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^{2}H NMR study of the self-assembly of an azo dye–cyclomaltooctaose (γ-cyclodextrin) complex

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Abstract

Orange II and γ-cyclodextrin form 2:1 and 2:2 complexes. These complexes self-associate, and microscopic viewing indicates the formation of a fibroid aggregate. The deuteron resonance (in ^{2}H NMR spectroscopy) from deuteron exchange and solvation between the aggregate and deuterium oxide exhibits quadrupole splitting ($\Delta\nu$) in the region of 0–1.2 KHz. The orientation behavior obtained from this splitting suggests the formation of a liquid-crystaloid substance. (1) The aggregate aligned by means of the super conductive and permanent magnetic fields shows a different NMR pattern and turbidity. (2) Dilution of the 0.12 M aggregate with deuterium oxide proceeds discontinuously via the nonoriented pattern to the oriented-pattern. (3) With an increase in temperature, the ^{2}H NMR spin–spin splitting values decrease continuously. A phase diagram that plots melting points in variable concentrations reveals separate solution and aggregate layers. (4) When the 0.06 M aggregate is diluted by deuterated solvents (water, methanol, ethanol, propanol, dimethyl sulfoxide, and acetone), increases in the amount added and the length of solvent molecules cause a regular reduction of $\Delta\nu$. The deuterium signal of the methyl group in dimethyl sulfoxide-d_6 exhibits a small $\Delta\nu$, suggesting that this solvent is included in the orientation system.

1. Introduction

Orange II [sodium *p*-(2-hydroxy-1-naphthylazo) benzenesulfonate, (1)] is readily soluble in water. It requires a considerable time for the formation of needle- and pine needle-like crystals from its 0.16 M saturated aqueous solution. But when 0.03

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M cyclomaltooctaose (γ-cyclodextrin, γ-CD) is added to an equivalent of **1**, the solution becomes viscous within an hour. The overall shape of the resulting gel-like mass becomes vague, and a pine needle-like aggregate appears. With time it becomes long and thick in texture, and increases in gloss after standing for three months or more. It does not crystallize, but keeps an associated colloid phase for several years. Such a phenomenon may be due to the self-association of the **1**–γ-CD complex. This morphological change is not observed in analogous azo dye–CD complexes examined, and **1** complexes with β-CD, DMβ-CD, and γ-epoxy-poly-CD [1–3]. This aggregate, when dried by the incident light in the polarized microscope, shows a rodlike, aligned texture. Induced circular dichroism (CD) of this aggregate appears at ~500 nm in the solution state, but in the aggregate state, it changes to the J-band (~547 nm) due to the head-to-tail stacking of **1** and the H-band (~353 nm) due to parallel stacking [4]. When the appropriately aged aggregate is put into a sandwich cell, and the upper glass is rubbed in the transverse direction, the change of the CD spectrum with time was measured. The band in the $\pi \rightarrow \pi^*$ region caused an increase in the θ-values due to an increase in the anisotropic part, and finally showed an almost forty-fold increase over that of the CD spectrum in the solution. Such facts indicate that the sticklike molecule **1**, which has a permanent dipole moment, orients upon rubbing.

The aim of this paper is to probe the orientation behavior of the aggregate quantitatively by ^{2}H NMR spectroscopy under conditions, in which the aligned substance exhibits splitting of the deuterium signal, and compare the results with those of a lyotropic liquid crystal. Changes of the orientation behavior induced into the aggregate by magnetic field, temperature, concentration, and solvent are all determined.

2. Materials and methods

A Jeol JNM-GSX (500 MHz) operating at 77 MHz and the deuteron internal lock system (continuous-wave sweep) of a Hitachi R-250 (250 MHz) instrument operating at 38 MHz were used for measurement of the deuteron nuclei. A Hitachi R-250 (250 MHz) instrument for SCM and a Hitachi R-24 (60 MHz) for the permanent magnet were used for investigation of the magnetic orientation of the aggregate.

1 and γ-CD form 2:1 and 2:2 complexes in aqueous solution [5]. An aggregate of variable concentrations was used only after three months or more had elapsed after preparation.

