Fluorescence Detected Circular Dichroism of a Red-shifted Exciton-coupling Chromophore *N,N'*-Carbonyl-bridged Dipyrrinone Derivative Using an Ellipsoidal Device

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Summary. A fluorescent red-shifted exciton-coupling chromophore, *N*,*N'*-Carbonyl-bridged dipyrrinone, was subjected to fluorescence-detected CD (FDCD) measurements as a primitive structure-elucidating probe with *trans*-1,2-cyclohexanediol template in several solvents under various instrumental conditions. With the help of a JASCO ellipsoidal mirror device FDCD465, a chloroform solution achieved the sensitivity enhancement by 50 times of the transmission CD and 5 times of the conventional FDCD. All FDCD spectra were completely free from the polarization artifacts.

Keywords. Circular Dichroism; Chromophore; Chirality; Configuration.

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Introduction

The CD exciton chirality method [1, 2, 3] is a powerful option when elucidation of the absolute configuration is required, especially if the X-ray crystallographic analysis is not executable for the molecule of interest. The exciton-coupled CD (ECCD) becomes observable when two or more chromophores with distinct transition moments interact one another through space. The coupling gives rise to a pair of intense Cotton effects with opposite signs reflecting the spatial relation of those transition moments. When the preferred conformation of the chromophores is clearly grasped, the sign of Cotton effects is unambiguously correlated to the absolute configuration for the molecule of interest. Chromophores can be intrinsic or synthetically introduced as long as the principle of exciton is fulfilled, and the method has been applied to a variety of compounds [2]. Developments for ECCD applications, that have been devoted to the course of enriching the list of options, include exploration for a new way to introduce chromophores [4], searches for new useful chromophores [5, 6], and survey for an alternative CD detection.[7]

It was reported that certain dipyrrinone derivatives can be good exciton-coupling chromophores [8] of the following features: (1) They possess absorption in a red-shifted region (~410 nm), (2) The internal carbonyl bridge makes the chromophore fluorescent, (3) The shorter belt results in the more intense ECCD, and (4) The derivatives possess potential for complementary CD studies. Particularly benzoyl analogue of xanthoglow shown in Fig. 1 was considered as an attractive exciton-coupling chromophore since it possesses the absorption at a long wavelength and the emission in high efficiency ($\phi_F = 0.65$ in chloroform).

<Figure 1>

When the emitted fluorescence is detected and used as a reporter of the difference in absorption between excitation by the left- and right-circularly polarized lights, the recorded signal is called fluorescence-detected CD (FDCD) and has been expected to be more sensitive and selective in virtue of the general characters of fluorescence technique [9]. Following the first appearance of the method, some theoretical outlooks had emphasized the valuable anticipations [10, 11] and other reports had introduced applications to protein structural studies [12, 13]. The instrumental developers, however, had begun struggling with the polarization artifacts [14] that are inevitable unless the ideal polarized-light generator is adopted, namely, even a slight impurity contained in the material can lead to the artifacts when the solution in use shows polarization in fluorescence. Consequently, the type of FDCD instrument should also be taken into account when discussing the experimental FDCD outputs.

One of the intriguing suggestions was an ellipsoidal mirror device [15]. The principle of ellipse that all the emission from one of the foci gets into the detector at the other focus allows both to cancel the artifacts in FDCD and to collect more lights. The idea actually led to a device that is well aligned on a sample mount and compatible with a commercially available CD spectrometer, the new generation of FDCD instrument [16]. The two problems, the polarization artifacts and the sensitivity drop, were simultaneously solved at least when measuring simple model compounds in solution.

Based on the upbuilt situation, the aim of this work is to provide the basic information for combining a new red-shifted ECCD fluorescing chromophore and an ellipsoidal FDCD device. Since the compound possesses a variety of quantum yields for several solvents [8], the sensitivity enhancement is also interesting, as well as the artifact elimination by the ellipsoidal device.

