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Biological functionalization of the amine-terminated Si(100) surface by glycine

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ABSTRACT

A peptide reaction of glycine on an amine-terminated Si(100) surface was investigated using C 1s, N 1s, O 1s, and Si 2p core-level spectroscopy, where the amine-terminated Si(100) surface was prepared using NH_3. In-situ thermal treatments at a mild temperature of 50 °C after the adsorption of glycine on a room-temperature amine-terminated Si(100) surface induced the peptide reaction between the carboxyl group of glycine and the amine group of the surface. This suggests that the amine-terminated Si(100) surface can be an excellent template for constructing a junction between a biomaterial and a Si surface using a dry process.

1. Introduction

The surface functionalization of an inorganic material with a biomaterial is essential for realizing a biocompatible device such as a biosensor [1]. In particular, the immobilization of a biomaterial on an inorganic material is one of the essential processes during the fabrication of a biocompatible device using bottom-up processes [2,3]. The immobilization was achieved by the formation of heterogeneous junctions between biomaterials and inorganic materials, which acts as a bridge in the signal transport of a biological signal or an electrical signal between the biomaterial and inorganic material. The formation of the heterogeneous junction has been achieved in a solution process on various inorganic materials such as Si [4], GaAs [5], silica [6], and Au [7], particularly using DNA, proteins, and peptides. However, a dry process is more compatible with the fabrication of semiconductor devices such a Si device. For this reason, it has been strongly required that a heterogeneous peptide junction with a linkage for sequential biological functionalization such as an amine ($-NH_2$) or a carboxyl (–COOH) group is realized on a semiconductor using a dry process. In the realization of a peptide junction between an amino acid and a surface, it is essential that the surface is terminated with an amine or a carboxyl group. In addition, it is desirable that the amine (carboxyl) group of the amino acid reacts only with the carboxyl (amine) group of the surface without multiple reactions [8].

For this reason, an amine-terminated Si(100) surface was used in this study, where the surface was prepared by the adsorption of NH_3 on a room-temperature (RT) Si(100)-(2×1) surface [9]. The amine termination can ensure a peptide reaction of an amino acid on the surface that reduces significantly a reaction between an amino acid and a Si atom. An amino acid composed of an amine group, a carboxyl group, and a side chain is the basic building block of DNA, proteins, and peptides that are produced by the sequent amide linkages between the amine and carboxyl groups of amino acids [10]. The simplest amino acid is glycine (CH_2NH_2COOH) which is a polar and uncharged bifunctional molecule, where its side chain is H [10]. Glycine has generally been used for studies of biomolecule reactions on various surfaces owing to its simple structure [11–13]. Accordingly, the reaction of glycine on the amine-terminated Si(100) surface can be a prototype in the study of a peptide reaction on a surface. The peptide reaction of glycine on the amine-terminated Si(100) surface was driven by thermal annealing at 50 °C after the adsorption of glycine at RT, as shown in Fig. 1. The reaction of glycine on a hydrogen-terminated Si(100) surface was also studied to clarify the reaction of glycine with the amine group on the amine-terminated Si(100) surface. In contrast to the amine-terminated Si(100) surface, glycine was not adsorbed on the hydrogen-terminated Si(100) surface. This supports that glycine reacts with the amine group on the amine-terminated Si(100) surface.

2. Experimental details

The experiments were performed in an ultra-high vacuum chamber equipped with a high resolution PHOIBOS 150 analyzer (SPECS,

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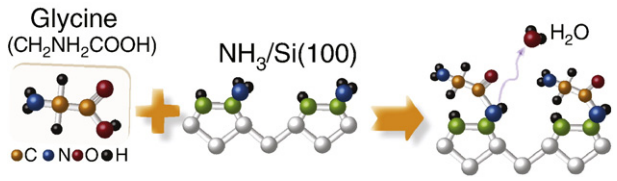

Fig. 1. The schematic diagram of the reaction process of glycine on the amine-terminated Si(100) surface.

