

Organization of Nucleosides Supported by Boronic-Acid-Appended Poly(L-lysine): Creation of a Novel RNA Mimic

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(Received September 28, 2000)

Boronic-acid-appended poly(L-lysine) (**1**) can form anionic boronate esters with diol derivatives in aqueous solution. One can thus expect that when nucleosides are mixed with **1**, the resultant complexes will satisfy the basic structural characteristics of RNA, viz., one-dimensionally organized nucleic bases and anionic charges. To obtain insights into this novel RNA mimic, the solution properties of adenosine (Ad) were estimated in the absence and the presence of **1**. The large absorption and CD spectral changes were observed at pH 6–10 where the poly(L-lysine) main chain tends to adopt an α-helix-rich conformation. On the basis of the fact that the magnitude of the spectral changes is much larger than the fraction of complexed Ad molecules, the polymer-supported organization mode was proposed: that is, Ad molecules complexed by covalent bonds with **1** act as clusters to induce further organization of uncomplexed Ad molecules by noncovalent interactions. The proposal was further confirmed by ^{1}H NMR spectroscopy and dynamic light scattering. On the other hand, none of these spectral changes were observed for 2′-deoxyadenosine. The **1**–Ad complex thus formed can interact with complementary poly(U) but not with noncomplementary poly(C), indicating that it can act as a novel RNA mimic.

The conformational changes in polypeptides are based on a subtle balance among several secondary forces, such as hydrogen-bonding interactions, electrostatic attraction and repulsion, hydrophobic forces, dipole-dipole interactions, etc.[1] It is expected, therefore, that the conformational transitions can be changed by a subtle change in the balance. One of the typical examples is the photocontrol of polypeptide higher-order structures by *cis–trans* photoisomerization of the azobenzene moiety appended in the side-chain.[2–4] It is known that a saccharide family frequently plays crucial roles in determining the higher-order structures of cell membranes and globular proteins.[5] It thus occurred to us that, if these higher-order structures can be controlled by saccharides, it would lead to a novel methodology to control their biological functions.[6] Recently, we and others have demonstrated that boronic acids act as a useful "*sugar-interface*" operative in water to recognize saccharides or to harness saccharides as a trigger function.[7–16] We thus expected that, if poly(L-lysine) is appropriately modified with a boronic acid group, the higher-order transitions of the resultant polypeptide chain would be controlled by the addition of saccharides.[17] With these objects in mind, we previously synthesized boronic-acid-appended poly(L-lysine) (**1**).[17] It was found that, when monosaccharides are added to the **1** solution, the helix content (monitored by CD spectroscopy) increases and the pH which gives the maximum helix content shifts to lower pH region.[17] In addition, these conformational changes can be readily detected by a fluorescence change in a probe introduced into the polypeptide chain.[18]

Here, it occurred to us that nucleosides might be organized in a one-dimensional manner along the **1** chain, because the boronic acid group can form a boronate complex with 2′,3′-diols in the D-ribose moiety.[19] In this system, the **1**–nucleoside complexes provide not only a one-dimensional nucleoside array but also anionic charges on the boron atoms, satisfying the basic structural characteristics of RNA. Furthermore, poly(L-lysine) is known to interact with RNA and DNA (mainly owing to the electrostatic interaction).[20] This suggests that nucleosides one-dimensionally organized along **1** may act as a novel RNA mimic, interacting with RNA and DNA (probably owing to the complementary hydrogen-bonding interaction) (Fig. 1). In fact, we have found that the aggregation of adenosine (Ad) is markedly facilitated by the presence of **1** and that the resultant **1**–Ad complex can interact with complementary poly(U) (Chart 1).

Results and Discussion

Aggregation of Ad. To dissolve both **1** and Ad homogeneously we employed a water:DMSO = 1:1 (v/v) mixture as a standard solvent. The "pH" values are shown with those of the buffered aqueous solution before mixing with DMSO.

In this mixed solvent Ad has an absorption maximum at 262 nm. In Fig. 2, A_{262} values are plotted against Ad concentrations. Figure 2 shows that the A_{262} values gradually deviate from the linearity, indicating that the aggregate is formed above [Ad] = 20 mmol dm^{-3}. We tried to precisely estimate the critical aggregate concentration (CAC) by a fluorescence method using pyrene[21,22] and a surface tension method[22] but failed: in these methods no clear break point assignable to the CAC appeared. Presumably, the aggregation process of Ad which does not have any long aliphatic chain does not show the discontinuous change in its aggregation properties. Judging from Fig. 2, therefore, one can only say that the aggregate