3. Results and discussion

Theory.—When a diluted and transparent isotropic deuterium oxide solution of this complex is put in a magnetic field, the ^{2}H NMR signal shows a sharp singlet with ringing. It belongs to the coalescence signal of the deuterium oxide solvent and deuterated hydroxy groups which are covalently bonded to the 2, 3, and 6 positions of the γ-CD in the complex. With an increase in concentration, the complex begins to aggregate, the solution becomes turbid, the signal becomes broad, and two peaks of equal intensity with a separation $\Delta\nu$ appear. $\Delta\nu$ gives the same value as measured by both 250 MHz and 500 MHz spectrometers: thus, the observed $\Delta\nu$ is not due to chemical shift, but due to the spin–spin splitting. Since in all systems investigated a simple splitting pattern without any central peak is observed, rapid exchange is assumed between oriented and isotropic deuterium nuclei. Generally, when amphiphilic molecules contain exchangeable protons, such as NH_2, COOH, and OH, $\Delta\nu$ may arise, not only from partially oriented D_2O, but also from deuteron nuclei covalently bound to partially oriented amphiphilic molecules. In the slow exchange process for instance in the octylamine $HCl–D_2O$ system [6], two separate $\Delta\nu$'s are observed and have been assigned to ammonium and water deuterons. In the present study, the discussion may be simplified by assuming only average $\Delta\nu$ of aggregate–deuterium oxide interaction. The splitting of the deuterium signal is characteristic of the aligned substance (e.g., a liquid crystal) and due to the interaction between a nuclear spin ($I = 1$) and an axially symmetrical electric field gradient. This phenomenon is called quadrupole splitting ($\Delta\nu$) and is expressed by the following equation [7].

$$\Delta\nu = |\Sigma P_i 3/4\chi \cdot S_i(3\cos^2\theta - 1)| \tag{1}$$

where P_i is the fraction of deuterons at site i, χ is the quadrupole coupling constant, S_i is an order parameter describing the average orientation of the molecules at site i with respect to the director and θ is the angle between the direction of the magnetic field and 1 giving rise to the orientation. For the nonoriented (powder) spectrum, the θ term in the above equation disappears and $\Delta\nu$ is directly proportional to S.

Magnetic orientation.—The magnetic orientation was examined as follows. The aggregate of the same lot (below 0.03 M concentration of host and guest molecules) was divided into three NMR tubes and shaken five times. The aggregates in the respective tubes were glossy and align diagonally with the axis of the tube. The deuterium signal gives a doublet with a splitting of 42 Hz, with a respective half-width of 19 Hz. Each tube was subjected to the following conditions for at least one week (Fig. 1). (1) When the NMR tube (1) was put in a permanent magnetic field (14.1 teslas) in which the direction of the external magnetic field is situated perpendicular to the long axis of the NMR tube, the aggregate sediments easily, is transparent, and gathers in the bottom of the NMR tube. It exhibits a glossy, fine stripe parallel to the direction of the external magnetic field in the plane of the tube that faces the magnet. (2) When the NMR tube (2) is put in a superconductive magnetic field (SCM, 58.7 teslas) in which the direction of its external magnetic field is situated parallel to the long axis of the NMR tube, the aggregate is likely to align parallel to the axis of the NMR tube, does not sediment, and becomes turbid: the SCM prevents the sedimentation of the aggregate. When the aggregate is removed from the SCM field, it gradually turns transparent and begins to spontaneously align. (3) When the sample (3) is left in the open air, its macroscopic appearance and the deuterium signal gradually change with time.

