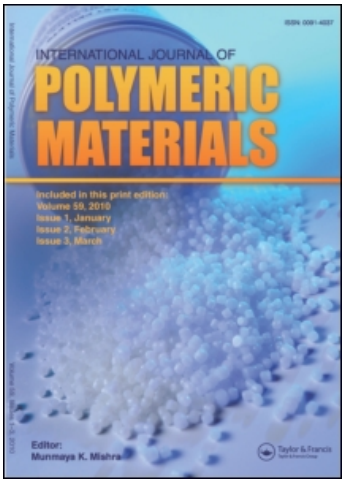

International Journal of Polymeric Materials
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713647664

Circular Dichroism and Circular Polarized Luminescence from a Green Fluorescent Protein—Initial Research for Chiroptical Emission of Biological Materials

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Online publication date: 09 August 2010

To cite this Article Goto, Hiromasa , Sawada, Isao and Nomura, Nobuhiko(2010) 'Circular Dichroism and Circular Polarized Luminescence from a Green Fluorescent Protein—Initial Research for Chiroptical Emission of Biological Materials', International Journal of Polymeric Materials, 59: 10, 786 — 792
To link to this Article: DOI: 10.1080/00914037.2010.483218
URL: http://dx.doi.org/10.1080/00914037.2010.483218

International Journal of Polymeric Materials, 59:786–792, 2010

ISSN: 0091-4037 print/1563-535X online
DOI: 10.1080/00914037.2010.483218

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Optical properties of a green fluorescent protein (GFP) are examined using optical absorption, circular dichroism (CD), and circular polarized luminescence (CPL) spectroscopies. The GFP has chiroptical activity and exhibits green circular polarized emission, although the g_{em}-factor is small. Poly(vinyl alcohol) (PVA)/GFP composite films are prepared to attempt long-term preservation of the GFP emission activity. After five years, the transparent PVA/GFP composite film still exhibits stable fluorescence that appears similar to the emission from the *Aequorea* jellyfish.

Keywords circular dichroism (CD), circular polarized luminescence (CPL), green fluorescent protein (GFP), photoluminescence, poly(vinyl alcohol) (PVA)

Received 18 January 2010; accepted 31 March 2010.
The authors thank the Engineering Workshop and the Chemical Analysis Center of the University of Tsukuba for glasswork. The authors express sincere appreciation to Prof. K. Akagi for continuing encouragement and for providing the CPL instrumentation.
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INTRODUCTION

Since green fluorescent protein (GFP) was isolated from *Aequorea* [1], it has been widely employed in biological studies, such as a bright fluorescent marker for gene expression. Blue emission from the protein *aequorin* excites GFP to exhibit green fluorescent light by a multichromophoric resonance energy transfer mechanism (Förster transition). In the research of fluorescent protein, the chemical structure of a papain-derived chromophore peptide was evaluated [2].

The optical absorption of GFP has been studied from both theoretical and experimental perspectives [3–5]. Circular dichroism (CD) measurements of fluorescent proteins were evaluated for a high β-sheet content of the peptide chain in the 3-D chiroptical structure [6,7]. The chiroptical property is derived from the chiral structure and the chromophore in the chiral environment.

We have developed a novel synthetic method for the preparation of chiral π-conjugated polymers in cholesteric liquid crystals as a chiral matrix. Cholesteric liquid crystals with structural chirality provide a chiral reaction field. A polymerization reaction within the asymmetric chiral field provides transcription of chirality to the synthesized polymers during the reaction. The polymers thus obtained in cholesteric liquid crystals exhibit both CD and circular polarized luminescence (CPL), although they have no asymmetric center in the chemical structure [8]. The chiral matrix effect for the production of chirality in synthetic polymers may relate to biological reactions, because the reactions occur within the chiral environment of a biological chiral field, and at enzymes as proteins with 3-D asymmetric structures.

Some natural life forms have optofunctional organs related to the polarization of light. For example, the African dung beetle *Scarabaeus zambesianus* detects the polarization of moonlight in the sky to orientate itself during the night [9]. Some beetles have exoskeletons with chiroptical cholesteric order that reflect circular polarized light. Recently, the circular polarized light detection properties, function, and system of mantis shrimp (*Stomatopod crustacean*) eyes were evaluated [10]. Such reports have accelerated research in bio-optics and biomimetic technologies.

From the aspect of materials science, learning from the biosystem and application of the biological functions provides effective methods to realize artificial functional materials with quantum performance.

In this study, we evaluate the optical properties of GFP using optical absorption, photoluminescence (PL), CD, and CPL spectroscopic measurements.

RESULTS AND DISCUSSION

Electrophoresis

Figure 1 shows an electrophoretogram of the GFP employed in this study, which indicates that the sample consists of various molecular weights.