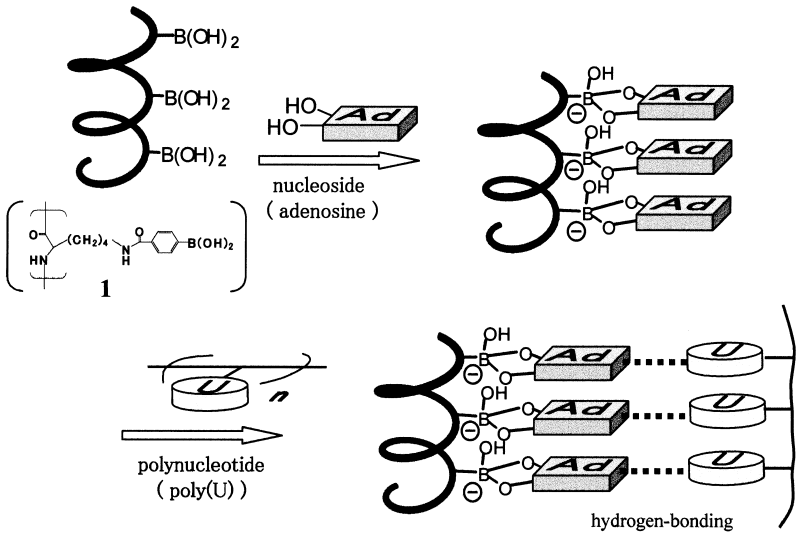


Fig. 1. Expected binding mode of RNA (or DNA) to complementary nucleosides one-dimensionally organized by **1**.

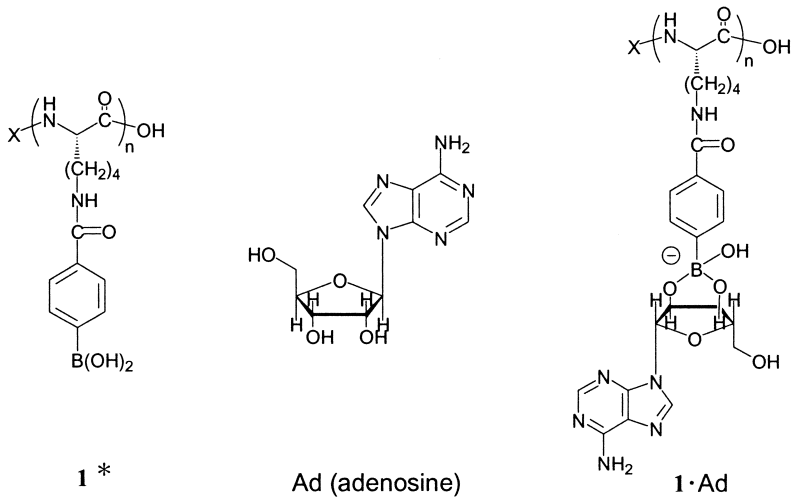


Chart 1. *It is presumed that the terminal amino group also reacts with a phenylboronic acid. However, this cannnot be differentiated from unreacted amino group (less than 1%). Hence, X in **1** is either –H or –CO–C_6H_4–$B(OH)_2$.

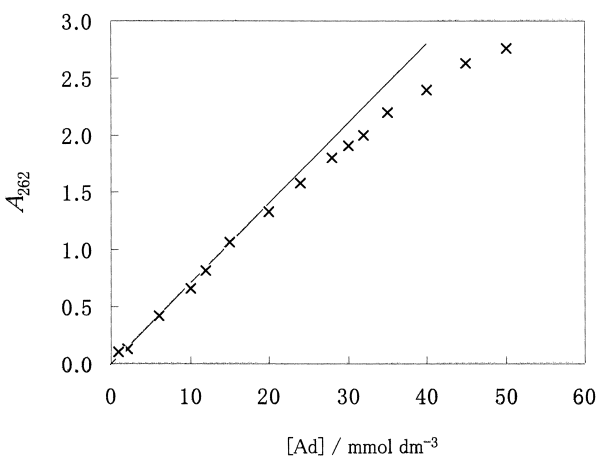


Fig. 2. Lineweaver–Burk plot for Ad in water:DMSO = 1:1 (v/v) at 20 °C: cell width 0.05 mm.

is formed at least above 20 mmol dm^{-3}.

Figure 3 shows the concentration dependence of the CD spectral change in Ad. One can easily explain this spectral change (A) by comparison with the authentic CD spectra (B)[23]: that is, the CD spectrum at [Ad] = 10 mmol dm^{-3} with a broad CD minimum (264 nm) is similar to that of monomer Ad, whereas the CD spectra gradually change into the exception-coupling-type band with increasing Ad concentration, which is similar to dimeric adenosine derivative (**2**, Chart 2) or poly(A).[23] These CD spectral results also show that Ad in water:DMSO = 1:1 (v/v) tends to aggregate above [Ad] = 20 mmol dm^{-3}.

