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Polyion-complexed assemblies of diacetylenic carboxylic acid with triblock polyamine carrying a boronic acid-functionalized segment

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The polyion complex of 10,12-pentacosadiynoic acid **1** and a novel ABA-type triblock polyamine **2**, containing phenylboronic acid groups as sugar-responsive sites, has been prepared. Monolayer properties of the complex **2**/**1** are examined by measuring surface pressure (π)–area (A) isotherms on water. The π–A curves are found to vary drastically by changing pH in the subphase, suggesting the conformational change of the copolymer segment of **2** containing phenylboronic acid groups and/or deformation of the polyion complex of **2**/**1**. Polymerizations of the complex monolayer of **2**/**1** are carried out upon UV irradiation, resulting in successful production of polydiacetylene with a blue form under optimum conditions of pH, temperature, substrate, surface pressure and so on. When the polymerized **2**/**1** LB film deposited on a quartz plate is immersed in aqueous solutions of various diol compounds such as sugars and nucleosides, a remarkable spectral variation due to blue to red color phase change of the polydiacetylene matrices is observed, implying binding of the diol compounds to the phenylboronic acid moieties located at the LB film surface. The extent of the spectral variation of the LB film seems to be proportional to the binding ability for the diol compounds.

We report the photopolymerization of mono- and multi-layers of polyion-complexed diacetylenic carboxylic acids with a triblock polyamine containing phenylboronic acid groups, and their interaction with diol compounds. Polyion complexation of ionic amphiphiles and oppositely charged polyions at the air–water interface is one of the most fascinating techniques for stabilization and facilitated deposition of monolayers.[1] Such polyion-complexed Langmuir–Blodgett (LB) films have been found to be stable in water. Another merit of the polyion complexation is to incorporate a functional polymer segment into the polyions.[1]

In this paper, we employed 10,12-pentacosadiynoic acid **1** as an ionic amphiphile and prepared freshly an ABA-type triblock polymer **2** composed of poly(2-dimethylaminoethyl methacrylate) (PDAM) segments and a copolymer segment made from DAM and *m*-methacrylamido phenylboronic acid (MPB) as a polyion (Fig. 1). The copolymer segment is expected to serve as a sugar-responsive site since boronic acid has been demonstrated to form stable complexes with diol compounds including poly(vinyl alcohol), glucose and sorbitol.[2–5] The introduction of tertiary amino groups into the copolymer segment is to facilitate sp^3-hybridization of the boron atom, which should enhance the complexation with these diol compounds.[5,6] The topotactic polymerization of diacetylene is well known to be acutely sensitive to the molecular order of molecular assemblies.[7] Thus, such a stringent requirement for efficient photopolymerization of diacetylenes provides an excellent test of the ordering in polyion-complexed mono- and multi-layer films. Additionally, the resultant polydiacetylene films have unique optical properties; the conjugated backbone of alternating double and triple bonds gives rise to intense absorptions in the visible spectrum. In single crystals or LB films,[8] these materials are known to undergo blue to red color transitions due to various environmental perturbations.[9–12] Recently, Reichert *et al.*[13] prepared a sialic acid-modified polydiacetylenic liposome that could specifically bind to influenza virus particles and could report the binding event by undergoing a visible color change. The present paper will describe the polymerization behavior of our polyion-complexed diacetylene mono- and multi-layers and their response to diol compounds.

