In Situ Photopolymerization of Methacrylic Acid at a Self-Assembled Xanthate Monolayer Surface on Gold. Formation of Poly(methacrylic acid) Brushes and Their Interaction with Cytochrome *c*

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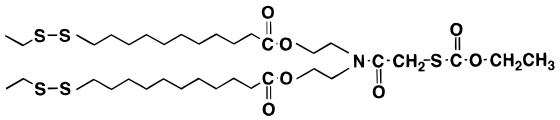
Received October 12, 1995; Revised Manuscript Received February 26, 1996[⊗]

ABSTRACT: Mixed monolayers of photoinitiatable xanthate-carrying, double-chained disulfide compound **1** and the corresponding xanthate-free compound **2** were spontaneously formed on a gold-coated quartz crystal microbalance (QCM). Self-assembling processes of these mixtures could be monitored as a frequency shift of QCM, and the composition of the resultant monolayers was found to be comparable to that of feed in solutions (f_1, mole fraction of **1** in the mixture). Photopolymerizations of methacrylic acid in aqueous solution were carried out upon UV irradiation initiated with xanthate groups of the monolayer-modified QCMs and immediately traced by measuring the frequency change. The polymerization rate was strongly dependent upon f_1 and gave a maximum at around $f_1 = 0.2$. In addition, the polymerization rate showed a marked pH dependence. Finally, an adsorption of cytochrome *c* to the poly(methacrylic acid) brushes thus prepared was examined by means of QCM and spectroscopy. As a result, cytochrome *c* was found to adsorb successfully to the poly(methacrylic acid) brushes through electrostatic interaction, depending upon the composition of the poly(methacrylic acid) chain grown at the monolayer surface.

Introduction

For many years, much effort has been dedicated to the study of macromolecules carrying ionized or ionizable groups. These synthetic polyelectrolytes are important as simplified models of natural polyelectrolytes such as protein and nucleic acid and also in many industrial applications. To fabricate the higher ordered structures of such biopolymers by using purely synthetic polyelectrolytes, we have devised a strategy in which polyelectrolytes are aligned on two-dimensional media such as water surface[1−7] and a solid substrate.[8−10] For the two-dimensional solid substrate, we have chosen gold-deposited plates since well-organized monolayers can be readily prepared on gold by spontaneous adsorption of organic thiols and disulfides.[11−19] Poly(methacrylic acid) (PMAA) has been employed as a typical polyelectrolyte because PMAA is well-known to show a marked pH-induced conformational change in water;[20] at low pH the macromolecule adopts a hypercoiled form to minimize the hydrophobic interactions, and at a higher degree of ionization (higher pH) the PMAA chain stretches to an expanded form. The novel polymeric amphiphiles, consisting of a PMAA segment and two long alkyl chains whose ends are modified with disulfide groups to attach to a gold surface, have been found to successfully form spontaneously adsorbed monolayers on gold substrates, and lateral molecular orientations as well as molecular thicknesses of the resulting monolayers have been governed by the conformational size and/or the chain length of the PMAA segment during adsorption.[9] The molecularly controlled surfaces of such PMAA "brushes" would be expected to be a useful environment for molecular interactions, and in fact they were found to have the ability to read out the chain length of guest polymers such as poly(ethylene glycol).[10]

Another approach to fabricate polyelectrolyte assemblies is to grow PMAA chains on an organized monolayer surface by the use of chemical reactions such as polymerization of methacrylic acid. For that purpose, we have designed a self-assembled monolayer whose surface is modified with a photoactive xanthate group. Xanthate derivatives were established to serve as a living radical initiator in photopolymerizations of vinyl monomers.[21] In the present paper, we describe the preparation of self-assembled monolayers (**1**) having a



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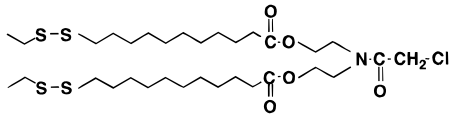

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photoactive surface on gold-coated quartz crystal microbalances (QCMs) and the photopolymerization of methacrylic acid on those monolayer surfaces. QCMs are known to provide very sensitive mass-measuring devices because of the resonance frequency changes upon the deposition of a given mass on the QCM electrode.[22−25] We have tried to use this technique to monitor in situ monolayer formation and photopolymerization processes. To characterize the surface property of the grown PMAA chains, an interaction with protein such as cytochrome *c* (cyt *c*) is also examined.