Results and Discussion

Concern about Polarization Artifacts

Elimination of the polarization artifacts had been one of the concerns in FDCD measurements for decades [14, 16, 17, 18, 19] and the issue was considered also in this paper. Relative intensities of the polarization artifacts of the model compound **1** were plotted against wavelengths through the output of linear dichroism (LD) by detecting the emission [20, 21] in the identical conditions to those of FDCD for each solution. The plot in Fig. 2 enables the polarization artifacts to be directly compared between the devices and their measuring conditions. The artifacts became the largest when the conventional FDCD405 attachment was used without a polarizer. Even with this primary setup of the device, 90° light collection with a single photomultiplier tube (PMT) system, this artificial intensity was dramatically reduced when a polarizer was set at the angle of 84.5°, where the strain from photo-elastic modulator (PEM) was presumably excluded [14, 21]. The ellipsoidal structure of an improved device FDCD465 reduced the artifacts to some extent and the residual degrees of artifacts were further suppressed with the help of balancing masks [16], by compensating the imbalance of the emission from the sample cell. This result demonstrates that either the standard FDCD405 with a polarizer at a proper angle or the ellipsoidal FDCD465 with balancing masks of appropriate sizes can eliminate considerably the polarization artifacts in FDCD measurements for a solution, even when the chromophore of interest possesses the polarized emission to any extent.

<Figure 2>

Measuring FDCD in Several Solvents

As the polarization artifacts are negligible when the ellipsoidal FDCD465 is used with appropriate balancing masks, these conditions were applied for FDCD measurements in several solvents. The resultant FDCD spectra are discussed here from the viewpoints of shape and smoothness as follows.

Chloroform was expected to be a useful solvent for compound **1** since the quantum yields were already reported [8] to be 0.65 for the monoester and 0.59 for the diester; the emission intensity was reasonably high and there was no significant loss of the efficiency between mono- and diesters. The FDCD spectrum obtained with the ellipsoidal mirror device in fact agreed well with its CD as shown in Fig. 3A, where the dotted lines (FDCD) looked less noisy to remind that the curve may be observable even with further diluted solutions.

<Figure 3>

The sensitivity improvement by FDCD was not distinct in methanol due to the relatively low fluorescence quantum yield (0.17) [8]. The shape of FDCD was not completely the same to CD for some reason as seen in Fig. 3B. This is a part of the question that we have been trying to answer since the preceding paper [21], where it was reported that FDCD can be different from CD even without the polarization artifacts that are preventable by employing either an ellipsoidal device or a polarizer on a standard device, but the difference may not be caused directly by the solvent effect. No obvious explanation has been given to the FDCD distortion at this stage and we have been inquiring what the general mechanism could be.

<Table 1>

Among the other solvents used, the fair sensitivity enhancement based on the fluorescence efficiency [8] and the slight shape deviation between CD and FDCD were also observed (Table 1). The sensitivity in dimethyl sulfoxide solution was slightly improved by FDCD, where the band at 380 nm was deformed in shape supposedly by the efficiency difference between the corresponding transitions in its fluorescing energy path. The FDCD curve in acetonitrile is another example that showed weaker Cotton effects at 430 and 380 nm, where the sensitivity improvement was not obvious. In cyclohexane, the difference between CD and FDCD was not negligible though subtle sensitivity increase was achieved. This example follows our empirical hunch that FDCD measurements in cyclohexane sometimes lead to deformation of the CD shape [19] especially when molecules possess polar chromophores. As was expected from the quantum yield (0.62) [8], FDCD in benzene gave a smoother FDCD curve, where the shape of CD had a different balance between the bands at 380 and 435 nm.

Comparison of Measurement Sensitivities

The chloroform solution was subjected further to measurements under more diluted conditions using both FDCD devices, and the outputs were compared with the transmission CD measurement. The experiments were performed under the conditions of no polarization artifacts, namely, the two conditions were employed, measurements by the conventional 405 mirror with a polarizer and the ellipsoidal device (465) with balancing masks. At the concentration of 4.7×10^{-7} *mol/L*, as shown in Fig. 4A, the CD curve was already too noisy for a practical use. The conventional 405 attachment, on the other hand, gave an acceptable shape of FDCD and the ellipsoidal

465 showed even clearer curve. When the solution was further diluted by 5 times, as plotted in Fig. 4B, although the conventional 405 device resulted in a very noisy spectrum, which did not seem trustful any more, the ellipsoidal 465 still led to a pragmatic curve. From those results, we can conclude that combination of our fluorescent red-shifted chromophore and the ellipsoidal device allows artifact-free CD detection even with a solution of as 1/50 low concentration as the experiments of transmission basis.