Results and Discussion

Preparation and characterization of polyion complex

We have shown in previous papers[14] that bis(isopropylxanthogen) disulfide (BX) serves as an photoinitiator–transfer agent–terminator for the photopolymerization of vinyl monomers and that the resulting polymers contain an isopropylxanthate group as a photoinitiatable site at each end of the polymer chain. When a second vinyl monomer is polymerized using these macro-photoinitiators, ABA-type triblock polymers are formed. In the same manner, a triblock polymer of **2** was freshly prepared. At first, a copolymer containing a photosensitive isopropyl xanthate group at each end of the copolymer chain was prepared by photopolymerization of MPB and DAM (1:1 in molar ratio) in the presence of BX. DAM was then polymerized by the copolymer as a macro-photoinitiator upon UV irradiation. As a result, a triblock polymer with segment lengths of $n = 39$ and $m = 19$ and with a copolymer composition of 1:1 was obtained. To elucidate the effect of incorporation of DAM unit into the copolymer segment, the pK_a value of the boronic acid moiety was measured in water by means of pH titration. From the titration curve (data not shown), the pK_a value was estimated to be 7.1. A phototitration method was also applied to this polymer to determine pK_a, since the absorption maximum of the phenylboronic acid moiety showed a red shift on going from a neutral to anionic species. The pK_a value (7.0) determined was consistent with that (7.1) obtained *via* pH titration. The results imply that the pK_a value of phenylboronic acid moiety is successfully lowered in the presence of tertiary amino groups, since the pK_a of monomeric phenylboronic acid is estimated to be 8.8. The interaction of the phenylboronic acid moiety of **2** with D-fructose, a typical diol compound, in water was subsequently examined *via* ^{11}B NMR spectroscopy. Fig. 2 shows ^{11}B NMR spectra of **2** before and after addition of D-fructose at pH 7.1. The spectrum obtained in the absence of D-fructose gives a peak at δ 10 that can be assigned to the free (sp^2-hybridized) boronic acid.[15] By adding a large excess of D-fructose, a new

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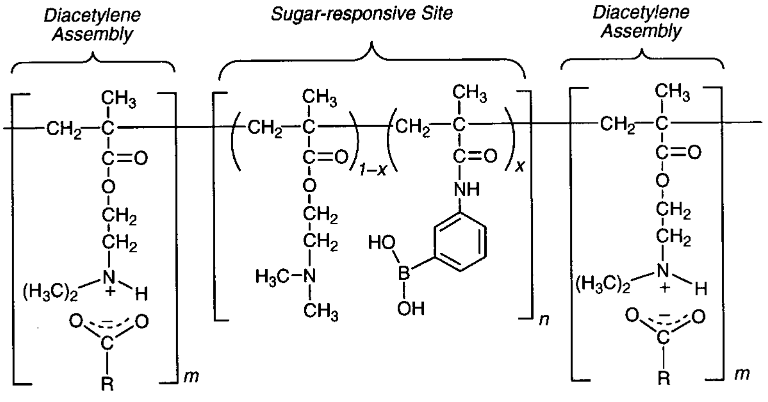

$R = —(CH_2)_8—C≡C—C≡C—(CH_2)_{11}—CH_3$

Fig. 1 Chemical structure of diacetylenic polyion complexes containing sugar-responsive sites

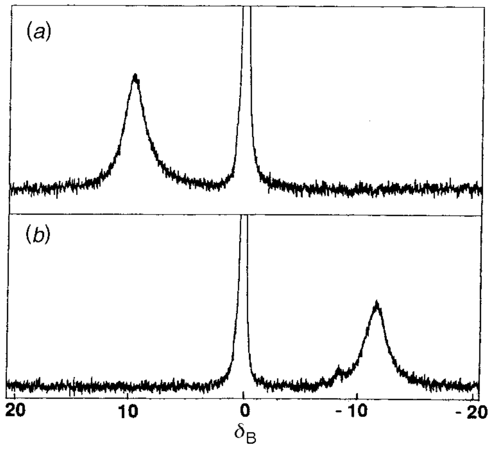

Fig. 2 ^{11}B NMR spectra of triblock polymer **2** (*a*) before and (*b*) after addition of D-fructose in $D_2O–H_2O$ (1:4 v/v) at pH 7.1 and 30 °C. The external standard is $B(OCH_3)_3$.

peak appears at δ −11.8 and simultaneously the peak at δ 10 disappears. Such a drastic upfield shift has been demonstrated to be due to formation of a boronate ester with the diol moiety of D-fructose.[16]

The polyion complex **2/1** was prepared by mixing equivalent amounts of methanolic solutions of **1** (20 mM) and **2** (20 unit mM of DAM in A segments). The chemical composition of the **2/1** complex thus obtained was estimated by ^1H NMR spectroscopy. The ratio of the content of **1** to that of DAM units in the A segments of **2** was calculated, by assuming that **1** would predominantly complex with DAM units of the A segments but not with DAM units of the B segment (copolymer segment), because the DAM units located in the B segment should complex with the vicinal MPB unit through formation of a charge transfer complex.[5,6] On the basis of the area ratio of the signal of ω-CH_3 in **1** to that of N-CH_3 in polymer **2**, the ratio of [**1**]:[DAM units in the A segment] was evaluated to be 0.97:1, which supports the molecular structure of the polymer complex **2/1** drawn in Fig. 1.