Experimental Section

Materials. The preparation of photoactive xanthate derivative *O*-ethyl-*S*-*N*,*N*-bis[(2-[(11-ethyldithioundecanoyl)oxy]-ethyl)carbamoylmethyl]dithiocarbonate (**1**) and xanthate-free *N*,*N*-bis[(2-[(11-ethyldithioundecanoyl)oxy]ethyl)carbamoylmethyl chloride (**2**) have been described elsewhere.[9] Cytochrome *c* from horse heart (cyt *c* type IV, Sigma) was used as purchased. Methacrylic acid was distilled under nitrogen atmosphere before use, and all other chemicals were reagent grade and used as received.

Method. Monolayers were spontaneously formed by immersing gold-mirror plates, which were prepared by evapora-

[⊗] Abstract published in *Advance ACS Abstracts,* April 15, 1996.

S0024-9297(95)01523-3 CCC: $12.00

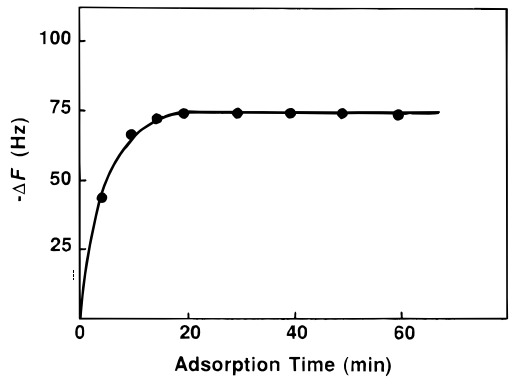

Figure 1. Frequency shift upon adsorption of **1** on a gold-coated QCM.

tion of gold onto glass plates or quartz crystal microbalances (QCM), into a 1 mM solution of **1** or mixtures of **1** and **2** in chloroform. After a prescribed period, the gold substrates were removed and rinsed with clean chloroform. The monolayer formation processes were monitored by QCM technique (USI System, Japan); only one side of the resonator was in contact with the surface of the solution, and the frequency change was recorded continuously.

X-ray photoelectron spectra (XPS) for the mixed monolayer on a gold plate were measured by a Shimadzu ESCA-1000 system with a Mg Kα X-ray source at a takeoff angle of 30°. The charging shift was corrected with the C_{1s} line emitted from neutral hydrocarbon.

Photopolymerization of methacrylic acid initiated with the monolayers thus obtained was carried out as follows. The monolayer-covered QCMs were immersed into the aqueous solution (2 mL) containing methacrylic acid (1 M) filled in a Pyrex glass cell, and then it was irradiated continuously with UV light of a low-pressure Hg lamp (160 W), which has a maximum light intensity at 253.7 nm. Photopolymerization processes were also monitored as the frequency change of QCMs. Water used in all experiments was purified with Milli Q System (Milli Pore Ltd.) and was bubbled with nitrogen gas due to purging oxygen gas that serves as an inhibitor for polymerization.

The interaction of these PMAA segment-carrying monolayers with cyt *c* was examined by means of QCM technique and UV−vis reflection spectroscopy (Otsuka Electronics, MCPO-1000, Japan). The monolayer-covered QCM was immersed into a phosphate buffer solution (4 mM, pH 7) containing 30 μM cyt *c*, and the frequency change was immediately recorded. The reflection spectra were measured for the samples before and after adsorption of cyt *c*.

