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A chiral Eu^{3+}–thienoyltrifluoroacetone complex on an avidin tetramer: luminescence and CD studies on the supramolecular protein–metal chelate complex

Masumi Taki, Hiroshi Murakami and Masahiko Sisido*

Department of Bioscience and Biotechnology, Faculty of Engineering, Okayama University, 3-1-1 Tsushimanaka, Okayama 700-8530, Japan. E-mail: sisido@cc.okayama-u.ac.jp

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A chiral Eu^{3+}–chelate complex was built into the biotin-binding sites of an avidin tetramer by the binding of biotin-linked thienoyltrifluoroacetones.

Eu^{3+}–β-diketone complexes are known to exhibit distinct luminescence when irradiated under UV light.[1] The emission properties have been intensively studied and have found a variety of chemical and biological applications, *e.g.* as sensitive fluorometric probes.[2] However, conventional bidentate β-diketone chelators form rather unstable complexes with Eu^{3+}, and their luminescence intensity is often very weak, especially in aqueous media. In order to overcome this problem, Matsumoto and coworkers synthesized a new type of Eu^{3+} chelate in which two bidentate β-diketone moieties are covalently linked.[3,4] The tetradentate ligand formed a very stable complex with Eu^{3+}, and the complex emitted strong luminescence.[4]

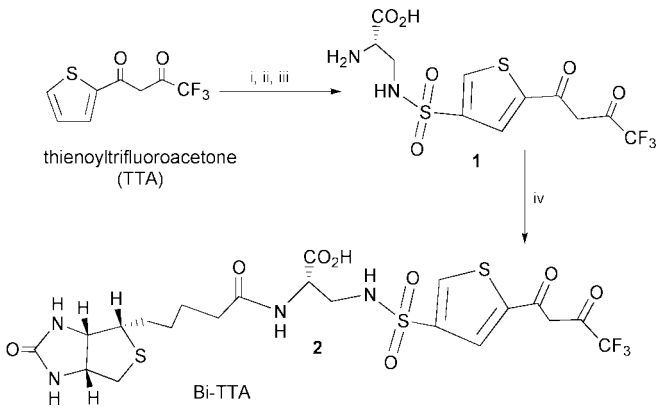
The finding of luminescence enhancement in the above tetradentate chelate motivated us to build a supramolecular Eu^{3+} complex of two bidentate ligand molecules arranged on a protein framework. A tetrameric avidin was chosen as the protein framework, since it strongly binds biotin[5] or biotin derivatives[6] through non-covalent interactions. The X-ray crystallographic data of the avidin tetramer[7] indicates that two of the four biotin-binding sites face one side of the tetramer and the two biotins in the binding sites take a right-handed screw configuration with a chirality factor of 0.90.[8] The dissymmetrically arranged biotin-binding pockets can be used to incorporate two biotin-labeled bidentate ligands in a chiral configuration. The structure and synthesis of the biotin-linked bidentate ligand **2** (Bi-TTA), are shown in Scheme 1.

Complexation of the Bi-TTA with avidin was confirmed by the fluorescence quenching of tryptophan in avidin. Fig. 1 shows the fluorescence spectra of avidin in the presence of different amounts of Bi-TTA. The peak position of tryptophan fluorescence shifted to a shorter wavelength[9] and decreased upon addition of Bi-TTA. The decrease stopped when the avidin units were saturated by the Bi-TTA molecules. The extent of fluorescence quenching was much larger than the quenching by biotin,[6] presumably owing to an energy transfer process from the singlet excited state of the tryptophan to the triplet state of the β-diketone group.

The formation of the Bi-TTA–Eu^{3+} complex on the protein framework was monitored by the increase of luminescence intensity of a solution of Bi-TTA and Eu^{3+} upon addition of avidin. As shown in Fig. 2, the narrow luminescence band at 613 nm increased with the addition of avidin, and the increase ceased after almost all the Bi-TTA molecules were bound to avidin. The luminescence intensity of the Bi-TTA–Eu^{3+} complex in the presence of avidin is 25 times larger than that of the free Bi-TTA and Eu^{3+} mixture in the absence of avidin. No luminescence band at 613 nm was observed for a mixture of Eu^{3+} and avidin in the absence of Bi-TTA.