Photopolymerization of polyion-complexed monolayers

The monolayer properties of the polymer complex **2/1** thus obtained were examined by measuring surface pressure (π)–area (*A*) isotherms on water. Fig. 3(*a*) displays a π–*A* isotherm of the **2/1** complex on pure water (pH 5.8) at 20 °C. The π–*A* curve of the **3/1** complex without the boronic acid-carrying copolymer segment is also shown for comparison. The monolayer of **2/1** is found to considerably expand, compared with that of **3/1**, probably due to the existence of the copolymer segment in **2**. Fig. 3(*b*) shows the pH dependence of the π–*A* curve of the **2/1** complex. The π–*A* curve measured at pH 10 almost fits that at pH 5.8. The pK_a value of **1** in water was 5.5 by pH titration. Therefore, at pH 10 electrostatic interactions would predominate between **1** and **2** because **1** is in the deprotonated carboxylate anion form at higher pH. At pH 5.8, where **1** might be in both carboxylate anion and protonated free carboxylic acid forms since the pK_a value of **1** is close to the subphase pH, hydrogen bonding as well as electrostatic interactions can be considered to contribute to the stability of the complex of **1** and **2**, as illustrated in Fig. 3. Lowering the pH in the subphase below the pK_a of **1** (pH 3) causes a drastic contraction and a drop in collapse pressure of the monolayer, which is very similar to those of a pure monolayer of **1** under the same conditions (data not shown), implying deformation of the complex due to protonation of the carboxylic acid of **1**.

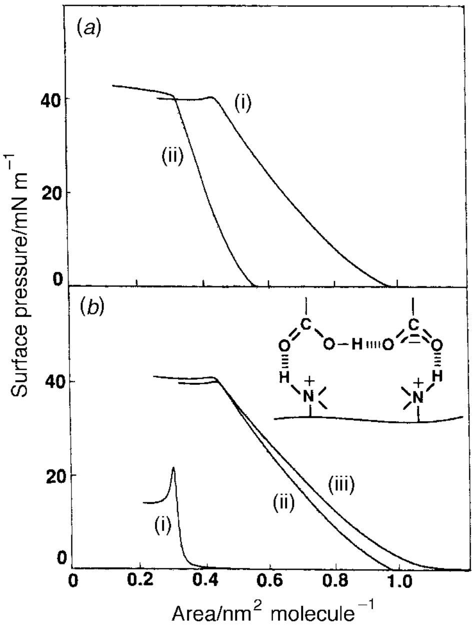

Fig. 3 (*a*) Surface pressure (π)–area (*A*) isotherms for (i) **2/1** and (ii) **3/1** complexes on pure water at pH 5.8 and 20 °C. (*b*) pH dependence of π–*A* isotherms of the **2/1** complex at 20 °C and (i) pH 3.0, (ii) pH 5.0 and (iii) pH 9.9.

Subsequently, photopolymerizations of the complex monolayer of **2/1** were carried out at pH 5.8. Fig. 4 depicts UV–VIS spectral changes of the monolayer on water upon UV irradiation at 5 °C and a constant surface pressure of

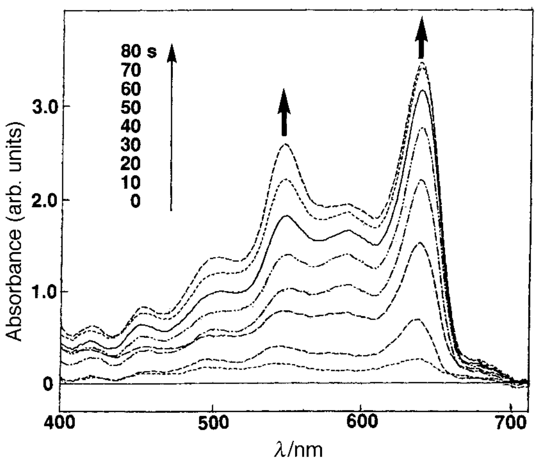

Fig. 4 Absorption spectral change of the **2**/**1** complex monolayer on pure water upon UV irradiation at 5 °C. The surface pressure was kept constant at 35 mN m^{-1} throughout irradiation.