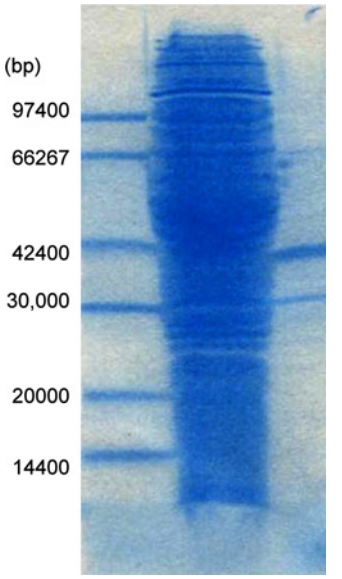

Figure 1: Electrophoretogram of the GFP employed in this study.

Optical Absorption and Circular Dichroism

Figure 2 (top) shows the optical absorption spectrum of GFP with absorption maxima at 463 nm (shoulder) and 486 nm. These absorption bands are due to π–π^* transitions and may be described as an almost pure HOMO-LUMO transition [11]. The CD spectrum of the GFP shows a positive Cotton effect at 400 and 420 nm, and a negative Cotton effect at 437 and 479 nm. Cody et al. reported that a denatured GFP exhibits no fluorescence and its absorption spectrum is significantly different from native GFP [2]. This research suggests that the base sequence in the protein changes the optical property of the luminescent protein, which may be related to the 3-D structure, because the base sequence in the protein influences the 3-D structure. The chiroptical properties of proteins are derived from both the primary molecular chiral structure and the 3-D chiral structure. However, it is unclear how the base sequence affects the chiroptical properties of GFP. The present results confirm chiroptical activity of the GPC.

Photoluminescence

Figure 3 shows PL spectra of the GFP. Excitation incident light at 273 nm allows a PL band at 337 nm (UV region). Excitation light at 390 nm affords an emission band at 504 nm in the visible region that has weak intensity compared to the emission at 337 nm. The fluorescence band at 470 nm is the green light emission from the GFP.

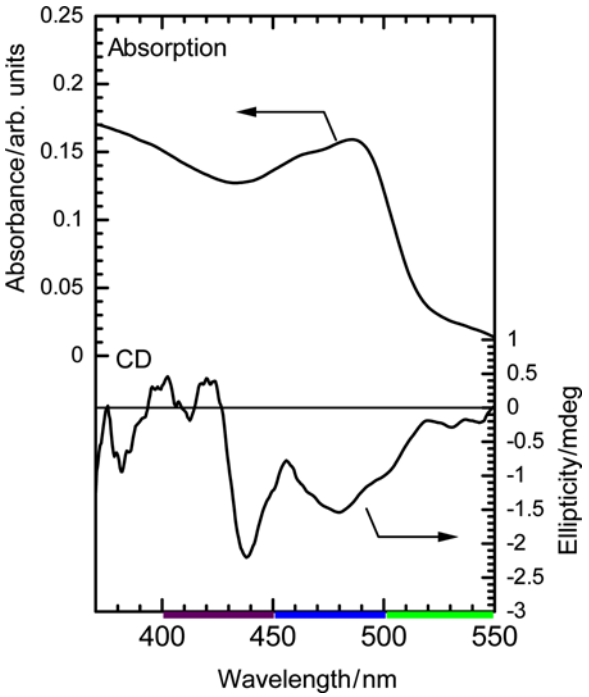

Figure 2: Optical absorption and CD spectra of GFP.

Circular Polarized Luminescence

Figure 4 shows the CPL spectrum and g_{em}-factor of the GFP. The radiative transition probabilities for left (L) and right (R) photons in spontaneous

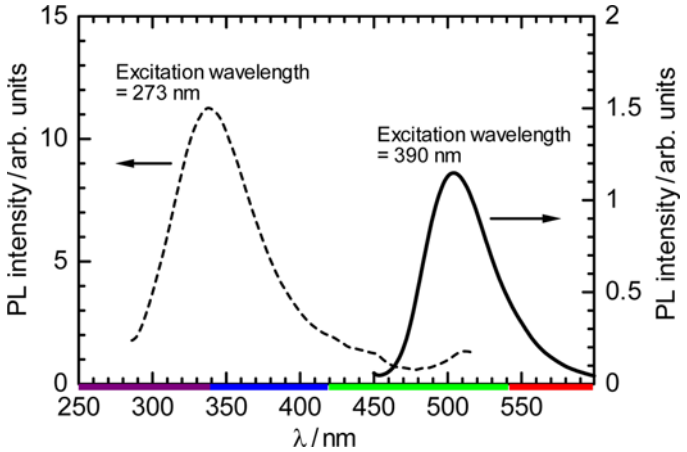

Figure 3: Photoluminescence spectra of GFP.