Results and Discussion

Self-Assembled Monolayer Formation on Gold-Coated QCM. The mass increase due to adsorption can be estimated from QCM frequency shift by using the Sauerbrey equation.[26] The following relation is obtained between adsorbed mass, Δm (ng), and frequency shift, ΔF (Hz), by taking into account characteristics of quartz resonators used in this study:

$$\Delta F = -0.184\Delta m/A \qquad (1)$$

where A is the surface area of the resonator. This value corresponds to an apparent area of gold deposited onto QCM, $A = 0.20 \pm 0.02$ cm^2, and then one finds that 1 Hz change in ΔF corresponds to 1.1 ng.

The self-assembling process of **1** was examined in situ in a chloroform solution in which the QCM sensor was immersed. After the frequency became unchanged with time in pure chloroform, the chloroform solution of **1** was added, and the frequency shift was recorded as shown in Figure 1. The concentration of **1** was 1 mM after the addition. The frequency steeply decreased upon the addition, reflecting adsorption of **1** on the gold surface of QCM. The frequency became constant around 40 min after the addition of **1**. A total frequency shift during adsorption of **1** was 75 Hz, which corresponds to 4.0×10^{-10} mol cm^{-2} on the basis of eq 1. By using this value, the occupied area per molecule on the gold surface can be calculated to be 0.37 nm^2, which is in good agreement with that (0.40 nm^2) for the cross-sectional area of two long alkyl chains aligned vertically to the surface normal. The monolayer is, therefore, considered to be an ideal film consisting of **1** molecules packed densely and oriented vertically to the gold surface. Single-chain thiols and disulfides have been found to form a monolayer with fully extended alkyl chains tilted for the gold surface normal by 20−30°.[27] The structural difference between them and our disulfide exists in the number of alkyl chain and thus the formation of such an ideal film may result from the double-chained structure of **1**. A similar situation has been observed in our previous work on ellipsometric estimation.[9]

Table 1. Total Mass Changes due to Adsorption of the Mixtures of 1 and 2 with Various Feed Compositions (f_1)

	total mass increase, Δm_t (ng)	
f_1	Δm_t(obs)[a]	Δm_t(theo)[b]
0	70	69
0.1	72	74
0.2	74	75
0.3	74	76
0.4	75	77
0.7	79	80
0.9	82	82
1.0	83	83

[a] Total mass changes calculated from the observed frequency shifts by using eq 1. [b] Theoretical values of total mass change estimated under the assumptions described in the text.

The assembling processes of the mixtures of **1** and **2** were subsequently examined in the same way. The composition of the mixture denoted as a molar fraction of **1** in a feed of solution (f_1) was varied over the wide range of $f_1 = 0-1.0$. Adsorption profile was similar to that for pure **1** and the adsorption equilibrium was found to be reached after about 40 min for all mixtures, while a total frequency shift during adsorption was slightly different among the mixtures. Total mass changes (Δm_t)(obs) calculated from the observed frequency shifts were summarized Table 1 in relation to the feed composition (f_1). In this table, a theoretical value of total mass change (Δm_t(theo)) is also listed that was calculated under the following assumptions: (1) molecular areas of **1** and **2** within the resultant monolayers on gold surfaces are the same due to similarity in their molecular structure; (2) compositions of the monolayers are the same as those of the feed solutions. It can be seen from the table that there is good agreement between the observed and theoretical values in total mass change. To obtain more direct information regarding the monolayer composition, an XPS spectrum was measured for a typical monolayer of $f_1 = 0.20$. From the elemental ratio of sulfur and chlorine referred to total carbon atom, which were estimated on the basis of S_{2p}, Cl_{2p}, and C_{1s} spectra, respectively, the monolayer composition was calculated to be 0.21, consistent with the feed composition ($f_1 = 0.20$). These results strongly suggest that self-assembled monolayers containing **1** and **2** are formed on gold surface with the same composition as that in feed solutions, and thus a two-