35 mN m^{-1}, just before the collapses pressure. The appearance of the absorption peaks in the UV–VIS spectra indicates that the photopolymerization of monomer took place upon UV irradiation, since there is no absorption peak of the monomeric **2**/**1** monolayer in an observed wavelength range of 400–700 nm before exposure to UV light. The remarkable absorption peak at 640 nm and the weak and broad shoulder at around 580 nm, which are assigned to the π–π* transition (excitonic absorption) and the phonon sideband of polydiacetylene, respectively,[17] were observed. Polydiacetylene, which shows an absorption peak at 640 nm, is designated as the blue form.[18] In the spectra, other characteristic absorption peaks at 540 and 500 nm were also observed, which are assigned to the π–π* transition and the phonon sideband of the polydiacetylene red form, respectively.[18] It has been reported on the basis of spectroscopic analysis that the electronic structure of the polydiacetylene red form is different from that of the blue form. Such a difference in the electronic structure has been demonstrated to stem from the delocalization length of the π-electrons; *i.e.* the red form has a shorter delocalization length than the blue form. Fig. 5 shows the UV irradiation time dependence of the absorbance at 640 nm, according to the absorption spectra in Fig. 4. The absorbance at 640 nm increases rapidly in the early stages of irradiation and saturates upon further irradiation, indicating that the polymerization reaction of **2**/**1** is almost complete after 80 s irradiation. It is significant that a diacetylenic monolayer complexed with a polyion such as **2** causes smooth and rapid polymerization upon UV irradiation and yields the more highly conjugated blue polymer, although the existence of the less conjugated red form cannot be excluded. In Fig. 5, the absorbance change upon UV irradiation is also shown for the **2**/**1** monolayer at a lower surface pressure of 25 mN m^{-1}. There is no absorption peak in the range of 400–700 nm even after 80 s irradiation, indicating that no polymerization reaction occurred, probably due to the less ordered diacetylene molecular packing in the monolayer to be polymerized. Such an ordered molecular packing in the monolayer is readily predicted to depend on temperature. In fact, when the same experiment under a surface pressure of 35 mN m^{-1} was performed at 15 °C, the polymerization did not proceed at all because of lowering of the crystallinity of the monolayer due to thermal molecular motion.

The photopolymerization of diacetylenic monolayers has been known to be considerably affected by interface conditions, and in particular the LB films deposited from the monolayers onto solid substrates have provided a blue form-rich polydiacetylene due to suppression of molecular motion.[19] Accordingly, our polyion complex monolayer was transferred onto a quartz plate, and its polymerization behavior was examined. The hydrophobic quartz plate, prepared by coating a thin layer (*ca.* 100 Å) of poly(dimethylsiloxane) prior to use, was lowered at a speed of 50 mm min^{-1} through the monolayer on a subphase at 35 mN m^{-1} and 5 °C, and a one-layer LB film, in which the polyion **2** of the complex is exposed to the water phase, was deposited on each side of the quartz plate. The transfer ratio was close to unity. Fig. 6 displays the absorption spectra of the **2**/**1** LB film as a function of irradiation time. Only the absorption peaks at 640 and 580 nm are found to appear with polymerization, implying that the produced polydiacetylene takes the highly conjugated blue form. This result is different from that of the monolayer on water. To elucidate the influence of the surface pressure at which the monolayer was transferred onto the substrate, the same irradiation experiment was performed for a LB film prepared at a lower surface pressure of 25 mN m^{-1}. Exposure of the film to UV light for times up to 180 s revealed no absorption peak due to polymerization in the wavelength range of 400–700 nm (data not shown), which is the same result as was observed for the monolayer on water. The temperature dependence of the polymerization was, however, different between the LB monolayer on a solid substrate and the monolayer on water. Fig. 7 shows the UV irradiation time dependence of the absorbance at 640 nm at 5 and 20 °C. Even at 20 °C, interestingly, the polymerization proceeds smoothly, following an almost identical profile to that at 5 °C. This result clearly demonstrates that the polyion-complexed diacetylene molecules in the deposited LB film have much reduced thermal molecular motion compared to those in the monolayer on