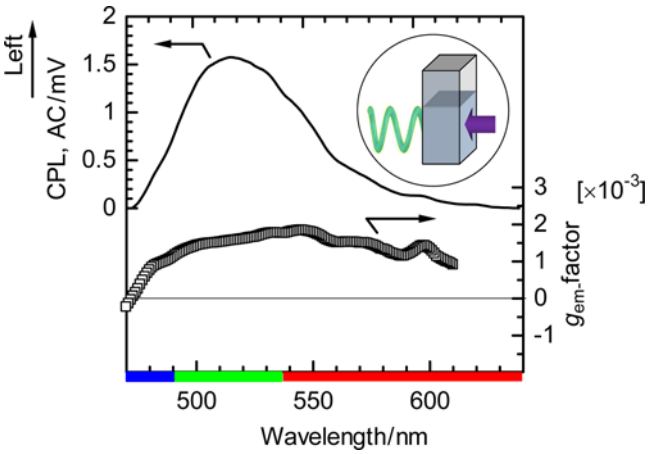

Figure 4: Circular polarized luminescence, and g_{em}-factor of the GFP. Inset shows a schematic for irradiation with incident excitation light and the circular polarized light emission.

emission are unequal in chiral molecules. The degree of circular polarization in the emission is defined by

$$g_{\mathrm{em}} = 2(I_{\mathrm{L}} - I_{\mathrm{R}})/(I_{\mathrm{L}} + I_{\mathrm{R}}) = V_{\mathrm{AC}}/V_{\mathrm{DC}}$$

where g_{em}, I, V_{DC} and V_{AC} are the dissymmetry factor in the emission, the intensity, the measured fluorescence, and the CPL, respectively. The GFP displays a left-handed (anticlockwise direction) circular polarized emission at 514 nm. This band can be derived from optical absorption (excitation) at short wavelengths (<426 nm, and positive sign in the CD). The g_{em}-factor of the GFP is between 0 and 1.8×10^{-3}, as shown in Figure 4. The inset shows a plausible schematic for the incident excitation light and circular polarized light emission from the sample in cuvette. A set of the results confirmed that the GFP exhibits the Cotton effect in both the CD and the CPL, although further investigation regarding the relationship between the sign of the Cotton effect and the 3-D structure, or the absolute configuration of the chromophore, is required.

Composite of GFP with Poly(Vinyl Alcohol)

A poly(vinyl alcohol) (PVA)/GFP composite film was prepared for long-term preservation of the GFP emission activity. The GFP was mixed well with PVA in water, because PVA has good solubility in water. The water in the mixture was then gradually evaporated at room temperature to produce a PVA/GFP composite film. Figure 5 shows a fluorescence microscopy image of the PVA/GFP composite film, which was taken five years after preparation of the composite (June 9, 2004–October 18, 2009). The GFP in the composite film consistently shows emission through the PVA matrix, which indicates

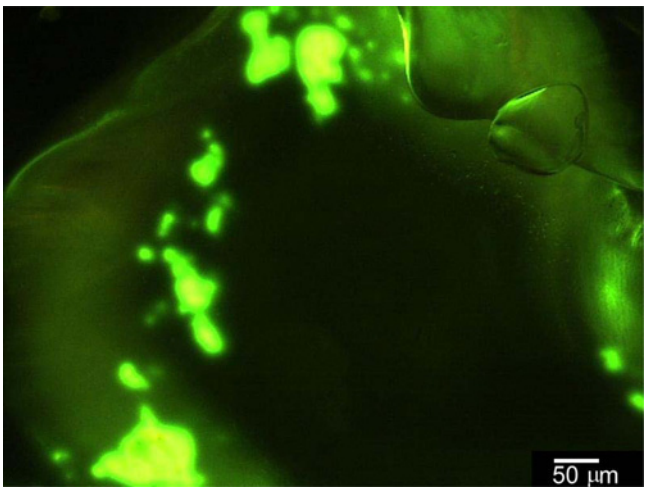

Figure 5: Fluorescence optical microscopy image of a PVA/GFP composite. This sample was prepared on the 9th of June, 2004, and the micrograph was taken on the 18th of October, 2009.

successful long-term preservation of the emission activity of the GFP within the transparent PVA matrix, and the composite exhibits luminescence similar to that from the *Aequorea* jellyfish. Preparation of a composite yields a stable bioluminescent film containing GFP, and this technique may be employed for industrial applications.

CONCLUSION

The optical properties of GFP were examined using optical absorption, PL, CD, and CPL spectroscopies. The GFP has chiroptical activity and has a green circular polarized emission, although the g_{em}-factor is small. This result suggests that living jellyfish may also emit circular polarized light. At present, it is not known whether circular polarized light plays a role in signaled communication between individual creatures in the natural world; however, other photogenic creatures, such as insects, might display circular polarized emission, because all life forms are mainly composed of chiral molecules and have hierarchal chiral structure from the molecular level to the macro-level.

Although further study is required, some of the chiroptical fluorescence properties of a light-emitting GFP protein were determined through this study.

Technique

Absorption spectra were obtained using a Hitachi U-2000 spectrophotometer, and circular dichroism measurements were performed using a Jasco

J-720 spectrometer for samples in 1.5 M potassium phosphate buffer. CPL spectra of the GFP were obtained with a Jasco CPL-200S spectrometer. All optical measurements were carried out at 25°C. Optical observations were conducted using a Nikon ECLIPS LV 100 high-resolution polarizing microscope with Nikon LU Plan Fluor and CFIUW lenses.

Materials

Enhanced GFP (EGFP; Clonetech, Co. Ltd) was employed in this study. More than 190 mutations were introduced into the wild-type GFP to produce EGFP by biological methods to improve the emission intensity and stability (PUCP20, *E. coli*).

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