

Exciton interactions in cyanine dye–hyaluronic acid (HA) complex: reversible and biphasic molecular switching of chromophores induced by random coil-to-double-helix phase transition of HA†

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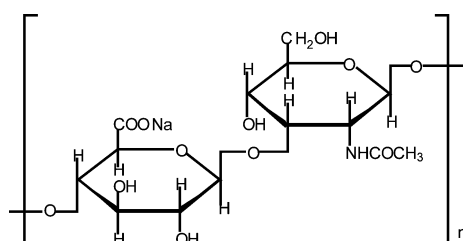
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Reversible and biphasic switching of monomer-to-*R*-chiral dimer transition and *R*-chiral-to-*S*-chiral dimer transition of cyanine chromophores were demonstrated by complex formation with hyaluronic acid.

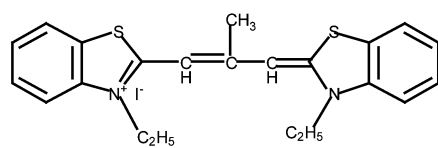
Regulated and reversible configurational changes and molecular motions in chromophoric supramolecular systems can form the basis of switching devices when they can be modulated by thermal and/or chemical means and translated into optical properties. In particular, the combination of a dye and polymer/self-assembling system will be useful in molecular device applications assuming the secondary structures can be easily controlled and the dye will respond to the changes in the secondary structure with a unique optical output to track, visualise, and quantify the different molecular interactions as a sensing probe.^{1–7} For example, poly(L-lysine) with anionic dyes shows dramatic spectral changes responding to random-to-helical conformational change in the peptide main chain.^{4,5} Similar specific spectral changes have been also realised in chiral lipid bilayer membrane systems.^{6,7} However, these systems can provide various mesophases because of their lability based on the fact that the residual groups on the peptide main chain are flexible and lipid aggregation allows the lateral diffusion of lipids. In this context, hyaluronic acids (HAs), a copolymer of D-glucuronic acid in a β-1,3 linkage strictly alternating with *N*-acetyl-D-glucosamine in a β-1,4 linkage (Scheme 1), promise to provide a specific microenvironment because of their rigid primary structure and versatile secondary structure.^{8–10} In this work, we present the complexation of cyanine dye NK-77 and HA as a simple functional

model for molecular switching devices, since cyanine dyes are by far the most frequently used chromophores in spectroscopic studies because of their high sensitivity and UV-vis response^{4–7,11} and induced circular dichroism (CD) characterization.^{4–7}

Addition of HA (degree of polymerisation: 110) to NK-77 and lowering the temperature led to gradual spectral changes in its absorption band as shown in Fig. 1(a). On lowering the temperature from 50 °C to 0 °C in aqueous-organic solvent, a decrease in the intensity of the band at 539 nm was observed. Interestingly, the spectral changes were biphasic at with the following two temperatures; there is one isosbestic point at 504 nm from 50 °C to 25 °C, and another at 467 nm, accompanied by a remarkable increase in the absorbance at 445 nm, on lowering the temperature from 25 °C to 0 °C. Cyanines derived from benzothiazolium units are known to form face-to-face dimers (H-aggregation mode), characterized by the appearance of an absorption band at around 420–455 nm, which is blue-shifted with respect to the original absorption band at around 540 nm.^{4–7,11} The trends observed in this case are comparable to previously reported results. Therefore, the broad band absorbing at 539 nm in Fig. 1(a) may correspond to the monomeric form of NK-77, and it disappears due to dimerization and a characteristic dimer band appears at 445 nm



Sodium hyaluronate (HA)



NK-77

Scheme 1 Hyaluronic acid (HA) and cyanine dye (NK-77).

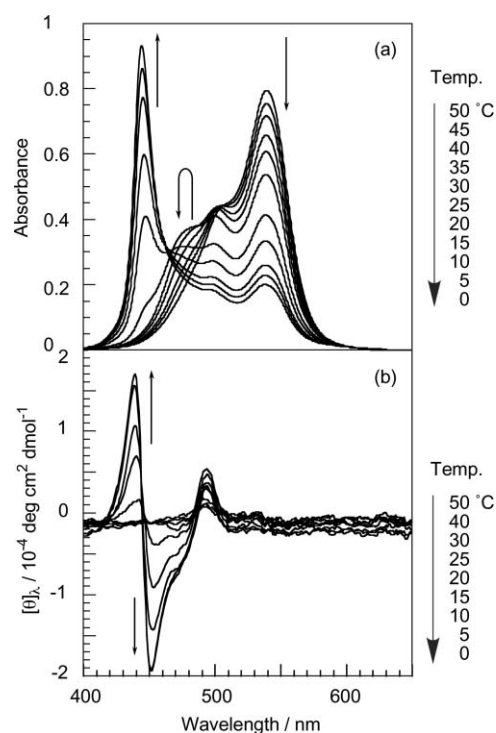


Fig. 1 Visible (a) and CD (b) spectra of HA (20 unit μM) and NK-77 (10 μM) mixture in 2.4 vol% MeOH–H₂O (pH 7.0) where unit is defined as the repeating chemical structure as shown in Scheme 1.