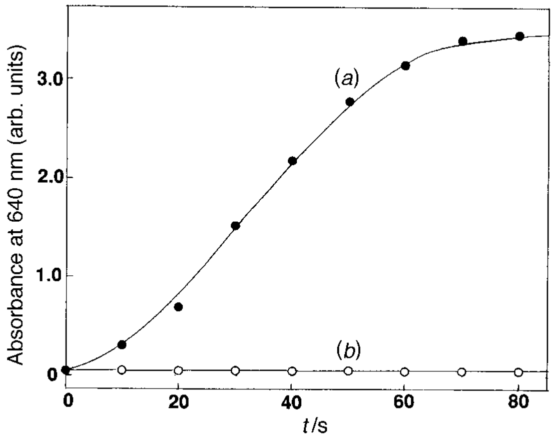

Fig. 5 UV irradiation time dependence of the absorbance at 640 nm for the **2**/**1** complex monolayer at 5 °C. The surface pressure was kept constant at (*a*) 35 or (*b*) 25 mN m^{-1}.

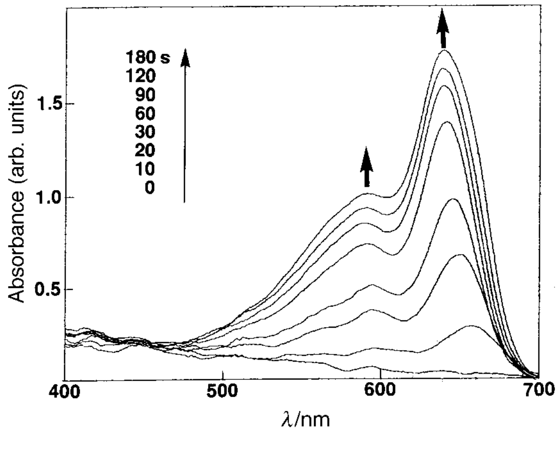

Fig. 6 Absorption spectra of the **2**/**1** complex LB film deposited on a quartz plate at 35 mN m^{-1} as a function of UV irradiation time at 5 °C

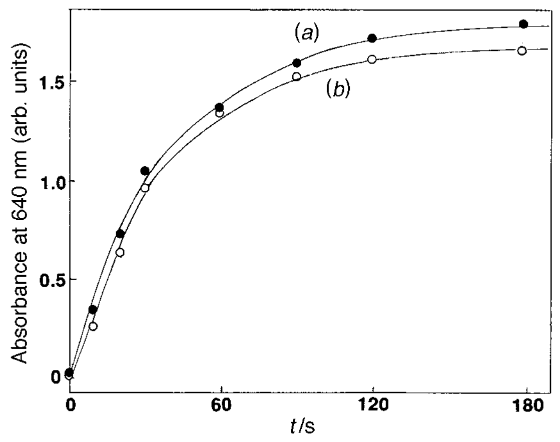

Fig. 7 UV irradiation time dependence of the absorbance at 640 nm for the **2**/**1** complex LB films at (*a*) 5 and (*b*) 20 °C

water, and are in a sufficiently crystalline state to allow effective topotactic polymerization.

Interaction of polymerized LB film with diol compounds

Fig. 8 shows the spectral change when the polymerized one-layer **2**/**1** LB film was immersed in an aqueous solution of D-fructose (10 mM) for 30 min at pH 7.1 and 20 °C. On the basis of ^{11}B NMR spectroscopy, D-fructose has been already found to form boronate esters with phenylboronic acid in water. In the spectrum before immersion, absorption peaks appear at 640 and 585 nm due to the highly conjugated blue polymer. Upon immersion, these peaks are found to decrease and the peak at 545 nm, based on the less conjugated red form, increases. When the same experiment was performed for the polymerized LB film of the **3**/**1** complex without the phenylboronic acid-containing copolymer segment, no spectral change was observed. Previous studies have also suggested that color transitions in polydiacetylenes arise from changes in the effective conjugation length of the polydiacetylene backbone[12,20] and that the electronic structure of the polymer backbone is strongly coupled to side chain conformation.[21,22] Therefore, the observed spectral change for our polydiacetylene triblock polyion complex **2**/**1** LB film must be considered as follows. First, D-fructose interacts with the boronic acid units of the copolymer segment, and then a specific binding of D-fructose causes a conformational variation of the copolymer segment that is transmitted to the polydiacetylene matrices along the polyion chain; as a result, this perturbs the effective conjugation length of the polydiacetylene.