Influence of Added 1 on the Absorption and CD Spectra of Ad. The absorption spectra of Ad were measured at pH 5–11 in the absence and the presence of **1**. As shown in Fig. 4, the absorption spectrum of Ad is basically independent of medium pH. At [Ad] = 10 mmol dm^{-3} where little aggregate is formed, no significant spectral change is not induced by the

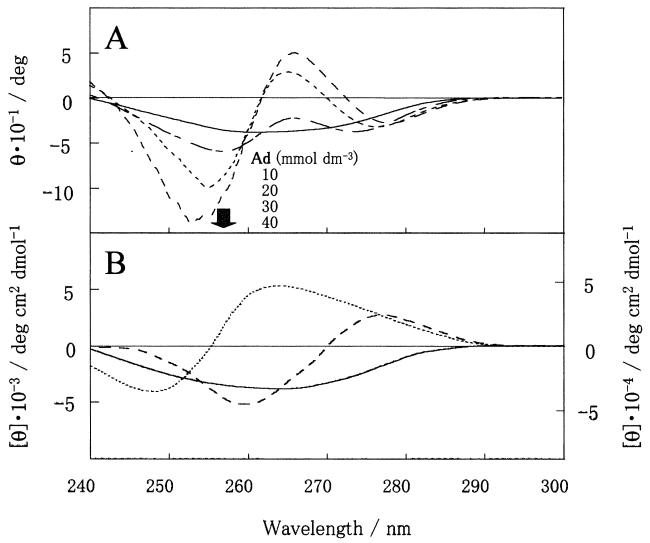
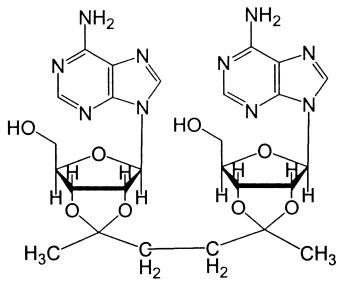


Fig. 3. (A) CD spectra of Ad (10–40 mmol dm^{-3}) in water:DMSO = 1:1 (v/v) with cell width 0.1 mm at 20 °C and (B) authentic CD spectra of Ad (———), **2** (---------) (all left scale), and poly(A) (·····) (right scale) in water.[23]



2 (2″S; 5″S)

Chart 2.

addition of **1**. At [Ad] = 50 mmol dm^{-3} where the aggregate is formed, in contrast, the absorbance significantly decreases at pH 6–10. The CD spectra also show the similar pH dependence (Fig. 5). The CD spectrum of Ad is scarcely affected by medium pH. At [Ad] = 10 mmol dm^{-3} the CD spectral change (λ_{min} 264 nm) is not induced by the addition of **1**, whereas at [Ad] = 50 mmol dm^{-3} the CD intensity minimum at 252 nm ($[\theta]_{252}$) further decreases at pH 6–10. These results consistently support the view that only when Ad is already aggregated, added **1** can change the aggregation mode. As such a CD spectral change is not induced for 2′-deoxyadenosine (50 mmol dm^{-3}: see Fig. 5) which does not have 2′-OH indispensable to complexation with a boronic acid group, it is undoubted that these spectral changes are due to 2′,3′-diol-boronic acid complexation.

Judging from the foregoing spectral change, one may regard the aggregated Ad molecules as being enforced to organize into a one-dimensional path along the **1** polymer chain. The monomer unit concentration of **1** is 0.17 mmol dm^{-3} and the concentration of Ad is 50 mmol dm^{-3}. This means that, even though all boronic acid groups are used for the complex formation, the spectral change can occur only by 0.34% (i.e., 0.17/50). Since the spectral changes are much greater than 0.34%, one must consider that the Ad molecules organized by com-