<Figure 4>

In summary, our fluorescent red-shifted exciton-coupling N,N’-carbonyl-bridged dipyrrinone can be used as a chromophore in FDCD measurements on compounds of unknown absolute configuration when incorporated in them by ester bonds. Chloroform is a suggested solvent in senses that FDCD agrees very well with CD and that the measurement sensitivity is by 50 times higher than the transmission CD. The results obtained by using the ellipsoidal FDCD465 device are much better than the conventional FDCD device. It reminds of positive outlooks both on the present molecule and the FDCD technique that the dipyrrinone scaffold has potential as a fluorophore to accept object-oriented designs by modifying the structure and that the device persuades to explore more fruitful utilization, respectively.

Experimental

The solvents used for all the measurements were purchased from Nakalai Tesque (chloroform, acetonitrile, cyclohexane, and benzene) and Sigma Aldrich Japan (methanol and dimethyl sulfoxide). All compounds for LD/CD/FDCD measurements were prepared as reported in the preceding paper [8].

CD, FDCD and LD measurements were performed on a JASCO J-720WI with either a standard FDCD405 (with a single PMT at 90° detection) or an ellipsoidal FDCD465 attachments. The angle of a polarizer on FDCD405 and the sizes of balancing masks on FDCD465 were chosen to minimize the degrees of fluorescence polarization (P_F), which were nicely monitored through the emission with the linear dichroism (LD) mode [16, 22]. The employed angle of polarizer was 84.5° in all related cases, and the best pairs of balancing masks were: upper/lower = 6.0/6.0 mm (chloroform), 6.0/6.5 (methanol), 5.0/5.0 (dimethyl sulfoxide), 6.5/7.0 (acetonitrile), 6.0/6.5 (cyclohexane), and 6.0/6.5 (benzene). A long pass filter L48 was employed to prevent contamination by the scattered light. UV-Vis spectra were recorded on Shimadzu UV-2500PC with the same intervals as CD/FDCD measurements and were used to convert the raw FDCD data into the CD comparable readings. The UV-Vis absorption and the reported extinction coefficients [8] were used to determine the solution concentrations in the solvents.

The FDCD raw data were obtained as $S = k(F_L - F_R)/(F_L + F_R)$, where F_L and F_R stand for the relative intensities based on $(F_L + F_R)$ values that were measured as DC voltage in volts. The unit of S value was adjusted by the derived instrumental constant k (+28684) as millidegrees (mdeg). Based on a standard method, FDCD spectra were obtained from the raw data [7, 10]: $\Delta\varepsilon = \varepsilon_L - \varepsilon_R = ((3.032 \text{ x } 10^{-5})S(1 - 10^{-A}))/(cd10^{-A})$, where A is UV-Vis absorbance, c is molar concentration (*mol*/*L*), and d is cell length (*cm*). This transformation enables direct comparison between shapes of the FDCD and transmission CD spectra.

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Figure Captions

Fig. 1. Structure of the present chromophore, benzoyl analogue of xanthoglow, and its diester of (1*R*,2*R*)-cyclohexanediol (**1**).

Fig. 2. Fluorescence-detected linear dichroism (LD), a relative scale for FDCD polarization artifacts, of (1*R*,2*R*)-**1** in chloroform at concentration of 4.8 x 10^{-6} mol/L. Plotted curves were observed by the following devices; a) FDCD405 (in black dotted lines) with no polarizer (dotted line with squares), b) FDCD405 with a polarizer at 84.5° (dotted line with no squares), c) ellipsoidal FDCD465 (in black solid lines) with no balancing mask (solid line with circles), d) ellipsoidal FDCD465 with 5.5/6.0 mm balancing masks (solid line with no circles). No distinct LD was observed for the solvent (gray dotted line) in the artifact-remaining condition.

Fig. 3. CD and FDCD curves of (1*R*,2*R*)- and (1*S*,2*S*)-**1** in two representative solvents, (A) chloroform and (B) methanol; Solid lines represent CD and dotted lines represent FDCD; Black lines are for (1*R*,2*R*)-**1** and grey lines are for (1*S*,2*S*)-**1**. All are directly comparable as $\Delta\varepsilon$ in the unit of $mol^{-1}dm^3cm^{-1}$.