† Electronic supplementary information (ESI) available: spectroscopic data. See <http://www.rsc.org/suppdata/cc/b4/b408032g/>

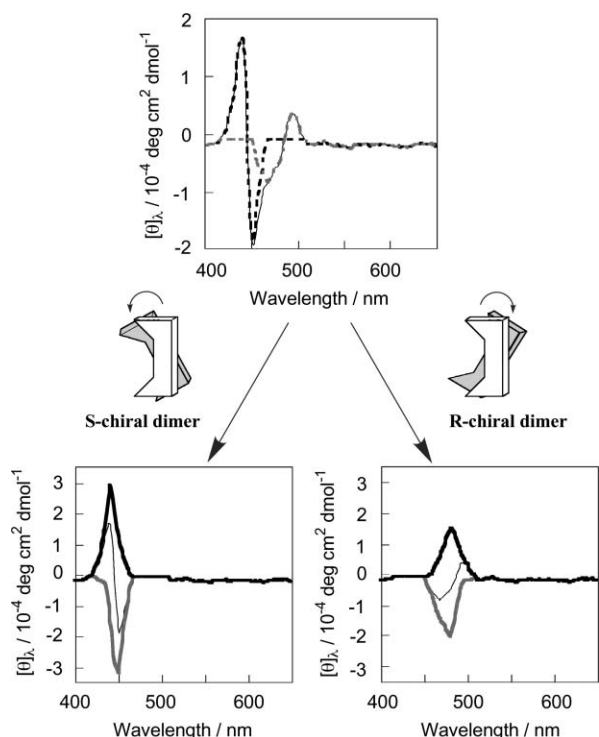


Fig. 2 Biphasic CD spectra of HA (20 unit μM) and NK-77 (10 μM) complex in 2.4 vol% MeOH–H₂O (pH 7.0).

that may be assigned to an H-aggregate, arising from the parallel stacking of the cyanine chromophores. A complete reversal of the original appearance was also observed with an increase in temperature, indicating that an equilibrium process is involved. It is also notable that a split-Cotton effect was observed at around 400–500 nm on lowering the temperature from 50 °C to 0 °C as shown in Fig. 1(b) in spite of using an achiral compound NK-77. These CD spectral changes are also able to be separated into two phases as shown in Fig. 2. The CD pattern centred at 445 nm was assigned to *S*-chiral dimer formation of NK-77 through head-to-head stacking in the temperature range from 25 °C to 0 °C, and another centred at 483 nm was assigned to *R*-chiral dimer formation from 50 °C to 25 °C. These results are consistent with the absorption spectral changes as shown in Fig. 1(a). HA is known to undergo random coil-to-double-helix phase transition at around 30 °C in aqueous–organic solvents.^{8–10} Therefore, monomer-to-*S*-chiral dimer transition of NK-77 with HA corresponds to the random coil-to-double-helix phase transition of HA. It is therefore presumable that the *R*-chiral dimer of NK-77 might be bound to the *meso*-form of the secondary structure of HA between the random coil and double helix phases. Moreover, the symmetrical split-Cotton effect of *R*- and *S*-chiral dimer formation (Fig. 2) implies that the phase transition of such a chiral dimer corresponds to the rotation along the *C*₂ axis of each NK-77 molecule.¹²

We monitored the effect of solvent composition on the absorption spectrum of the mixture of NK-77 (10 μM) and HA (20 unit μM) at 0 °C in mixed aqueous–organic solvent in the range of 2.4–18 vol% MeOH. The intensity of the 445 nm band is markedly higher at lower MeOH content.[†] Although MeOH is

essential to dissolve NK-77 since the cyanine dye is insoluble in water *per se*, the smallest amount of organic solvent is preferable in order to form the *S*-chiral dimer of the chromophores. In other words, the *R*-chiral dimer is preferentially formed in 18 vol% MeOH. Addition of varying amounts of HA (10–100 unit μM) to a solution of NK-77 (10 μM) in 2.4 vol% MeOH–H₂O at 0 °C led to a gradual red shift in its absorption band from 445 nm to 483 nm.[†] When the molar ratio of [HA]/[NK-77] was 10, its CD spectrum showed clearly an *S*-chiral pattern centred at 483 nm.[†] Therefore, configurational control of the chiral dimer was achieved by varying the polarity of the solvent and the molar ratio of NK-77 and HA.

No NK-77 dimerization was observed from the absorption and CD spectra in the combination with the monomer unit of D-glucuronic acid, and/or *N*-acetyl-D-glucosamine *per se*.[†] This result indicates that the macromolecular structure of HA is essential to form the chromophoric dimer. When 0.02 unit mM of poly(acrylic acid) was added to a solution of NK-77 (0.01 mM), chromophoric dimerization was confirmed from the appearance of sharp absorption band at 470 nm, however, no chirality was observed from its CD spectrum.[†] This result implies that the highly ordered secondary structure of HA is necessary to form the chiral dimer of NK-77. Various degrees of polymerisation and several different types of origins of HAs[‡] were examined and compared to one another;[†] however, there was no critical difference among them.

In conclusion, the complex of a cyanine dye and HA possesses the unique property of molecular switching of monomer-to-*R*-chiral dimer and *R*-chiral-to-*S*-chiral dimer transition of NK-77 and the interconversion was thermally responsive and regulated by varying the molar ratio of NK-77 and HA. Such assembly and configurational switching of chromophores could lead to the design of novel types of molecular switching devices by using a combination with the simple glycosaminoglycan of HA as a functional and macromolecular medium.

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Notes and references

[‡] Five different types of HAs were used from Chisso Corporation (degree of polymerisation: 72, 110 and 143 from *Streptococcus* genus) and Q. P. Corporation (degree of polymerisation: 53–133 for HA-L and 4–9 for HA-Q from commercial broiler chickens).

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