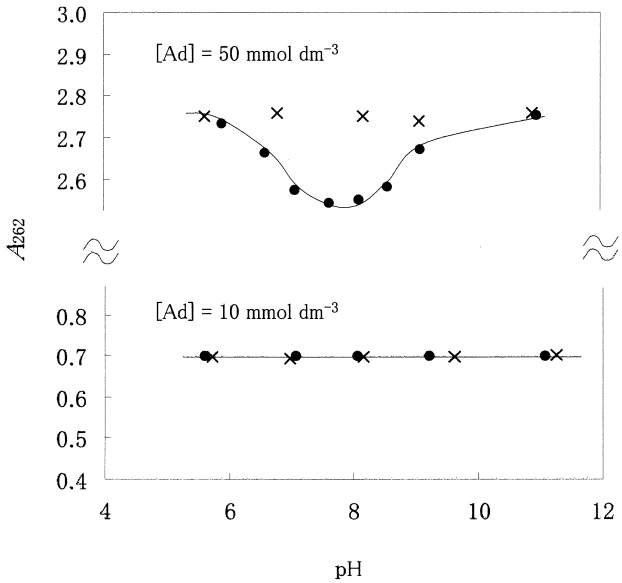


Fig. 4. pH Dependence of A_{262} in the absence (×) and the presence (●) of **1** (0.17 monomer unit mmol dm^{-3}); lower plots, [Ad] = 10 mmol dm^{-3}; upper plots, [Ad] = 50 mmol dm^{-3} ; water:DMSO = 1:1 (v/v) with cell width 0.05 mm at 20 °C; pH was adjusted with aqueous NaOH and HCl solutions in the presence of KCl (1.0 mmol dm^{-3}).

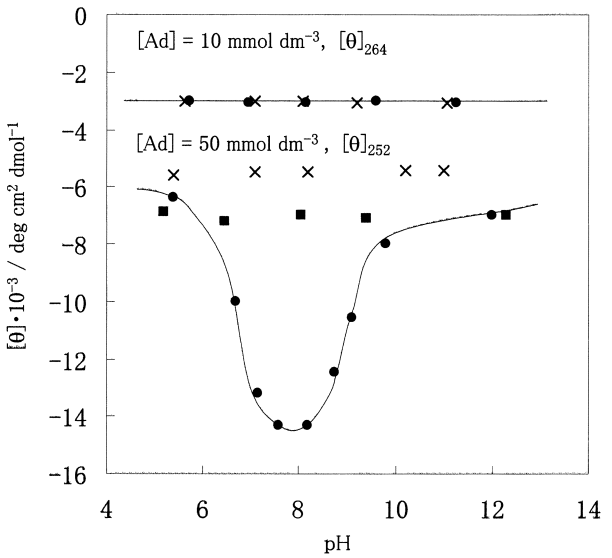


Fig. 5. pH Dependence of $[\theta]_{264}$ (at [Ad] = 10 mmol dm^{-3}) or $[\theta]_{252}$ (at [Ad] = 50 mmol dm^{-3}) in the absence (×) and the presence (●) of **1** (0.17 monomer unit mmol dm^{-3}); lower polts, [Ad] = 50 mmol dm^{-3}; upper plots, [Ad] = 10 mmol dm^{-3}; water:DMSO = 1:1 (v/v) with cell width 0.1 mm at 20 °C; pH was adjusted with aqueous NaOH and HCl solutions in the presence of KCl (1.0 mmol dm^{-3}). ■ denotes the plot for [2′-deoxyAd] = 50 mmol dm^{-3}.

plexation act as clusters to further organize uncomplexed but self-aggregated Ad molecules. In other words, the cluster formation is effected by the covalently-bonded complexation with the polymer and the additional organization is effected by the noncovalent intermolecular interaction.

Then, what kind of a conformational change is induced in the main chain of **1** upon Ad complexation? Usually, the conformational change in polypeptides is estimated by the CD band at around 200 nm. In the present system, this wavelength region is totally masked by the absorption bands of Ad. We thus used "transparent" 1,4-anhydroerythritol (**3**) as a model compound of Ad. The pH-dependent CD spectra showed that, as already observed for D-fructose or D-glucose,[17,18] the conformation changes in the order of β-sheet $\rightarrow$ α-helix $\rightarrow$ random coil with increasing medium pH (Fig. 6).[24] The $[\theta]_{208}$, which is useful as a measure of the α-helix content, is plotted against medium pH in Fig. 7. It is seen from Figs. 6 and 7 that, at neutral to slightly basic media where the significant absorbance and CD spectral changes are induced by Ad, the content of α-helix is increased: at the optimum pH 9.0 ($[\theta]_{208} = -1.89 \times 10^4$ deg cm^2 dmol^{-1}) the α-helix content reaches 52%.[24] Although the influence on the main chain conformation should be somewhat different between Ad and **3** (Chart 3), we consider that basically the α-helix conformation is utilized to organize Ad molecules along the poly(L-lysine) chain.

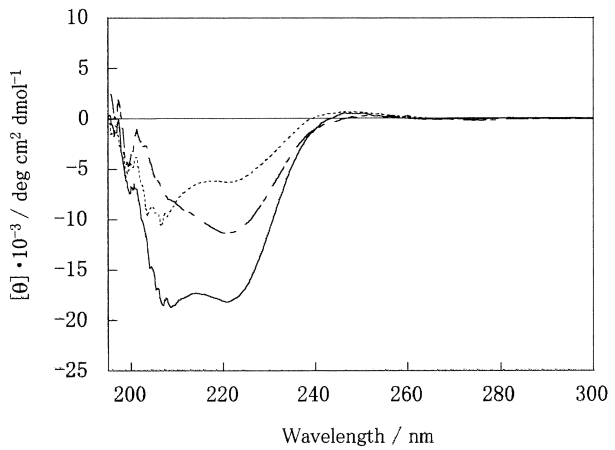


Fig. 6. CD spectra of **1** at selected medium pH's: [**1**] = 0.17 monomer unit mmol dm^{-3}, [**3**] = 50 mmol dm^{-3}, water with cell width 1.0 cm: pH 7.41 – – – –, 9.03 ——, 10.49 -------.