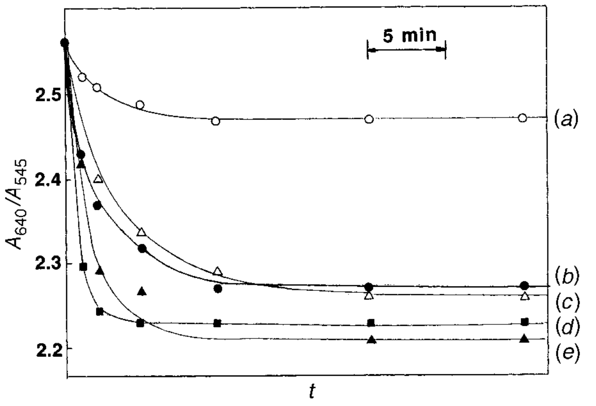

Fig. 9 The absorbance ratio of A_{640}/A_{545} of the polymerized **2**/**1** complex LB film as a function of immersion time into various nucleoside solutions ([nucleoside] = 10 mM, pH 7.1): (*a*) thymidine; (*b*) adenosine; (*c*) guanosine; (*d*) cytidine; and (*e*) uridine.

Subsequently, the interaction of the polymerized **2**/**1** LB film with nucleosides of adenosine, uridine, cytidine and guanosine was examined, since boronic acid is known to interact specifically with nucleosides having *cis*-diol moieties. Fig. 9 summarizes the immersion time dependence of the absorbance ratio at 640 and 545 nm (A_{640}/A_{545}). The data for thymidine, which has two OH groups in a *trans*-conformation on the ribose residue, were included for comparison. The value of A_{640}/A_{545} is found to show only a slight decrease upon immersion into the thymidine solution. On the other hand, the ratios for nucleosides containing *cis*-diol moieties decrease drastically, reflecting specific interaction of the boronic acid units of the copolymer segment with these nucleosides. The changes in A_{640}/A_{545} reach a plateau after 20 min. There is a small but significant difference in the total change of the ratio of A_{640}/A_{545} among nucleosides, *i.e.* the total ratio change for nucleosides having a pyrimidine ring seems to be slightly larger than that for nucleosides having a purine ring. We do not have any conclusive evidence so far to explain this phenomenon.

Finally, the reversibility of such an interaction between the boronic acid units and nucleosides was evaluated *via* treatment with an acidic solution (pH 3.0), since at such a low pH the boronic acid adopts the sp^2 hydrized free form, and the boronate ester with the nucleoside should be hydrolysed. Fig. 10 displays the ratio change upon immersing the LB film with adenosine, a typical nucleoside, into water at pH 3.0. The ratio is clearly found to return immediately to its original value, implying that adenosine desorbs from the copolymer

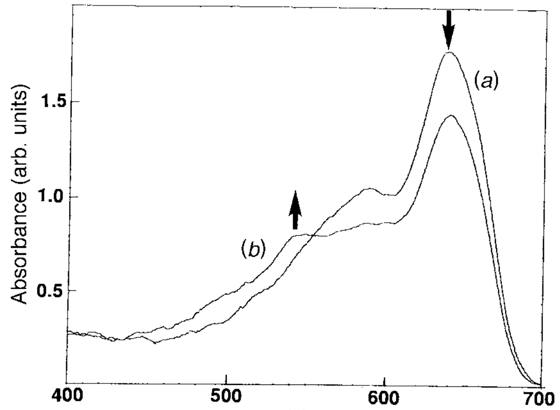

Fig. 8 Absorption spectra of the polymerized **2**/**1** complex LB film (*a*) before and (*b*) after immersion into the D-fructose solution ([D-fructose] = 10 mM, pH 7.1) for 30 min