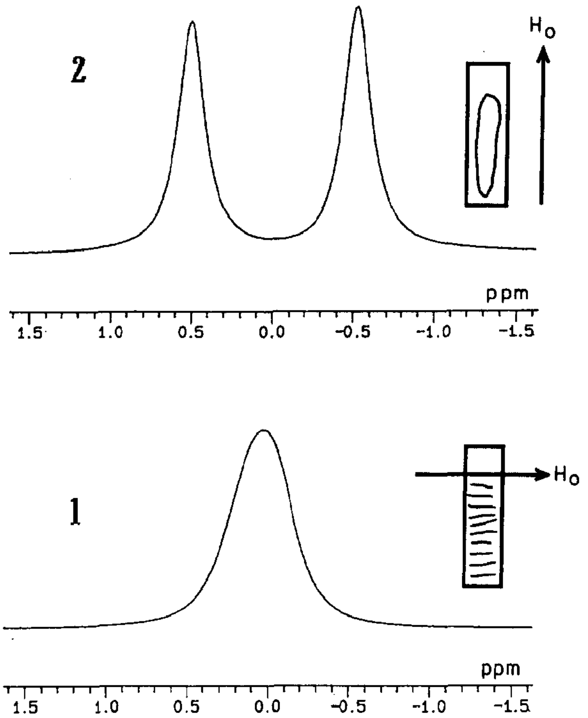

Fig. 1. ^{2}H NMR spectrum of the aggregate aligned in different magnetic fields and its appearance macroscopically aligned in the NMR tube. (1) Permanent magnetic field (14.1 T). (2) Superconductive magnetic field (58.7 T). The ~0.03 M aggregate was measured on a Jeol JNM-GSX(77 MHz) instrument.

Each tube was measured with the SCM spectrometer. Sample 1 showed a singlet with a half-width of 33 Hz. Sample 2 showed a doublet with a separation of 79 Hz and a half-width of 15 Hz. From Eq. 1, $\Delta\nu$ becomes largest when θ is 0°, reduces to one-half when θ is 90°, and vanishes when θ is 54.8° (the magic angle). In Fig. 1, sample 1 is shown to exhibit a broad singlet. As the θ value approaches the magic angle, the broad half-width may cause the narrow splitting of the signal to disappear. Sample 2 exhibits a clear $\Delta\nu$. Here the θ value approaches 0° judging from the appearance of the texture.

It is reported that when a thin layer of turbid *p*-azoxyanisole is arranged perpendicular to the magnetic field, it becomes transparent instantly [8], and when it is removed from the magnetic field, it returns to the turbid condition after a rather long time. The transparent state is due to the fact that the light axis of the liquid crystal aligns parallel to the direction of the magnetic field. In the present case, when transparent sample 1 was put in the SCM field, it became turbid, perhaps indicating that the SCM magnet disturbs the direction of **1** aligned perpendicular to the NMR tube by the permanent magnet.

The magnetic alignment discussed above is dramatically observable at low concentration. With an increase in the concentration, the orientation of the

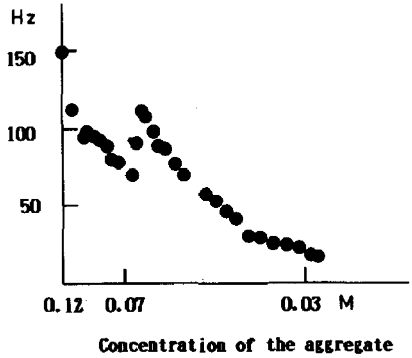

Fig. 2. Concentration dependency of the quadrapole splitting values.

aggregate itself acts more strongly. Once the texture has been formed, the direction of **1** was unaffected by the magnetic field, and the pattern did not change to a measurable degree. In this case, the contents became very viscous.

Concentration dependency of deuterium signal.—The 0.12 M aggregate was diluted with deuterium oxide by shaking five times in the longitudinal direction of the NMR tube, and the concentration dependency on the orientation of the aggregate was measured by $\Delta\nu$. The 0.12 M aggregate was very viscous, so the signal obtained with separation $\Delta\nu$ (150 Hz) was broad and did not change very much on shaking.

In Fig. 2 it is shown that increasing the amount of deuterium oxide causes a reduction of $\Delta\nu$, indicating the presence of a special interaction between deuterium oxide and the aggregate molecules. The curve does not continue, but a reproducible minimum value appears at ~ 0.07 M.

