.5) were added to the deprotected silica and the suspension was shaken overnight. The silica was then filtered, washed four times with 100 mM sodium acetate buffer (pH 5.5) containing 1% of Tween 20, four times with 100 mM sodium acetate buffer (pH 5.5), and three times with water, and dried in a dessicator over P_2O_5. Fmoc loading: 34.8 $\mu mol\,g^{-1}$. Yield: 96.7%.

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