Sensitivity Enhancement of Exciton Coupling by Fluorescence Detected Circular Dichroism (FDCD)

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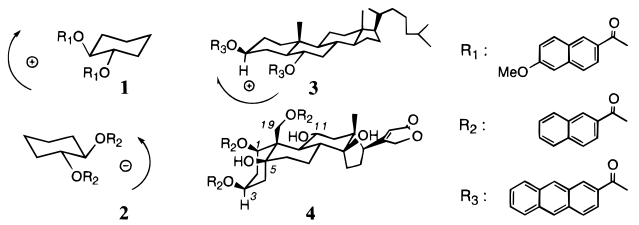
Exciton coupled circular dichroism,[2,3] characterized by split Cotton effects, is a microscale chiroptical method that determines the absolute configurations or conformations of a variety of compounds. This method becomes particularly powerful when complex experimental curves agree with calculated couplets, e.g., vinblastine.[4] A critical experimental finding was that the exciton split CD of molecules consisting of multiple interacting chromophores, identical[5a] or different,[5b,c] can be reproduced by pairwise summation of interacting chromophores, a principle which has been confirmed by theoretical calculations.[6] Since the amplitude of the split CD (*A* value, A_{CD}) is proportional to ϵ^2, chromophores with intense absorptions such as porphyrins with sharp Soret bands at ca. 415 nm (ϵ 350 000) exhibit couplings at a distance of 40−50 Å.[7] Fluorescent chromophores further facilitate ng $\sim$ μg scale handling of sample and enhance their HPLC detection,[8] e.g., 2-naphthoic acid, λ_{max} 234 nm (ϵ 57 500), and fluorescence emission λ_{max} 360 nm upon irradiation at 234 nm.

However, the sensitivity of conventional CD detection (obtained in transmission) remains limited because it depends on the intensity of dichroic absorption. In contrast, in fluorescence detected circular dichroism (FDCD), the sensitivity is greatly enhanced because it is based on direct mearurement of emitted radiation against a zero background. Moreover, in FDCD, only the CD-active and fluorescent transitions give rise to a signal,[9,10] thus enabling the selective measurement of the CD of fluorophores in a multichromophoric molecule, e.g., proteins with single fluorescent tryptophans.[11] FDCD using a modified phase-modulation spectrofluorometer[12] has been used to determine the enantiomeric excess without physical separation of enantiomers.[13] However, due to technical difficulties, high sensitivity has been achieved so far only when FDCD was combined with other techniques, such as laser-based detection for HPLC[14] and on-column detection in capillary electrophoresis.[15] Moreover, the FDCD of multichromophoric systems are not necessarily straightforward and have led to various observations, i.e., no exciton coupling due to stacking in the case of ethenoadenosines,[16,17] weak coupling arising from nonradiative deactivation with poly(1-pyrene-alanine),[18] and a complex CD pattern with polytryptophan.[19] In the following we report extension of FDCD to exciton coupled systems consisting of identical chromophores with well-defined and intense electric transition moments, *1(S),2(S)-trans*-cyclohexanediol bis(6-methoxy-2-naphthoate) (**1**), *1(R),2(R)-trans*-cyclohexanediol bis(2-naphthoate) (**2**), a steroidal 3β,6α-bis-(2-anthroate)[8b] (**3**), and ouabagenin 1,3,19-tris-(2-naphthoate) (**4**). This FDCD measurement results in 50−100-fold sensitivity enhancement over conventional CD.



The measurements were performed with a modified JASCO-720 CD instrument provided with a prototype attachment for fluorescence detection. 6-Methoxy-2-naphthoate derivative **1**, 2-naphthoate derivative (**2**, **4**), and 2-anthroate derivative (**3**) exhibit intense fluorescence, with emission maxima at 392, 361, and 437 nm when excited at 240, 231, and 262 nm, respectively. The quantum yields for **1**, **2**, and **3** were, respectively, 0.64, 0.29, and 0.24 (in MeCN) relative to 9,10-biphenylanthracene as reference (quantum yield 0.95 in polar solvent).[20]

The initial 6-methoxy-2-naphthoate (**1**) solution ($c = 4.37 \times 10^{-6}$ M, UV absorbance $A = 0.39$ at $\lambda_{max} = 237$ nm) was diluted 10-fold each time down to the lowest detectable fluorescence level; the intensity of the positive CD couplet becomes weaker at lower concentrations with the S/N ratio approaching 2 at a concentration of 4.37×10^{-8} M, which is the detection limit for the conventional CD in this case (Figure 1a). In contrast, the exciton coupled FDCD exhibits a conspicuous bisignate curve at 4.37×10^{-8} M (Figure 1b), the S/N ratio becoming 2 only after a further 100-fold dilution; the detection limit for this compound is thus ca. 200 pg/mL under the instrumental parameters used in this study.