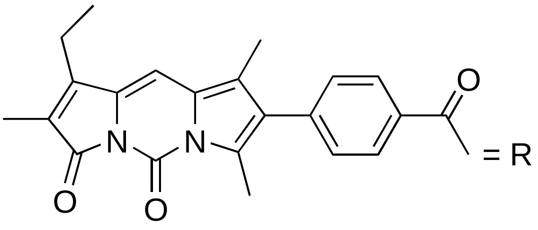
Fig. 4. CD and FDCD of (1*S*,2*S*)-**1** in chloroform at concentrations of A) 4.7 x 10^{-7} *mol/L* and B) 9.4 x 10^{-8} *mol/L*; CD (gray line a), FDCD by model-405 (dotted line b), and FDCD by ellipsoidal-465 (solid line c); all are directly comparable as $\Delta\varepsilon$ is the unit of $mol^{-1}dm^{3}cm^{-1}$.

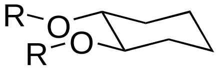
Table 1. Comparison of FDCD and CD Spectral data for the xanthoglow benzoate analog diester of (1*R*,2*R*)-cyclohexanediol **1**.

Solvent		$\Delta\varepsilon_{1\ \max}$ (λ_1)	λ at $\Delta\varepsilon = 0$	$\Delta\varepsilon_{2\ \max}$ (λ_2)
Chloroform	FDCD	-14.0 (436)	402	+1.2 (391)
	CD	-14.3 (437)	403	+2.2 (392)
	CD (lit[b])	*-15.2 (432)*	*403*	*+3.9 (380)*
Methanol	FDCD	-12.3 (434)	394	+1.1 (380)
	CD	-16.5 (404)	404	+5.8 (387)
	CD (lit[b])	*-16.7 (429)*	*402*	*+6.3 (381)*
Dimethyl sulfoxide	FDCD	-13.6 (435)	398	+1.5 (384)
	CD	-14.2 (433)	399	+3.1 (382)
	CD (lit[b])	*-15.1 (432)*	*400*	*+3.4 (380)*
Acetonitrile	FDCD	-15.7 (429)	395	+1.6 (378)
	CD	-17.3 (427)	395	+3.0 (387)
	CD (lit[b])	*-17.2 (426)*	*394*	*+4.0 (388)*
Cyclohexane	FDCD	-21.0 (436)	401	+4.3 (386)
	CD	-19.2 (433)	404	+9.0 (390)
	CD (lit[b])	*-19.0 (429)*	*400*	*+8.8 (380)*
Benzene	FDCD	-18.9 (439)	401	+2.1 (390)
	CD	-16.7 (433)	402	+3.5 (383)
	CD (lit[b])	*-17.4 (431)*	*401*	*+3.9 (381)*

[a] $\Delta\varepsilon$ in $dm^3mol^{-1}cm^{-1}$, λ in nm; [b] data from ref [8].

Fig. 1





(1*R*,2*R*)-**1**

Benzoyl analogue of xanthoglow

Fig. 2

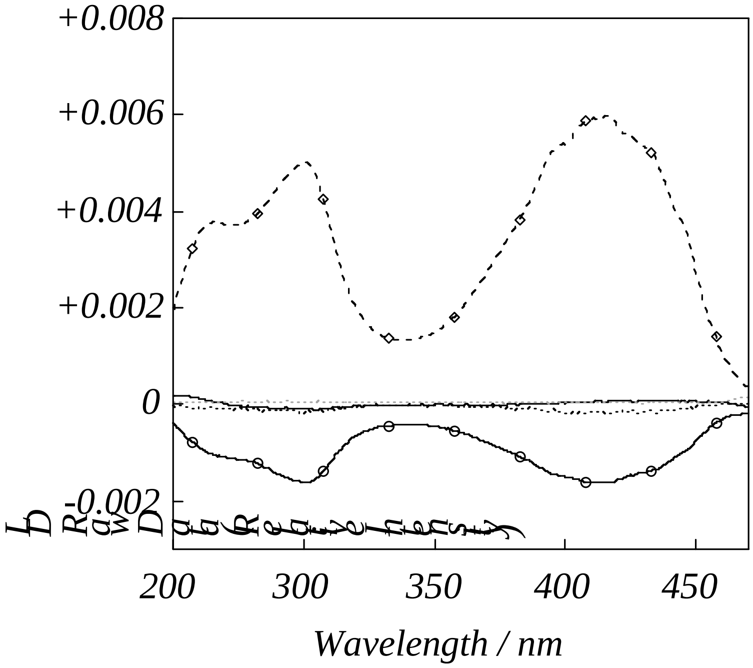

+0.008
+0.006
+0.004
+0.002
0
-0.002
LD Raw Data (Relative Intensity)
200
300
350
400
450
Wavelength / nm