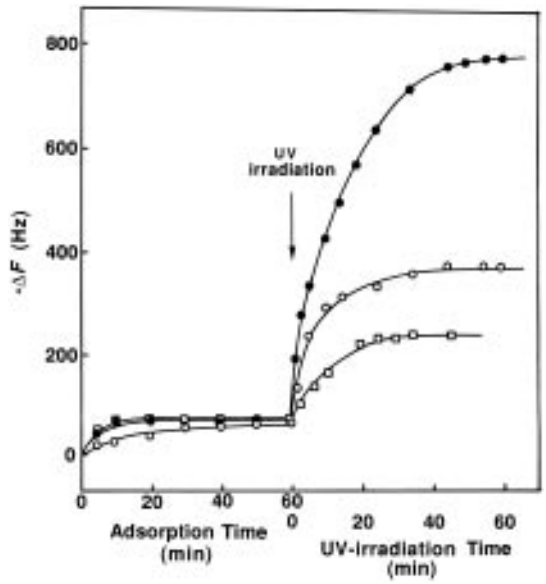

Figure 2. Frequency shift upon polymerization of methacrylic acid initiated with the immobilized monolayers of **1** and **2** (○, $f_1 = 0.1$; ●, $f_1 = 0.2$; □, $f_1 = 0.4$) on QCMs.

dimensional distribution of photoinitiatable (xanthate) groups on these mixed monolayer surfaces would be readily regulatable by varying the feed composition.

Photopolymerization of Methacrylic Acid on the Mixed Monolayer Surface. Photopolymerizations were carried out upon UV irradiation at the monolayer-covered QCMs, which were immersed in aqueous solutions containing methacrylic acid (1 M). The polymerization process was monitored as the frequency shift of QCM. Figure 2 shows frequency changes upon UV irradiation for typical monolayers with various compositions of $f_1 = 0.1$, 0.2, and 0.4, which were prepared by immersing the gold-coated QCMs into the corresponding chloroform solutions for 60 min. For each monolayer, the frequency is found to steeply decrease upon irradiation, followed by a gradual decrease, and then becomes constant around 60 min after irradiation. Such a frequency decrease must be derived from a mass increase due to the growing PMAA chains initiated with xanthate groups attached to the monolayer surface. There are marked differences both in the initial rate of frequency decrease and in the total frequency shift during irradiation among the monolayers with various composition. An apparent polymerization rate (R_p) is thus estimated from the initial frequency decrease and plotted as a function of the monolayer composition, f_1 in Figure 3. At the monolayer composition of $f_1 = 1.0$ (pure **1** monolayer), the R_p shows a smallest value. With decreasing f_1, R_p gradually increases and gives a maximum value at $f_1 = 0.2$. We do not have any conclusive evidence so far to explain this phenomenon, but one interesting possible explanation is as follows. First the initiators (**1**) immobilized on QCM photodissociate upon UV irradiation to generate primary radicals that can initiate polymerization, and subsequently the resultant growing polymer radicals progressively pick up monomers as shown schematically in Figure 4. Since methacrylic acid as a monomer would be gathered around the growing PMAA chains due to attractive interactions such as hydrogen bonding and hydrophobic interaction, an effective monomer concentration must become higher than that in bulk and then the polymerization would be accelerated. In that case, a distribution of xanthate groups at the two-dimensional surface should also become important for an effective polymerization. Actually, in the case of pure **1** monolayer ($f_1 = 1.0$) the polymerization hardly proceeded (see Table 2), probably because many initiator radicals generated at the two-dimensional surface could not readily pick up apparently large monomers that would be in a hydrated form and/or because a crowded growing radical would suppress an effective propagation reaction even though initiator radicals could capture monomers. As a result, there existed a most appropriate monolayer composition (i.e., two-dimensional initiator distribution) at around $f_1 = 0.2$.