At this concentration, the doublet becomes broad, and it becomes impossible to measure the half-width. This phenomenon can be observed only in a small region, and after that, the aggregate begins to become glossy and orients longitudinally. Upon adding additional deuterium oxide, the stripe pattern parallel to the long axis of the NMR tube begins to appear, its half-width becomes narrow, and the two signals achieve baseline separation. The signal height increases and $\Delta\nu$ decreases continuously by dilution and becomes a singlet below 0.02 M. The discontinuous curve in Fig. 2 may indicate that above 0.07 M the orientation of the aggregate itself (maybe the texture of the three dimensions) predominates and that at concentrations < 0.07 M the orientation to the longitudinal direction of the NMR tube that is achieved by shaking is predominant. The overall texture moves from the powder pattern to the orientation pattern.

Temperature dependence on $\Delta\nu$ under different concentrations.—Plots of the temperature up to the melting points of the aggregate vs. $\Delta\nu$ are shown in Fig. 3 for different concentrations. $\Delta\nu$ is usually lower at the higher temperatures. The behavior shown in Fig. 3 may be due to a diminished degree of the orientation of deuterium oxide and the aggregate molecules, and/or an increased degree of

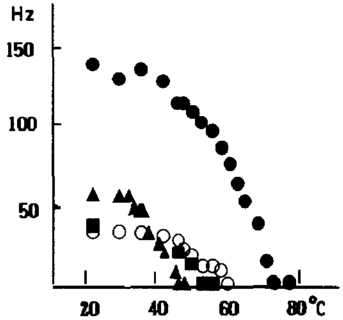

Fig. 3. Quadrupole splitting as a function of temperature at different concentrations. The concentration of the aggregate was as follows: ●, 0.12; ○, 0.08; ■, 0.06; and ▲, 0.03 M.

solvation of the aggregate. $\Delta\nu$ of the 0.12 M aggregate in the powder pattern at 23°C is extraordinarily large (150 Hz) compared to those of other concentrations. Maybe this concentration has a greater influence on $\Delta\nu$ than does the mode of the orientation. With an increase in the temperature, the value continuously decreases. At 77°C the signal becomes a singlet, and the aggregate becomes an isotropic solution. At other concentrations, the $\Delta\nu$'s show almost the same values [35 Hz at 0.08 M, 35 Hz at 0.06 M, and 58 Hz at 0.03 M (all at 23°C)], but singlet signals appear depending on the concentration (61°C at 0.08 M, 58°C at 0.06 M, and 49°C at 0.03 M). The 0.03 M aggregate shows larger $\Delta\nu$ than those of 0.06–0.08 M aggregates. At this concentration, the aggregate is easy to orient parallel in the NMR tube, and the oriented pattern may be predominant.

Phase diagram.—In Fig. 4, the melting points of the aggregate at various concentrations were plotted against the temperature. Each aggregate gives a singlet in region I (isotropic solution), and a doublet in region M. In areas where the temperature is lower and the concentration is higher, generally larger $\Delta\nu$'s appear.

Solvent effects.—A change of the orientation upon addition of the solvent was

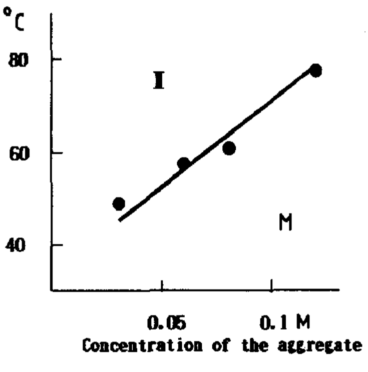

Fig. 4. Temperature dependency on quadrapole splitting of the aggregate at varying concentrations.