Provided the molecule lacks other nonfluorescent chromophores[21] and the absorption and emission mechanisms of the bichromophoric molecules are similar to those of monochromophoric systems (which we assume is valid for **1** and **2**), then the above FDCD spectra can be converted to normal CD ($\Delta\epsilon$) spectra by the equation given by Tinoco *et al.*,[9b]

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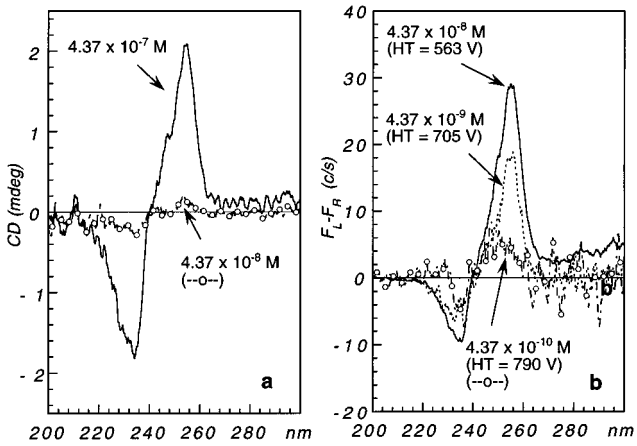

Figure 1. (a) CD of **1** (in MeCN) at different concentrations. Signal to noise ratio becomes low (S/N = 2) at 4.37×10^{-8} M. (b) FDCD spectra of **1** (in MeCN) using a standard UV32 filter at different concentrations and voltages of the photomultiplier. Signal to noise ratio becomes low (S/N = 2) at 4.37×10^{-10} M.

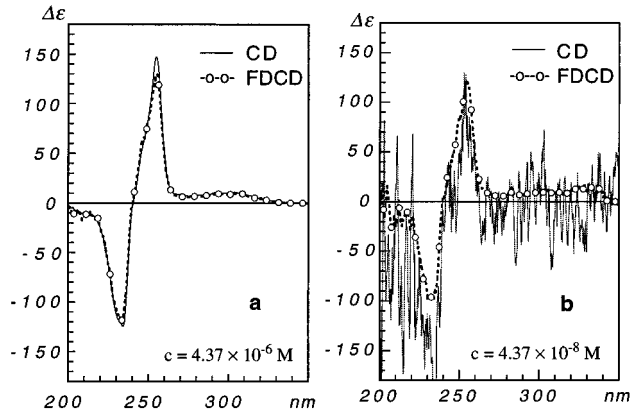

Figure 2. (a) CD of **1** (in MeCN), $c = 4.37 \times 10^{-6}$ M. Solid line shows conventional CD; dotted line shows CD derived from FDCD and eq 1. (b) CD of **1** (in MeCN), $c = 4.37 \times 10^{-8}$ M. Noisy line shows conventional CD, S/N = 2; the CD derived from FDCD (Figure 1b) and eq 1, dotted line, still exhibits clear exciton coupling.

$$\Delta\epsilon = \epsilon_L - \epsilon_R = \frac{2S(1 - 10^{-A})}{cd10^{-A}\ln 10} \qquad (1)$$