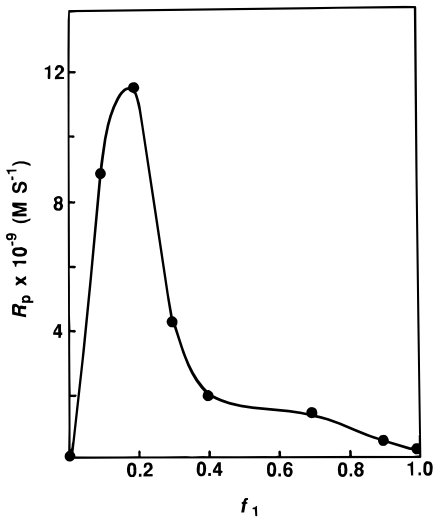

Figure 3. Monolayer composition (f_1) dependence of the apparent polymerization rate (R_p) of methacrylic acid.

The total mass increase (Δm_t) during polymerization, calculated from the observed frequency shift in Figure 2, is summarized in Table 2 together with R_p. The monolayer composition dependence of Δm_t is found to be very similar to that of R_p, and Δm_t gives again a maximum at around $f_1 = 0.2$.

All polymerization experiments described above were carried out in acidic solutions of pH 2.3. Subsequently, we performed polymerizations at various pHs (2.3−7.0) by using a monolayer of $f_1 = 0.2$ in the same way since the monomer and the resultant polymer have ionizable COOH groups. Figure 5 shows relationship between polymerization rate, R_p and pH in the solutions. With elevating pH from 2.3, the R_p drastically decreases, and at pH 7.0 the polymerization is completely depressed. The values of pK_a for methacrylic acid and PMAA have been reported to be 3.5[28] and 7.3[28] (apparent pK_a), respectively. In the low-pH region below the pK_a of methacrylic acid, the monomer, whose carboxylic acid groups is completely protonated, can be polymerized smoothly as mentioned above. Beyond the pK_a (pH > 3.5), on the other hand, an electrostatic repulsive force works among monomers and then it becomes difficult for them to gather around the growing radical, leading to a decrease in R_p. When the pH is elevated to 7.0, the polymerization is almost inhibited because ionization of carboxylic acid groups of the growing polymer chains is also promoted in addition to that of the monomers.

Interaction of Cyt *c* with the PMAA Brushes. To characterize the surface property of thus-grown PMAA brushes, interaction with cyt *c* has been examined by using a QCM technique. Cyt *c* is a typical redox protein, whose surface is known to be covered with positively charged lysin residues at neutral pH.[29,30] At that pH,