Germany), which is installed at the 7B1 beamline in Pohang Accelerator Laboratory (PAL). The long-range orders of amine-terminated and clean Si(100)-(2×1) surfaces were observed by low energy electron diffraction (LEED). Chemical bonds before and after the reactions of glycine on the amine-terminated Si(100) surface were characterized by photoemission spectroscopy. A clean Si(100) surface was prepared by repeated resistive heating, which resulted in a 2×1 LEED pattern. The cleanness of the Si(100)-(2×1) surface was confirmed by measuring C 1s, O 1s, and Si 2p core-level spectra. Glycine (Sigma-Aldrich) was evaporated by heating a tungsten filament rapping a carbon crucible at 150 °C. The C 1s, N 1s, O 1s, and Si 2p core-level spectra were measured at a normal emission angle at RT before and after the reactions of glycine on Si samples [14]. The binding energies (E_b) of the core-level spectra were calibrated using the Si 2p core-level spectra of Si samples and the Au 4f core-level spectra of Au foil attached to a sample holder.

3. Results

An amine-terminated surface was prepared by exposing NH_3 on a RT Si(100)-(2×1) surface [9]. NH_3 is decomposed into NH_2 and H on the Si (100)-(2×1) surface and NH_2 and H saturate the dangling bonds of Si dimers, as shown in Fig. 1. A hydrogen-terminated surface was produced by the adsorption of atomic hydrogens on the Si(100)-(2×1) surface at 400 °C [15], where a hydrogen cracker was used to decompose hydrogen molecules into atomic hydrogens. The dangling bonds of the Si(100)-(2×1) surface are saturated only by H. The hydrogen-terminated Si(100) surface is thus useful in determining whether glycine reacts only with NH_2 on the amine-terminated Si(100) surface.

Fig. 2(a) and (b) show the core-level spectra after the adsorption of glycine on the hydrogen- and amine-terminated Si(100) surfaces at

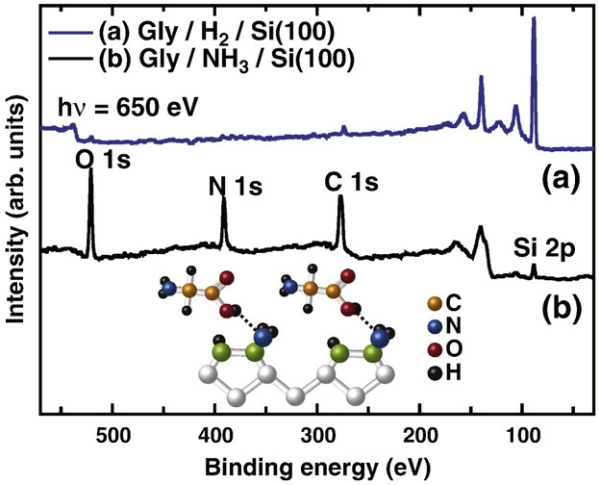

Fig. 2. The core-level spectra of (a) glycine on the RT hydrogen-terminated Si(100) surface and (b) glycine on the RT amine-terminated Si(100) surface. The schematic diagram at the bottom represents a hydrogen bond between glycine and the amine group on the amine-terminated surface. The core-level spectra were measured with a photon energy ($h\upsilon$) of 650 eV.