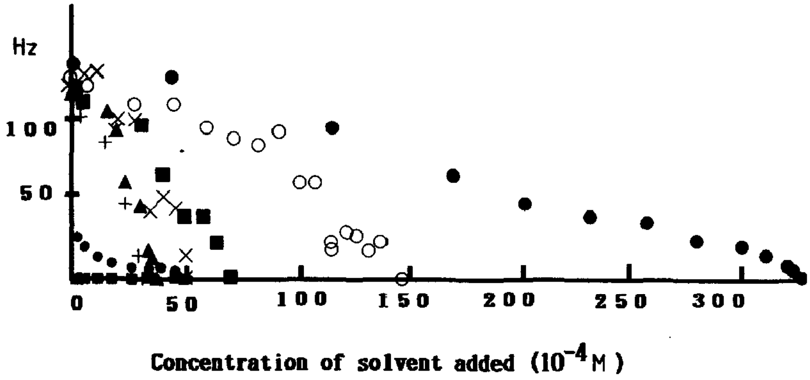

Fig. 5. Change of $\Delta\nu$ against the amount of solvents added. The concentration of the aggregate is 0.06 M. The solvents were: +, n-PrOD; ▲, $(CD_3)_2SO$; ×, $(CD_3)_2CO$; ■, EtOD; ○, MeOD; ●, D_2O; ○, $(CD_3)_2SO$; and ■, $(CD_3)_2CO$.

checked by $\Delta\nu$. An aliquot of the 0.06 M aggregate of the same lot was diluted by dimethyl sulfoxide-d_6, acetone-d_6, deuterium oxide, methanol-d_1, ethanol-d_1, and propanol-d_1, respectively. After mixing the solvents, the NMR tubes were shaken five times, and the measured $\Delta\nu$'s were plotted against the concentrations of the solvents added (Fig. 5).

Fig. 5 indicates the following. (1) Increases in the amount added and the overall length of the solvent molecule cause continuous and quick reduction of $\Delta\nu$: propanol causes the orientation of the aggregate to disappear about tenfold more quickly than does deuterium oxide. An increase of one methylene group in the alcohol series decreases $\Delta\nu$ by half. (2) Dimethyl sulfoxide-d_6 and acetone-d_6 have similar structures and $\Delta\nu$'s, but the methyl-d_3 signals of both solvents show quite different behaviors. The former exhibits a small $\Delta\nu$, and the latter maintains a singlet under all conditions. This means that in the aggregate–dimethyl oxide-d_6 system, the methyl-d_3 signal also exhibits orientation behavior. It is reported that dimethyl sulfoxide forms hydrogen bonding with the hydroxy group at the 2-position of the CD [9]. In the present aggregate, there may exist a γ-CD channel. When this channel is partially cut off by this solvent, this may also be included in the orientation system which causes its methyl-d_3 signal to split.

4. Conclusions

This aggregate is an assembly of **1** piled in the γ-CD channel. **1** is included quickly, but loosely fitted into the cavity of the γ-CD as a 1:1 complex. The second **1** is included slowly but into the same cavity and forms a 2:1 complex. The exposed part of the second **1** is included quickly into the cavity of the second γ-CD and forms a 2:2 complex. The benzenesulfonate part, which is the exposed portion of the first **1**, is included into the the cavity of the third γ-CD which has already formed a 1:1 complex, and forms a 3:3 complex, and so on. The aggregation expands, not only in the direction of the symmetry axis of the CD, but also in the other two dimensions. It has lyotropic liquid crystal-like character as it is soluble in H_2O, makes a stereoregistical polymer, it keeps the arrangement of the monomer unit, and is thought that the strain due to the growth process of the polymer chain is small. The present research was carried out following the formation of the lyotropic mesophase. With a surface-active agent, $\Delta\nu$ of 0–11 KHz can be observed [6], and the deuterium signal changes from a typical powder pattern to an oriented one after 40 min in the magnet [10]. In the present aggregate, the largest $\Delta\nu$ is only 0.2 KHz, and it requires time to orient in the magnetic field. The thick cavity of the γ-CD which encloses **1** may prevent the orientation of the azo dye molecules with each other, thus making $\Delta\nu$ very small. Nevertheless, changes of the orientation behavior induced in the aggregate by magnetic field, temperature, concentration, and solvent were definitely detected. The precise conformation of the fragile aggregate, including the solvent interaction, is now the subject of molecular dynamics calculations.

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