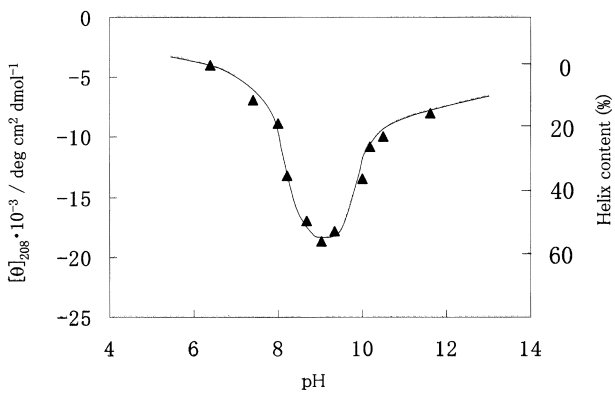


Fig. 7. Plot of $[\theta]_{208}$ vs medium pH. The measurement conditions are shown in a caption to Fig. 6.

As a summary of the forgoing findings, one can now propose an organization mode shown in Fig. 8. The main chain of **1** changes its conformation in the order of β-sheet $\rightarrow$ α-helix $\rightarrow$ random coil; meanwhile, the 2′,3′-diol-boronic acid complexation, which can occur with the aid of OH^- adduct formation, becomes possible in pH region above 6. Thus, only in the neutral to slightly basic pH region where the main chain adopts the α-helix conformation, Ad molecules are complexed to the pendent boronic acid groups and induce the further binding of uncomplexed Ad molecules to organize them, presumably, in a one-dimensional way. In case Ad molecules are not aggregated by themselves, complexed Ad molecules are not sufficient to induce further organization of uncomplexed Ad molecules.

Further Evidence to Support the Polymer-Supported Organization Mode. Several lines of evidence collected so far are all related to the absorption and CD spectroscopic data. To strengthen this polymer-supported organization mode in Fig. 8, we collected the ^{1}H NMR and the dynamic light scattering (DLS) data.

Figure 9 shows the ^{1}H NMR spectra of Ad in the absence and the presence of **1** measured in D_2O:DMSO-d_6 = 1:1 (v/v). The 8-H and 2-H protons in the adenine moiety and the 1′-H in the D-ribose moiety (δ 8.48, 8.33, and 6.03, respectively) shift to higher magnetic field in the presence of **1** (δ 8.38, 8.23, and 6.00, respectively). The upper-field shift supports the view that the π–π-stacking interaction among Ad molecules is facilitated by **1**. As mentioned above, the concentration of the boronic acid group is only 0.34% of the Ad concentration. Hence, these measurable changes in the chemical shifts cannot be explained by 1:1-type complexation between Ad and the

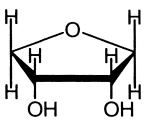


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Chart 3.

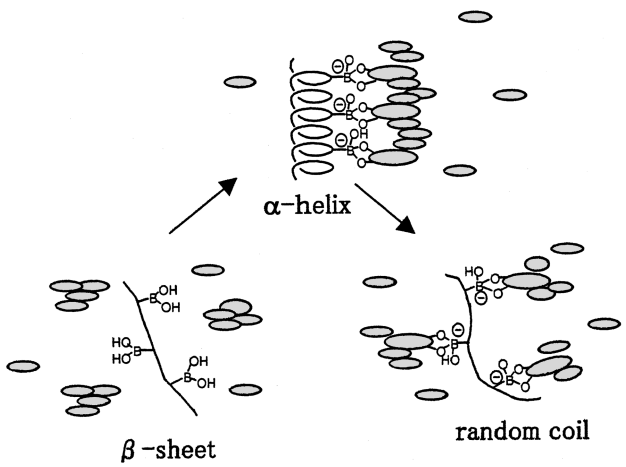

Fig. 8. Schematic representation of the Ad binding to **1**.