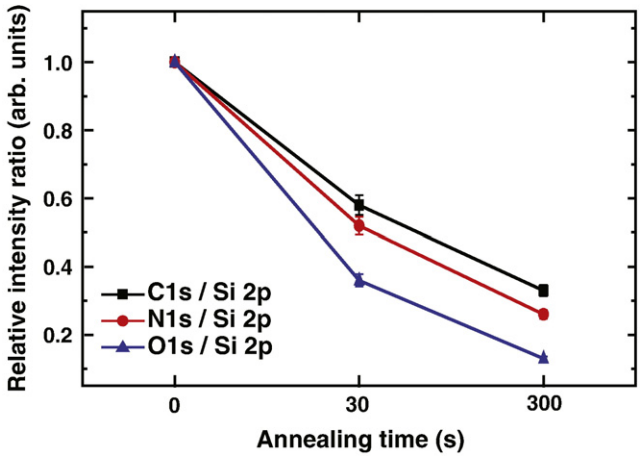

Fig. 3. The intensity ratios of C 1s, N 1s, and O 1s core-level spectra relative to Si 2p core-level spectra of glycine on the amine-terminated Si(100) surface as a function of t_a at 50 °C. The C 1s, N 1s, O 1s, and Si 2p core-level spectra were measured with $h\upsilon = 400$, 540, 650, and 140 eV, respectively.

RT, respectively. The C 1s, N 1s, and O 1s core-level spectra were not observed from the hydrogen-terminated Si(100) surface. In contrast to the hydrogen-terminated surface, C 1s, N 1s, and O 1s core-level spectra were clearly measured from the amine-terminated Si(100) surface. The distinctive difference of core-level spectra suggests that glycine does not react with the hydrogen-terminated Si(100) surface, but it is weakly bonded to the amine group on the amine-terminated Si(100) surface through a hydrogen bond [13], as illustrated at the bottom in Fig. 2. The intensity ratio of N 1s and Si 2p spectra after the adsorption of glycine on the amine-terminated surface suggests that glycine forms multiple layers on the amine-terminated Si(100) surface.

The chemical reaction of glycine on the amine-terminated Si(100) surface induced by annealing the surface at 50 °C was studied. The changes in the C 1s, N 1s, and O 1s core-level spectra as increasing annealing time (t_a) were measured. Fig. 3 shows the intensity ratios of the C 1s, O 1s, and N 1s core-level spectra relative to the Si 2p core-level spectra, where the intensity of each spectrum was normalized to the intensity before annealing ($t_a = 0$ s). The intensities of the C 1s, N 1s, and O 1s core-level spectra decreased gradually as increasing t_a. Especially, the intensity of the O 1s core-level spectrum decreased more rapidly than those of the C 1s and N 1s core-level spectra. This suggests that glycine is desorbed continuously from the amine-terminated Si(100) surface accompanied with a chemical reaction related to an oxygen dissociation from glycine. A possible origin for the oxygen dissociation from glycine on the surface is the formation of an amide bond between glycine and the amino group of the amine-terminated Si(100) surface [10], as illustrated in Fig. 1. Therefore, the changes in the intensities of the core-level spectra suggests that glycine without a direct bond with the amine-terminated Si(100) surface is desorbed under the annealing condition, whereas glycine weakly bonded to the amine-terminated Si(100) surface forms an amide bond with the elimination of H_2O.

The reaction of glycine on the amine-terminated Si(100) surface was further studied by applying curve fitting to the C 1s, O 1s, and N 1s core-level spectra, as shown in Fig. 4 and Table 1. The C 1s core-level spectrum before the annealing is composed of two components (C1 and C2) originating from the carboxyl carbon and α-carbon of glycine, respectively [16,17], as shown in Fig. 4(a). The N 1s core-level spectrum has a single component (N1) with $E_b = 401.8$ eV, which shifts 3.2 eV towards a higher binding energy with respect to that of the amine-terminated Si(100) surface, as shown in Fig. 4(d). Such a large binding