When an excess of avidin was quickly added to a fresh mixture of Bi-TTA and Eu^{3+} up to a tetrameric avidin : Bi–TTA ratio of 5 : 1, the luminescence emission at 613 nm decreased to 17% of the intensity for the tetrameric avidin : Bi-TTA ratio of 1 : 4. The reduced Eu^{3+} emission in the presence of an excess of avidin suggests that the emission enhancement at 613 nm originates from interactions between the metal chelates on the avidin tetramer and Eu^{3+} that are most effective when the tetrameric avidin : Bi-TTA ratio is 1 : 4.

The ligand : Eu^{3+} ratio was determined to be 1 : 1 from the luminescence titration curve of the mixture of tetrameric avidin:Bi-TTA (1 : 4) with the addition of Eu^{3+}. The luminescence intensity at 613 nm increased with the addition of Eu^{3+}, and the increase ceased when the Eu^{3+} : Bi-TTA ratio reached



Scheme 1 *Reagents and conditions*: i, chlorosulfuric acid, 0 °C → room temp., 30%; ii, *N*-α-Boc-L-α,β-diaminopropionic acid, DMF, carbonate buffer (pH 9.3), r.t., 80%; iii, TFA, 0 °C, 100%; iv, *N*-hydroxysuccinimidobiotin, TEA, DMF, r.t., 100%.

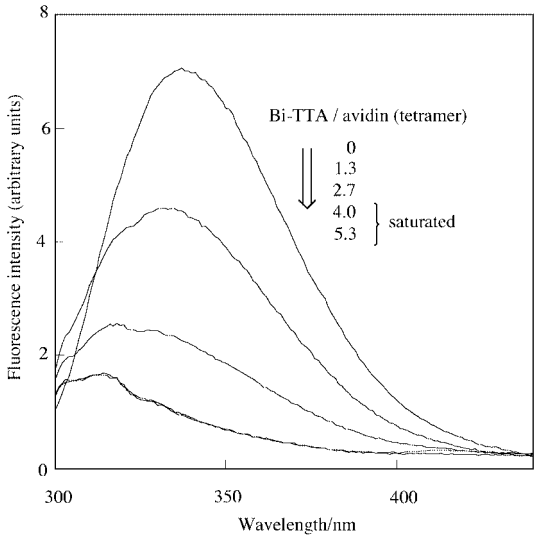


Fig. 1 Fluorescence spectra of avidin–Bi-TTA mixtures in PIPES–NaCl buffer at pH = 6.5. λ_{ex} = 290 nm. The molar ratios are indicated: [avidin (tetramer)] = 7.8×10^{-8} M.

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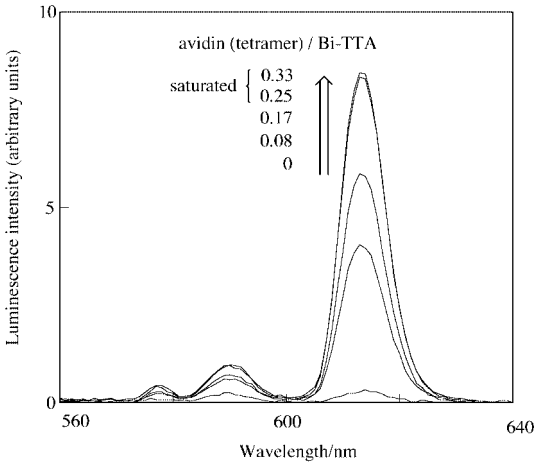

Fig. 2 Luminescence spectra of avidin–Bi-TTA mixtures in the presence of $EuCl_3$ in PIPES–NaCl buffer at pH = 6.5. The molar ratios of avidin (tetramer)/Bi-TTA are indicated: λ_{ex} = 343 nm. The TTA moiety is selectively excited under the conditions: [Bi-TTA] = 3.1×10^{-7} M, [$EuCl_3$] = 1.2×10^{-4} M.