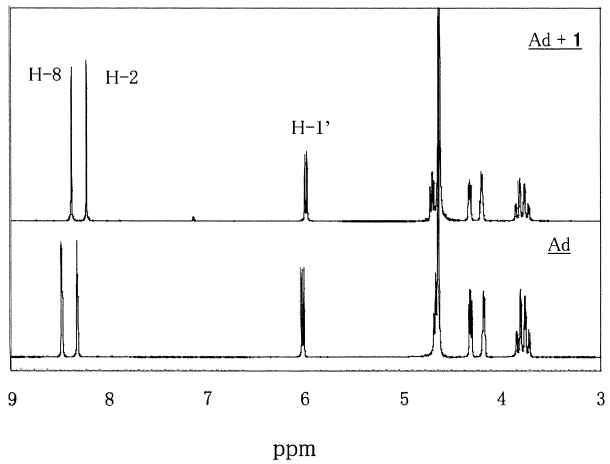

Fig. 9. ^{1}H NMR spectra of (A) Ad (50 mmol dm^{-3}) and (B) Ad (50 mmol dm^{-3}) plus **1** (0.17 mononer unit mmol dm^{-3}) in D_2O:DMSO-d_6 = 1:1 (v/v), pD 8.4 (corrected), 20 °C.

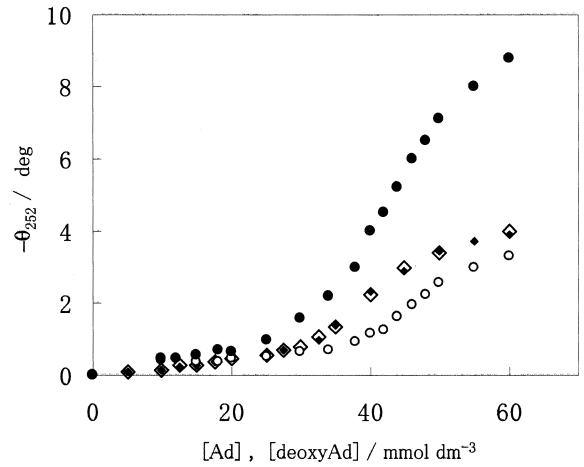

Fig. 10. Ad concentration dependence of the CD spectral intensity (252 nm): [**1**] = 0.17 monomer unit mmol dm^{-3}, pH 8.0, water:DMSO = 1:1 (v/v), [KCl] = 1.0 mmol dm^{-3}, cell width 0.1 mm, 20 °C: ○ Ad only, ● Ad + **1**; ◇ 2′-deoxyadenosine only, ◆ 2′-deoxyadenosine + **1**.

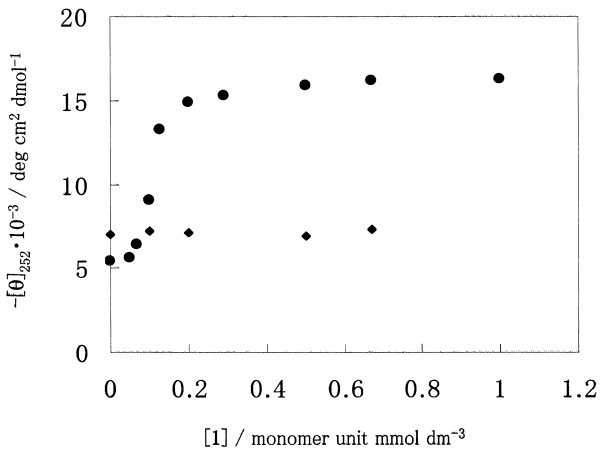

Fig. 11. **1** concentration dependence of the CD spectral intensity (252 nm): [Ad] = [2′-deoxyadenosine] = 50 mmol dm^{-3} (constant). Other measurement conditions are recorded in a caption to Fig. 10.

pendent boronic acid group. It is obvious, therefore, that the complexed Ad molecules act as a cluster to induce the further organization of uncomplexed Ad molecules.

Under the standard measurement conditions (water:DMSO = 1:1 (v/v), pH 8.0, [Ad] = 50 mmol dm^{-3}), the particle of Ad could not be detected by DLS. Since the particle with 2.5 nm diameter is sufficiently detectable, one may consider that the size of the Ad aggregate is relatively small (judging from the molecular size, less than octamer). From the solution of **1** (0.17 monomer unit mmol dm^{-3}), we obtained D (diffusion coefficient) = 1.3×10^{-8} cm^2 s^{-1} and r (diameter) = 507 nm. When **1** (0.17 monomer unit mmol dm^{-3}) was mixed with Ad (50 mmol dm^{-3}), these parameters were changed to $D = 6.5 \times 10^{-9}$ cm^2 s^{-1} and r = 1000 nm. The results clearly indicate that Ad molecules are enforced to aggregate through the interaction with **1**.

Influence of the Concentration Changes on the CD Spectra. So far, the concentrations of **1** and Ad have been fixed to 0.17 monomer unit mmol dm^{-3} and 50 mmol dm^{-3}, respectively, in order to compare the different spectral data under the identical measurement conditions. One may obtain more concrete insights into the organization mode from the concentration dependences.