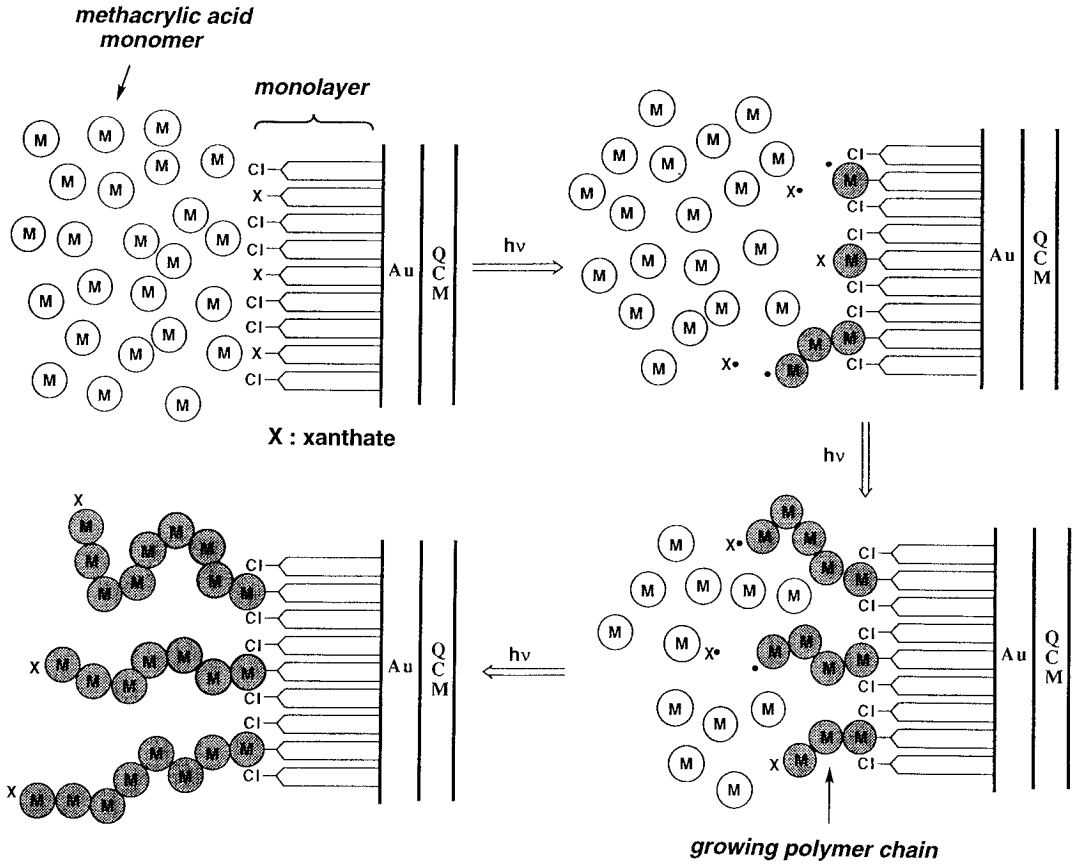

Figure 4. Possible illustration for the polymerization of methacrylic acid at the monolayer surface.

Table 2. Monolayer Composition Dependence of the Total Mass Increase during Polymerization (Δm_t) and the Mean Degree of Polymerization of a PMAA Brush (n)

f_1	$10^9 R_p$ (M s^{-1})	Δm_t[a] (ng)	n[b]
0	*c*	*c*	*c*
0.1	8.9	326	358
0.2	12.0	751	399
0.3	4.3	153	60
0.4	1.9	186	54
0.7	1.4	110	19
0.9	0.4	64	8
1.0	0.2	11	1

[a] Total mass changes during polymerization calculated from the observed frequency shifts by using eq 1. [b] Mean degree of polymerization of the PMAA brush estimated by using the values of Δm_t and f_1. [c] No mass increase was observed during 1 h of UV irradiation.

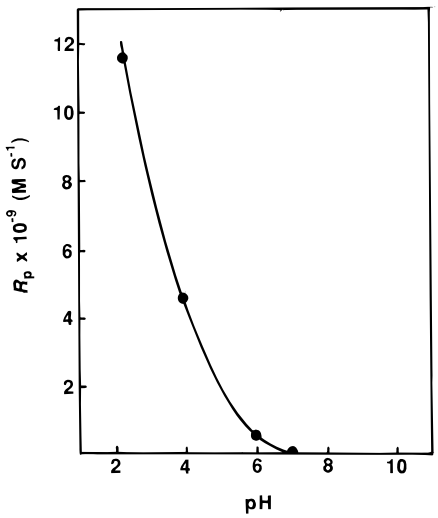

Figure 5. pH dependence of the apparent polymerization rate (R_p) of methacrylic acid.

in contrast, the monolayer surface is expected to have negative charges of the deprotonated PMAA brush, which would lead to attractive interaction with cyt *c*. The cyt *c* adsorption process was performed by immersing the PMAA monolayer-modified QCM in a solution with 30 μM cyt *c* in 4 mM, pH 7 phosphate buffer and immediately monitored by a frequency shift. Figure 6 displays the frequency shift for the PMAA monolayer with $f_1 = 0.4$. Upon the immersion, the frequency steeply decreased, followed by a gradual decrease, and then leveled off at around 80 min. When the same experiment was performed for the PMAA brush-free ($f_1 = 0$) monolayer, there was no significant frequency shift (shown as a dashed line in Figure 6). Thus, the observed frequency decrease must be due to adsorption of cyt *c* on the PMAA chains through electrostatic binding. Rinsing the cyt *c*-bound PMAA monolayer with buffer four additional times resulted in a nearly identical frequency value, which implies that desorption is negligible. Further evidence for binding of cyt *c* on the monolayer would be obtainable by spectroscopy. When a reflection−absorption spectrum (not shown here) was measured for the monolayer ($f_1 = 0.4$) after 80 min adsorption of cyt *c*, there was a new absorption peak at 412 nm, which should be ascribable to the heme moiety of cyt *c* since cyt *c* in buffer solution exhibited an absorption maximum at 409 nm.