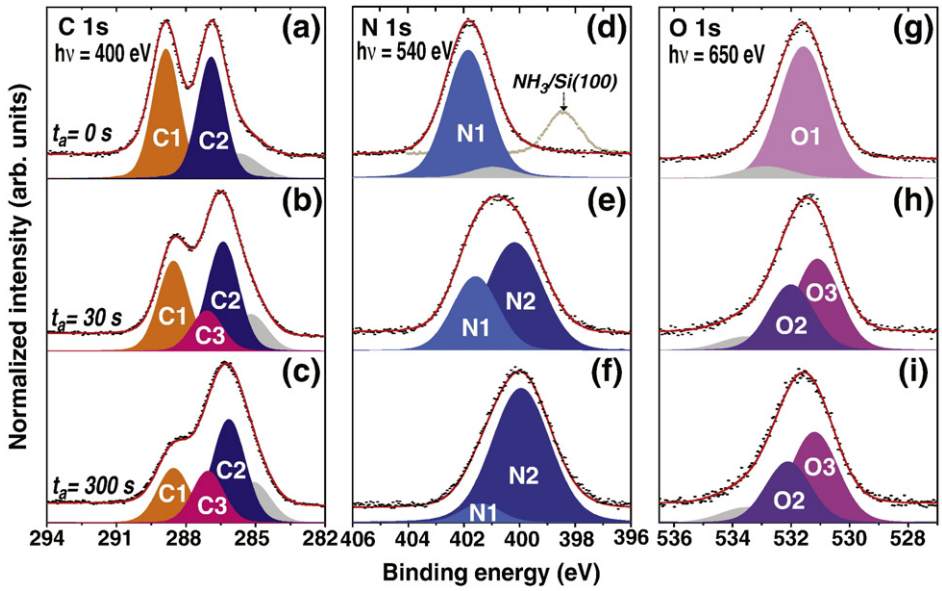

Fig. 4. Decomposed (a)–(c) C 1s, (d)–(f) N 1s, and (g)–(i) O 1s core-level spectra as a function of t_a, respectively. The arrow in (d) indicates the E_b (398.4 eV) of the N 1s core-level spectrum of the amine-terminated Si(100) surface. The C 1s, N 1s, and O 1s core-level spectra were measured with $h\upsilon = 400$, 540, and 650 eV, respectively.

energy shift of the N1 component is due to the protonation of NH_2 of glycine to NH_3^+ [10,18]. The O 1s core-level spectrum [see Fig. 4(g)] also has a single component (O1) with $E_b = 531.6$ eV, which is close to that of a carboxylate [13,18,19]. The spectra suggest that glycine exists in the form of a zwitterion on the amine-terminated Si(100) surface before the annealing which is the form of glycine in the solid state [18].

Fig. 4 shows the changes in the C 1s, O 1s, and N 1s core-level spectra as increasing t_a at 50 °C. In the C 1s core-level spectra, the intensity of the C1 component decreased with the appearance of the C3 component with $E_b = 287.4$ eV after the annealing, as shown in Fig. 4(b) and (c). The E_b of the amide bond of a peptide is between 287.4 and 288.4 eV, which is approximately 1 eV higher than that of C–N and approximately 1 eV lower than that of O = C–OH [6]. Therefore, the C3 component is able to be assigned to an amide bond (–O = C–N–). The difference in the intensity of the C1 component before and after the annealing was similar to the intensity of the C3 component. This suggests that the carboxyl group of glycine is involved in the formation of the amide bond.

In the N 1s core-level spectra, the N1 component shrank and shifted toward a lower binding energy down to 401.5 eV after the annealing, whereas the N2 component with $E_b = 400.1$ eV began to increase, as shown in Fig. 4(e) and (f). Since glycine is desorbed by the annealing, as described above, the remaining glycine in the form of a zwitterion becomes close to a neutral molecule. The neutral molecule has a lower E_b than the zwitterion. The change in the N1 component thus can be explained in terms of the transformation of glycine from the zwitterion to the neutral molecule [20,21]. On the other hand, when the amine (carboxyl) group of glycine directly reacts with a Si surface, the E_b of its N 1s core-level is 398.7 (400.6) eV [12]. This suggests that glycine does not bond to a Si atom after the annealing. In addition, the E_b of the N2 component is different from that of the polypeptide of glycine, where the E_b of the polypeptide is approximately between 400.8 and 401.0 eV [18]. This excludes polycondensation between glycines by the annealing. Another origin is the formation of the amide bond of glycine with the amine group on the surface. The N 1s core-level of the amide bond (–O = C–N–Si) on the Si surface can have a higher E_b than that of the amide bond (–O = C–N–C) of a polypeptide because of the difference in an electronegativity between Si and C. Therefore, the E_b's of the C3 and N2 components support that glycine forms an amide bond with the amine-terminated Si(100) surface leaving a neutral amino group, as illustrated in Fig. 1.