where $S = (F_L - F_R)/(F_L + F_R)$, F_L and F_R are the fluorescence intensities for left and right circularly polarized light, ϵ_L and ϵ_R are the corresponding extinction coefficients, A is the UV/vis absorbance of the fluorescent moiety of the molecule, c is concentration, and d is pathlength. As shown in Figure 2a, using eq 1, the experimental exciton split CD of **1** ($c = 4.37 \times 10^{-6}$ M), λ_{ext} ($\Delta\epsilon$), 254.8 nm (+147.4)/234.4 nm (−124.3), $A_{CD} = +271.7$, can be simulated satisfactorily from the experimental FDCD (at the same concentration) giving rise to a CD with extrema at 255.4 nm (+129.4) and 233.8 nm (−120.2), $A_{CD} = +249.5$. The very close intensities (A values) of the CD and FDCD bisignate curves of **1** (Figure 2a) suggest that the split energy level arising from exciton coupling is preserved in the emission stage as well. At a 100-fold dilution where the S/N ratio of CD is too low, the FDCD can still reproduce a satisfactory CD although of slightly lower intensity, with λ_{ext} ($\Delta\epsilon$) 254.0 nm (+120.8)/233.2 nm (−98.3), $A_{CD} = +219.1$ (Figure 2b).

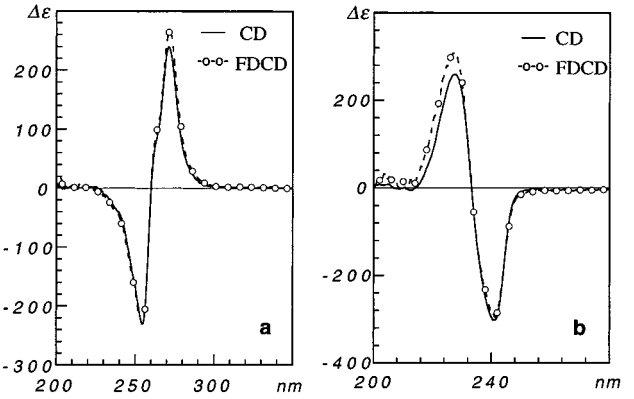

Figure 3. Comparison of conventional CD with the CD converted from FDCD for bis- and trischromophoric molecules: (a) **3** (in MeCN) at 2.92×10^{-6} M, using a L38 filter; (b) **4** (in MeCN) at 2.10×10^{-6} M, using a UV32 filter.

2-Naphthoate derivative **2** also exhibits similar CD and FDCD curves with a typical negative couplet. In this case the detection limit of FDCD is 4.12×10^{-8} M, or 50 times higher than normal CD. Since the estimation of detection limit in the FDCD measurements of **1** and **2** has been performed under similar instrumental conditions, the sensitivity ratio of $\Delta F(\lambda)$ over normal CD $\Delta A(\lambda)$ is directly related to the molecular quantum yield q, $\Delta F(\lambda)/\Delta A(\lambda) = q \times$ constant.[22] The quantum yield of **1** (0.64) is almost twice that of **2** (0.29); it is thus reasonable that **1** and **2** show, respectively, >100-fold and ca. 50-fold increases in sensitivities of FDCD over normal CD. As shown in Figure 3, excellent agreement is also seen between conventional CD and FDCD for bis-2-anthroate **3** and tris-2-naphthoate **4**.

As described, FDCD correctly measures the exciton coupling between two or more identical strongly absorbing fluorescent chromophores under conditions too dilute for CD measurements. FDCD thus provides a sensitive tool for structural studies requiring only picogram levels of sample under favorable conditions. However, in the case of coupling between two different chromophores, especially between strong and weak fluorophores, complications due to energy transfer are observed; this aspect is under further study.

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Supporting Information Available: CD and FDCD spectra of **2** at various concentrations, excitation and emission spectra of **1** and **2**, experimental details for measurement of quantum yields, derivatization of the FDCD equations, and block diagram of the modified JASCO J720 CD with FDCD attachment (12 pages). See any current masthead page for ordering and Internet access instructions.

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(22) Beer's law and eq 1 give the relationship between $\Delta F(\lambda)$ [$= F_L(\lambda) - F_R(\lambda)$] and q: $\Delta F(\lambda) = GqI_o(\lambda)10^{-A(\lambda)}\Delta A(\lambda)\ln 10$, where G is the gain of the instrument including the PMT voltage, q is the quantum yield, $I_o(\lambda)$ is the incident light (kept constant as I_o for all wavelength range by an instrumental controller), $A(\lambda)$ is UV absorbance, and $\Delta A(\lambda)$ is the difference in absorbance between left and right circularly polarized lights. For a very dilute solution, the term $10^{-A(\lambda)}$ can be considered as unity. A comparison of the sensitivity between FDCD and normal CD should be based on $\Delta F(\lambda)/\Delta A(\lambda)$ [$= GqI_o\ln 10$].