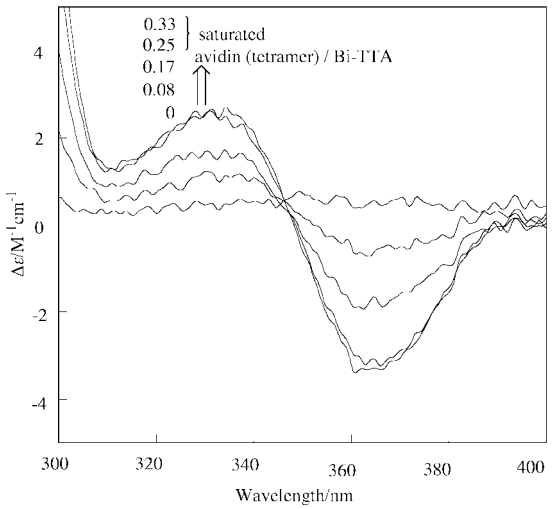

Fig. 3 CD spectra of Bi-TTA in the presence of $EuCl_3$ and different amounts of avidin in PIPES–NaCl buffer at pH = 6.5. The molar ratios are indicated; [Bi-TTA] = 4.0×10^{-4} M, [$EuCl_3$] = 3.0×10^{-3} M.

4:4. The Eu^{3+}:ligand ratio indicates that each TTA moiety binds a single Eu^{3+} ion, and the luminescence intensity increases when two TTA–Eu^{3+} complexes self-assemble on one side of the tetrameric avidin framework.

The formation of the two TTA–Eu^{3+} complexes was also confirmed from CD spectra. As shown in Fig. 3, a marked CD doublet appeared at 333 and 365 nm with the addition of avidin to a mixture of Bi-TTA and Eu^{3+}. The CD peak was saturated when all the Bi-TTA molecules were bound to avidin. No CD signal was observed in a tetrameric avidin:Bi-TTA (1:4) solution in the absence of Eu^{3+}. When Eu^{3+} was added to the latter solution, a similar CD spectrum as in Fig. 3 appeared, indicating the formation of the ligand–Eu^{3+} complex.

The luminescence and CD data indicate the formation of a chiral dimeric ligand–Eu^{3+} complex on the protein framework. The chiral lanthanide complex may find applications in enantioselective catalysis for the cleavage of nucleic acids[10] or proteins.[11]

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Notes and references

1 S. I. Weissman, *J. Chem. Phys.*, 1942, **10**, 214.
2 F. S. Richardson, *Chem. Rev.*, 1982, **82**, 541.
3 J. Yuan and K. Matsumoto, *Anal. Sci.*, 1996, **12**, 695.
4 J. Yuan, K. Matsumoto and H. Kimura, *Anal. Chem.*, 1998, **70**, 596.
5 N. M. Green, *Adv. Protein Chem.*, 1975, **29**, 85.
6 M. Kuragaki and M. Sisido, *Bull. Chem. Soc. Jpn.*, 1997, **70**, 261.
7 P. C. Weber, D. H. Orlendorf, J. J. Wendoloski and F. R. Salemme, *Science*, 1989, **243**, 85.
8 The chirality factor is defined by $\zeta = \boldsymbol{r}_{12} \cdot (\boldsymbol{m}_1 \times \boldsymbol{m}_2)$, where $\boldsymbol{r}_{12}$ is a unit vector from the center of first biotin site to the second biotin site and $\boldsymbol{m}_1$, $\boldsymbol{m}_2$ are unit vectors that are fixed on the two biotin molecules, respectively. ζ takes a value from +1 for a right-handed screw configuration to −1 for a left-handed one. ζ = 0 for an achiral configuration: see, N. Harada and K. Nakanishi, *Circular Dichroic Spectroscopy, Exciton Coupling in Organic Stereochemistry*, University Science Books, New York, 1982; Y. Inai, M. Sisido and Y. Imanishi, *J. Chem. Phys.*, 1990, **94**, 8365.
9 G. P. Kurzban, G. Gitlin, E. A. Bayer, M. Wilchek and P. M. Horowitz, *Biochemistry*, 1989, **28**, 8537.
10 M. Komiyama, N. Takeda and H. Shigekawa, *Chem. Commun.*, 1999, 1443.
11 Y. Yamamoto and M. Komiyama, *Nucleic Acids, Symp. Ser.*, 1992, **27**, 33.