It is meaningful to reveal the relation between composition (distribution) of the PMAA brush on the monolayer and cyt *c* adsorption behavior. The total frequency decrease upon adsorption of cyt *c* is plotted against f_1 in Figure 7. Here we assume that f_1 corresponds to a surface composition of the PMAA chain; i.e.,

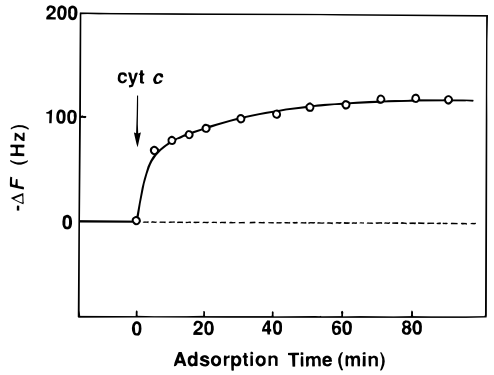

Figure 6. Frequency shift upon adsorption of cyt *c* on the PMAA brushes grown at the mixed monolayer ($f_1 = 0.4$, solid line) and on the PMAA brush-free monolayer ($f_1 = 0$, dashed line) immobilized on QCM.

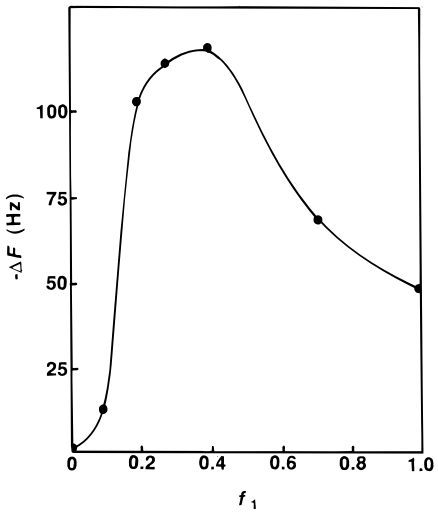

Figure 7. Monolayer composition dependence of the frequency shift upon adsorption of cyt *c* on PMAA brushes.

each xanthate initiator produces exactly one bound PMAA chain. With increasing the number of PMAA chain on the monolayer surface (f_1), a drastic increase in the amount of adsorption of cyt *c* is observed, and it gives a maximum at around $f_1 = 0.4$, followed by a decrease with further increase in f_1. It may be difficult to discuss precisely such an adsorption behavior of cyt *c* only in terms of the surface composition of PMAA chains (f_1), because the chain length (n, see Table 2) and the total number of methacrylic acid unit of PMAA brushes were considerably different in f_1. Despite such a difficulty, it must be certain that the surface distribution of PMAA brushes plays a major role for an effective cyt *c* adsorption.

Conclusions

In the present study, we have demonstrated that (1) self-assembled monolayers, composed of photoinitiatable xanthate-carrying **1** and xanthate-free **2**, on gold-coated QCMs can be formed, in which the compositions of monolayers are the same as those of solutions, (2) photopolymerizations of methacrylic acid initiated with these monolayers are successfully monitored as a frequency shift of QCM, and (3) the resultant PMAA brushes grown on the monolayers give a favorable field for accepting guest macromolecules such as protein, depending upon the surface composition of PMAA chains. These findings will provide a novel methodology to construct a "polymer brush" on a molecularly ordered monolayer surface.

Acknowledgment. Financial support from the aid of Doshisha University's Research Promotion Fund (1995) is gratefully acknowledged.

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MA9515238