Fig. 3

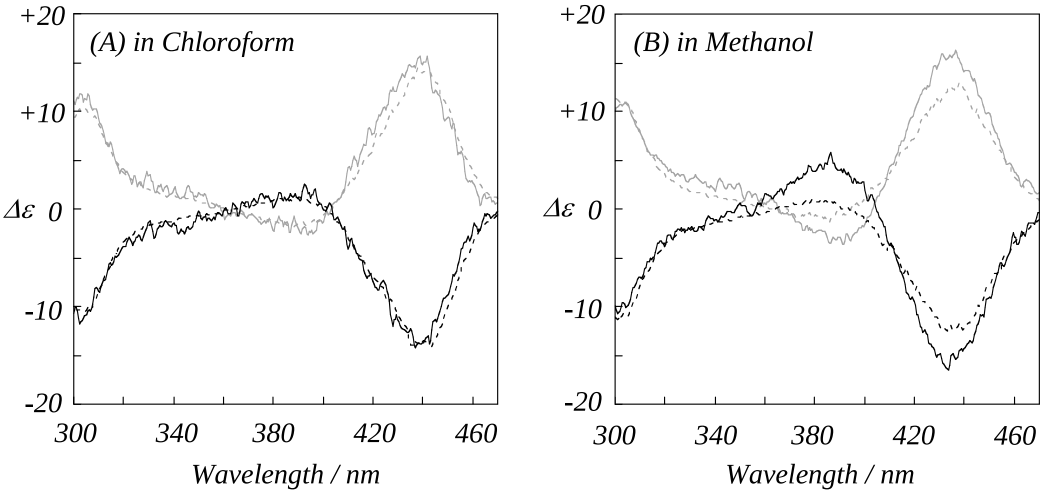
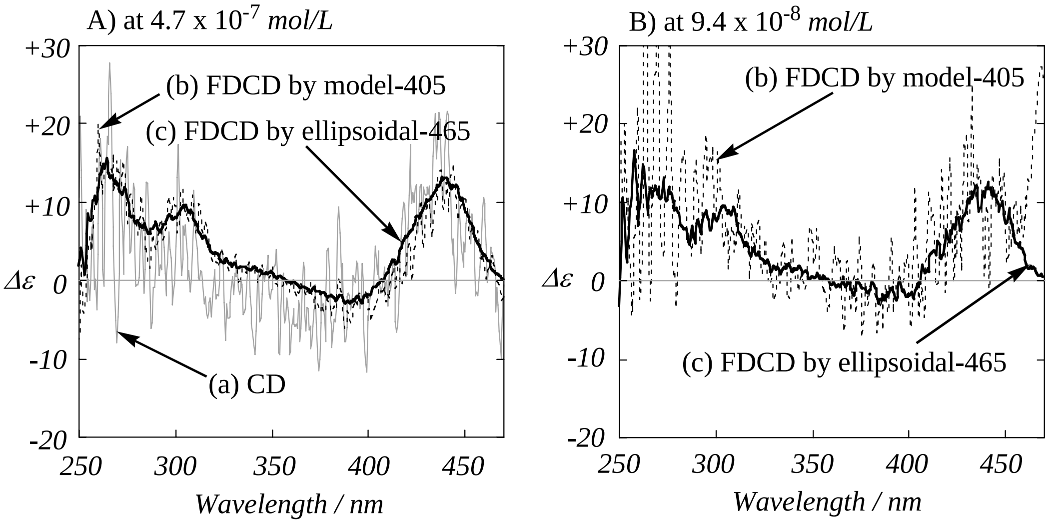

(A) in Chloroform
(B) in Methanol
+20
+10
Δε 0
-10
-20
300
340
380
420
460
Wavelength / nm

Fig. 4



A) at 4.7×10^{-7} *mol/L*

B) at 9.4×10^{-8} *mol/L*