In the O 1s core-level spectra, the spectrum with a single O1 component became asymmetric as increasing t_a, as shown in Fig. 4(h) and (i). The asymmetry in the O 1s core-level spectra does not originate from an inelastic scattering due to a vibrational excitation of glycine because the asymmetry was not observed before the annealing. This suggests that there are at least two components with different chemical bonds. The asymmetric O 1s core-level spectra were fitted with two components, where the E_b's of the O2 and O3 components are 532.2 and 531.2 eV, respectively. As reported in previous experiments [16,17], when a molecule with a carboxyl group was adsorbed on a semiconductor surface, components in an O 1s core-level spectrum were not resolved. The origins of the O2 and O3 components were thus determined by the O 1s core-levels of glycine in the gas phase, where the O 1s core-level of the hydroxyl (–OH) group of glycine in the gas phase has a higher E_b by 1.5 eV than that of the keto (C = O) [18]. For this reason, the O2 component is able to be assigned to the hydroxyl group of glycine without a reaction with the amine group on the surface and the O3 component to the keto of both the amide bond between glycine and the amine group on the surface and the carboxyl group of glycine without the reaction. The E_b's of the two components are away from those of Si suboxides, where the E_b's of Si–O and Si–O–Si are 533.5 and 532.6 eV, respectively [22]. This suggests that the Si surface was not oxidized by the annealing.

Table 1
The fitting parameters of the C 1s, N 1s, and O 1s spectra shown in Fig. 4. The units of the E_b and Gaussian width (GW) are eV.

Core-level	Component	E_b(GW)		
		$t_a = 0$ s	$t_a = 30$ s	$t_a = 300$ s
C 1s	C1	288.80 (1.28)	288.50 (1.45)	288.50 (1.55)
	C2	286.80 (1.28)	286.40 (1.53)	286.30 (1.60)
	C3		287.40 (1.50)	287.40 (1.50)
N 1s	N1	401.80 (1.65)	401.50 (1.80)	401.50 (2.00)
	N2		400.10 (2.00)	400.10 (2.40)
O 1s	O1	531.60 (1.85)		
	O2		532.20 (1.70)	532.20 (1.70)
	O3		531.20 (1.70)	531.20 (1.80)