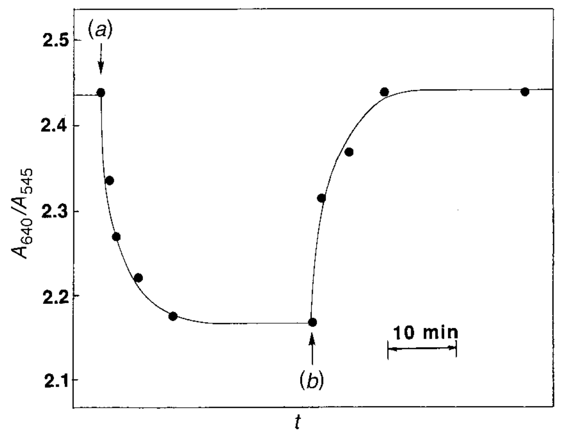

Fig. 10 Reversible change of the ratio of A_{640}/A_{545} of the polymerized **2**/**1** complex LB film by immersion into (*a*) adenosine solution at 7.1 and (*b*) water at pH 3.0

segment, which recovers the original conformation and effective conjugation length of the polydiacetylene backbone.

Conclusions

We have demonstrated that polymerized LB films complexed with a phenylboronic acid-functionalized triblock polycation are biomolecular materials that contain both a molecular recognition function (phenylboronic acid group-incorporated copolymer segment) and a detection moiety (polydiacetylene segment). The binding of diol compounds is transmitted to the polydiacetylene matrices along polyion chains and transduced to a visible color change detected by absorption spectroscopy. It is noteworthy that such a color change can take place *reversibly* in response to the binding of diol compounds. These findings are due to the combined properties of the functional segment-carrying polyion and the perturbation-sensitive electronic structure of polydiacetylene.

Experimental

Materials

DAM, 10,12-pentacosadiynoic acid **1**, D-fructose and nucleosides were purchased from Wako Chemical Co. (Japan). MPB was prepared by reaction of *m*-aminophenylboronic acid and methacryloyl chloride.

The triblock polymer **2** was prepared according to the manner reported previously.[1] The photopolymerization of MPB and DAM was carried out in the presence of bis(isopropylxanthogen) disulfide (BX) upon UV irradiation in acetone at 30 °C for 9 h. The precipitate was washed with acetone several times and dried *in vacuo* to give a white powder. The copolymer composition (*x*) and segment length (*n*) was determined by ^{1}H NMR spectroscopy. By using the copolymer thus obtained as a macrophotoinitiator, DAM was polymerized upon UV irradiation in MeOH at 30 °C for 8 h. The resultant triblock copolymer was purified by repeated reprecipitation from acetone (solvent) and hexane (nonsolvent) and dried *in vacuo*. The segment length (*m*) was determined by ^{1}H NMR spectroscopy. The homopolymer of DAM **3** was prepared by polymerization initiated with AIBN in acetone at 60 °C.

Polyion complexes were prepared as follows. Equivalent amounts of a methanolic solution of **1** (10,12-pentacosadiynoic acid) and of **2** or **3** were mixed at 40 °C and then cooled to room temperature. The mixtures were poured into benzene and the precipitates produced were washed with benzene several times and dried *in vacuo* to give a white powder. The stoichiometric composition of these complexes was evaluated by ^{1}H NMR spectroscopy.

Instruments and measurements

A low pressure Hg lamp was used as a source of UV light. UV–VIS spectra were recorded on a UV-2100 spectrophotometer (Shimadzu Co. Ltd.,). ^{11}B NMR spectra were recorded at 30 °C using a JEOL JNM GX-400 spectrometer. External $B(OCH_3)_3$ was used as a reference.

The monolayers were obtained by spreading a benzene solution (about 1 mg cm^{-3}) of diacetylenic compound on purified water (Milli-Q system, Millipore Ltd.). Twenty minutes after spreading, the monolayer was compressed continuously with a rate of 1.20 cm^2 s^{-1}. Wilhelmy's plate method and a Teflon-coated trough with a microprocessor-controlled film balance [FSD-50 (USI system Ltd., Japan) with a precision of 0.01 mN m^{-1}] were used for surface pressure measurements. The pH in the subphase was adjusted with aqueous HCl and KOH.

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