Figure 10 shows the influence of Ad and 2′-deoxyadenosine concentrations on the CD spectral intensity. The CD intensity increases (negatively) with increasing Ad and 2′-deoxyadenosine concentrations according to a sigmoidal curvature. This suggests that some CAC (although not so sharp as in conventional surfactants) exist at 10–20 mmol dm^{-3}. Important herein is the finding that the distinct (negative) increase in the CD intensity is observed for the Ad + **1** system, but not at all for the 2′-deoxyadenosine + **1** system. This confirms again that the 2′,3′-diol-boronic acid complexation plays an essential role in the polymer-supported organization. It is also seen from Fig. 10 that the clear CD intensity difference between Ad only and Ad + **1** occurs above the 20 mmol dm^{-3}, indicating that the self-aggregate formation of Ad is a prerequisite.

Figure 11 shows the influence of **1** on the CD spectral intensity. Again, the CD spectrum of 2′-deoxyadenosine is scarcely changed by addition of **1**. In contrast, the CD intensity for Ad sharply increases at [**1**] = 0.07 monomer unit mmol dm^{-3} and is saturated above 0.3 monomer unit mmol dm^{-3}. This sharp cooperativity indicates that both transitions (a β-sheet-to-α-helix transition in the polymer main chain and an aggregate-to-one-dimensional organization transition in Ad molecules) have an auto-accelerative characteristic.

Interaction of the Ad + 1 System with Complementary and Noncomplementary Polynucleotides. The foregoing findings suggest an intriguing idea that Ad molecules one-dimensionally organized along a poly(L-lysine) polymer chain may behave like poly(A) and interact with complementary poly(U) but not with noncomplementary poly(C). To evaluate the reality of this idea, we measured the CD spectra of Ad in the presence of **1** and/or polynucleotides.

As already confirmed, the CD spectrum of Ad is unaffected at [Ad] = 10 mmol dm^{-3} (or less than 10 mmol dm^{-3}) by add-

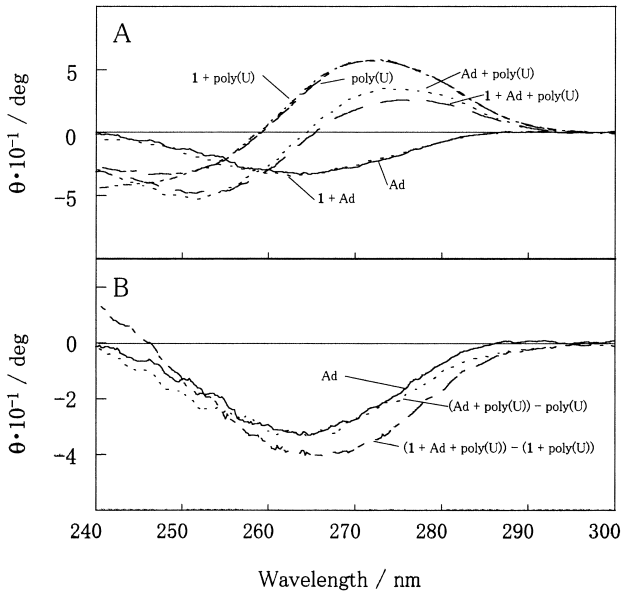

Fig. 12. (A) CD spectra of Ad and additives (**1** and/or poly(U)): [Ad] = 5.0 mmol dm^{-3}, [poly(U)] = 2.5 monomer unit mmol dm^{-3}, [**1**] = 2.5 monomer unit mmol dm^{-3}: (B) differential CD spectra of Ad and additives: pH 9.0, cell width 0.1 mm, 20 °C.

ed **1**. We confirmed here (Fig. 12A) that the CD spectrum of poly(U) is not affected either by added **1**, indicating that there exists no significant interaction between poly(U) and **1**. A mixture of Ad and poly(U) gives the CD spectra which are exactly the same as the summed CD spectra of Ad and poly(U), illustrated using a computational method (Fig. 12A). This means that, although poly(U) has a complementary hydrogen-bonding site for Ad, it is not strong enough to organize monomeric Ad along the poly(U) polymer chain. In contrast, when **1** was added to the Ad + poly(U) mixture, the CD intensity in 260–280 nm region decreased (Fig. 12A).# The presence of the difference is more clearly shown by the differential CD spectra (Fig. 12B): the (negative) increase in the CD intensity at 260–280 nm implies that the stacking interaction among Ad molecules is further intensified by the coexistence of **1** and poly(U).[23]

These CD spectral results support the view that although the Ad–poly(U) interaction is not so strong as to enforce the one-dimensional organization of Ad molecules along the poly(U) polymer chain, those one-dimensionally "preorganized" by complexation with **1** do interact with complementary poly(U). On the other hand, such a CD spectral change was not observed for noncomplementary poly(C). As shown in Fig. 13, the CD spectrum of Ad + poly(C) is almost same as that of **1** + Ad + poly(C). The results mean that even though Ad molecules are one-dimensionally preorganized by **1**, the interaction with noncomplementaly poly(C) does not take place.