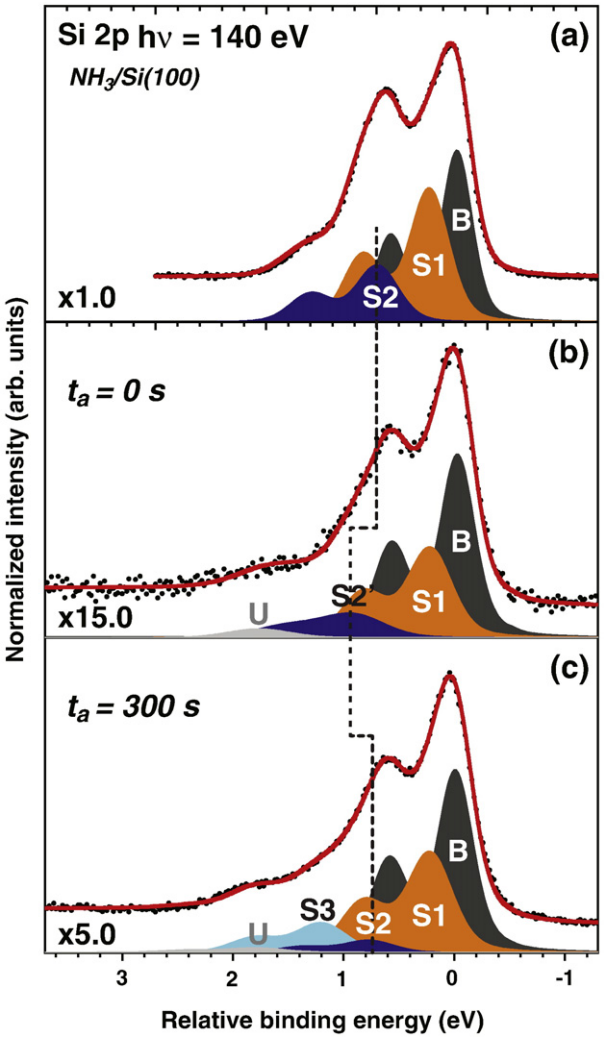

Fig. 5. The Si 2p spectra of the amine-terminated Si(100) surfaces (a) before and (b)–(c) after the adsorption of glycine, where the t_a of (b) and (c) are 0 and 300 s, respectively. The Si 2p spectra were measured with $h\upsilon = 140$ eV.

Fig. 5(a) shows the Si 2p spectrum of the amine-terminated Si(100) surface. The Si 2p spectrum is composed of three components. The B, S1, and S2 components originate from bulk Si, H-terminated Si, and, NH_2-terminated Si, respectively [14]. The E_r's of the S1 and S2 components are 0.25 and 0.72 eV, respectively, as shown in Table 2, where E_r is the relative binding energy of each component to the binding energy of the B component. Fig. 5(b) shows the Si 2p spectrum after the adsorption of glycine on the RT amine-terminated Si(100) surface. The S1 component keeps the same E_r, which suggests that H on the amine-terminated Si (100) surface does not react to glycine. In contrast, the S2 component shifts to a higher binding energy by 0.23 eV, which is assigned to the S2′ component in Fig. 5(b). This supports that glycine reacts with NH_2 on the amine-terminated Si(100) surface and the S2′ component originates from NH_2 bonding weakly to the zwitterion of glycine. The U component with a E_r of 1.80 eV can be assigned to a Si suboxide, but the U component does not originate from a Si suboxide because of the following reasons. Even after the annealing at 50 °C, the intensity of the U component was maintained by keeping its E_r, as shown in Fig. 5(c). If the origin of the U component is a Si suboxide, the U component has to shift to a higher binding energy and its intensity is expected to increase because of further oxidation by the annealing. This suggests that the origin of the U component is not a Si suboxide and Si oxides were not formed by the annealing. The S3 component with a E_r of 1.21 eV is able to be assigned to the amide bond (–O = C–N–Si) between glycine and NH_2 on the amine-terminated Si(100) surface. This is because electrons are transferred from N to C.

Table 2
The fitting parameters of the Si 2p spectra shown in Fig. 5. The units of the E_r and Gaussian width (GW) are eV.

Surface	Parameter	Component					
		B	S1	S2	S2′	S3	U
NH_3/Si(100)	E_r	0	0.25	0.72			
	GW	0.27	0.35	0.47			
$t_a = 0$ s	E_r	0	0.25		0.95		1.80
	GW	0.30	0.40		0.60		0.60
$t_a = 300$ s	E_r	0	0.24	0.75		1.21	1.80
	GW	0.30	0.40	0.45		0.45	0.45

4. Conclusion

A peptide junction between glycine and an amine-terminate Si(100) surface was successfully achieved using a dry process. Glycine was adsorbed on a RT amine-terminated Si(100) surface and sequently annealed at 50 °C, which was compared with the adsorption of glycine on a hydrogen-terminated Si(100) surface. The C 1s, N 1s, O 1s, and Si 2p core-level spectra were measured before and after the thermal treatment as a function of annealing time. Glycine was weakly bonded to the amine-terminated Si(100) surface at RT and its form was similar to a zwitterion in the solid state. The thermal treatment induced the carboxyl group of glycine to form an amide bond with the amine group on the Si surface. The achievement of a peptide reaction using a dry process is important because the dry processes employed in the fabrication of semiconductor devices can be applied to biocompatible devices.

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