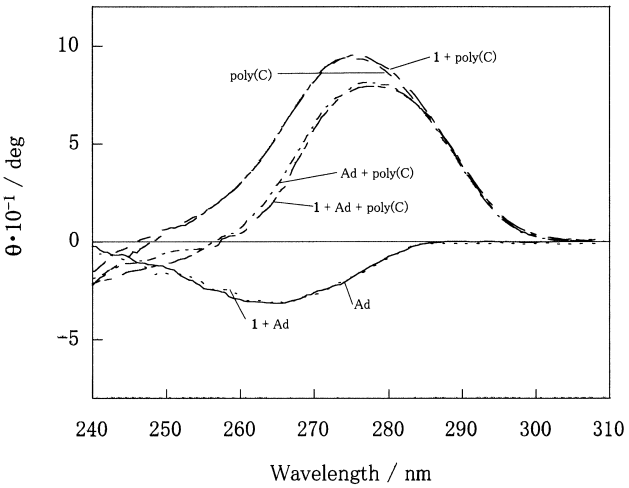

Fig. 13. CD spectra of Ad and additives (**1** and/or poly(C)): the measurement conditions are similar to those recorded in a caption to Fig. 12.

As a summary of the foregoing findings, one can reasonably justify the **1**-supported selective complexation of Ad with poly(U), as illustrated in Fig. 1, which acted as the motivation of the present study.##

Conclusion

The present study established that utilizing the boronic acid–diol interaction, one can organize nucleosides in an one-dimensional way along poly(L-lysine). The **1**–Ad complex thus formed can behave as a novel RNA mimic. It was also shown, however, that the polymer-supported organization is also affected by self-aggregation of Ad molecules and the conformation of the polymer main chain. It is undoubted that the largest characteristic of the present system is that the RNA mimic can be constructed "reversibly" by the boronic acid–diol interaction. This will enable further applications of the present system, for example, to RNA transport, temporary suppression of transcription processes, etc.

Experimental

Materials. Hydrobromide salt of poly(L-lysine) (mf 50000) was purchased from Wako Pure Chemical Industries, Ltd. The preparations of **1** were reported previously.[17] The percentage of the unreacted lysine residue of **1** was determined by the reaction with sodium picrylsulfonate. From the absorbance of the picrylamine chromophore (λ_{max} 350 nm, ε_{max} 15000 dm^3 mol^{-1} cm^{-1}), it was found that less than 1% was unreacted.

Miscellaneous. ^{1}H NMR spectra were measured in CD_3OD with a Bruker ARX300 apparatus. IR spectra were recorded on a Shimadzu FT-IR 8100M. CD spectra were measured on a Jasco J-720WI spectropolarimeter. Each CD spectrum was measured three times and the averaged spectra are shown in Figs. (measure-

The CD intensity in this wavelength region is affected by two factors: that is, 1) Ad concentration which decreases the CD intensity in the low concentration region and increases it in the high concentration region (Fig. 3) and 2) conformational change in poly(U) which decreases the CD intensity when the characteristic hair-pin conformation is disordered. The Ad concentration used herein is low but can disorder the poly(U) conformation. Thus, it is reasonable to decrease the CD intensity.

When two polymeric chains form a duplex, it is indispensable for these two chains to have the same (or similar) helical pitch. In the present system, unbound Ad molecules are inserted into the clefts formed by **1**-bound Ad residues. Hence, the coincidence of the helical pitch is not an important factor.

ment conditions: scan speed 20 nm min^{-1}, response 2 s, band width 2.0 nm, sensitivity 10 mdeg). The CD spectral changes observed for poly(U) were relatively small: we show the averaged raw CD spectra in (A) and the difference CD spectra in (B) in Fig. 12. By comparison with the noncomplementary Ad + poly(C) system in Fig. 13, it is clear that there is a significant CD change, larger than the error range, in the complementary Ad + poly(U) system. The absorption spectra and fluorescence spectra were measured by Jasco V-570 and Hitachi F-4500 spectrophotometers, respectively. The surface tension was measured by Kyowa Scientific. Co. ESB-IV. Diameter and diffusion coefficient were measured by Otsuka Electronics Co. DLS-7000. The medium pH was adjusted by adding aqueous HCl solution or NaOH solution.

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