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Exciton circular dichroism couplet arising from nitrile-derivatized aromatic residues as a structural probe of proteins

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ABSTRACT

Exciton coupling between two chromophores can produce a circular dichroism (CD) couplet that depends on their separation distance, among other factors. Therefore, exciton CD signals arising from aromatic sidechains, especially those of tryptophan (Trp), have been used in various protein conformational studies. However, the long-wavelength component of the commonly used CD couplet produced by a pair of Trp residues is typically located around 230 nm, thereby overlapping significantly with the protein backbone CD signal. This overlap often prevents a direct and quantitative assessment of the Trp CD couplet in question without further spectral analysis. Here, we show that this inconvenience can be alleviated by using a derivative of Trp, 5-cyanotryptophan (Trp_{CN}), as the chromophore. Specifically, through studying a series of peptides that fold into either α-helical or ß-hairpin conformations, we demonstrate that in comparison with the Trp CD couplet, that arising from two Trp_{CN} residues not only is significantly red-shifted but also becomes more intense due to the larger extinction coefficient of the underlying electronic transition. In addition, we show that a pair of *p*-cyanophenylalanines (Phe_{CN}) or a Phe_{CN}–Trp_{CN} pair can also produce a distinct exciton CD couplet that can be useful in monitoring conformational changes in proteins.

Circular dichroism (CD) spectroscopy is one of the most commonly used techniques in assessing the secondary structural content of proteins [1,2]. This is because the far ultraviolet (UV) CD spectrum in the region of 190–250 nm [3], arising from the backbone of a polypeptide, depends on the exciton couplings among the individual π–π* and *n*–π* transitions of its amide units [4]. Similarly, when two amino acids with aromatic sidechains (chromophores) are in close proximity, exciton coupling between their π–π* transition bands ($^1A_1$–^1B_b transition) could also lead to formation of unique CD signatures in this spectral region [5,6]. For example, the tryptophan (Trp) residues in the Trpzip ß-hairpins give rise to a positive CD band at around 228 nm, providing a convenient spectroscopic feature to monitor ß-hairpin formation [7–9]. Similarly, a recent study by Gasymov and coworkers [10] showed that a pair of Trp residues separated by less than 10 Å in a protein can produce an observable exciton CD band at around 230 nm. In addition, Khan and coworkers [11] observed the formation of a CD band resulting from the exciton coupling between two different aromatic sidechains in the enzyme phospholipid/lipid A palmitoyl transferase PagP.

The interaction potential (V_{AB}) between the electronic transition dipole moments (μ_A and μ_B) of two chromophores (A and B) can be expressed as

$$V_{AB} \propto \frac{\mu_A \mu_B}{r_{AB}^3}(\cos\gamma - \cos\alpha\cos\beta), \quad (1)$$

where $\vec{r}_{AB}$ is the distance between A and B, α (ß) represents the angle between $\vec{\mu}_A(\vec{\mu}_B)$ and $\vec{r}_{AB}$, and γ is the angle between $\vec{\mu}_A$ and $\vec{\mu}_B$. The extinction coefficient ($\Delta\varepsilon$) of the CD couplet arising from this exciton coupling is proportional to V_{AB} [12], as shown below:

$$\Delta\varepsilon \propto V_{AB} R_{AB}, \quad (2)$$

Abbreviations used: CD, circular dichroism; UV, ultraviolet; Trp, tryptophan; Trp_{CN}, 5-cyanotryptophan; Phe_{CN}, *p*-cyanophenylalanine; IR, infrared; HPLC, high-performance liquid chromatography; MALDI, matrix-assisted laser desorption/ionization; TFE, 2,2,2-trifluoroethanol; UV–Vis, UV–visible; DPC, dodecylphosphocholine; Ala, alanine.

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where R_{AB} is the rotational strength [12], which is defined as

$$R_{AB} \propto \pm \vec{r}_{AB} \cdot (\vec{\mu}_A \times \vec{\mu}_B). \quad (3)$$

Combining Eqs. (1)–(3) leads to

$$\Delta\varepsilon \propto \pm \frac{\mu_A^2 \mu_B^2}{r_{AB}^2} \Theta(\alpha, \beta, \gamma). \quad (4)$$

This equation indicates that $\Theta(\alpha, \beta, \gamma)$, which depends on the spatial orientations of $\vec{\mu}_A$ and $\vec{\mu}_B$, determines the sign of the long-wavelength component of the CD couplet. A convenient way to determine this sign is to first project $\vec{\mu}_A$ and $\vec{\mu}_B$ onto a common plane and then rotate the transition dipole moment of the front chromophore onto the one in the back. If a clockwise/counter-clockwise rotation is needed in order to superimpose the two transition dipole moments, then accordingly the exciton CD couplet will have a positive/negative chirality; in other words, the long-wavelength component of the exciton coupling band will have a positive/negative value of molar ellipticity [12].

Eq. (4) indicates that the amplitude of an exciton CD couplet arising from two identical chromophores depends on the square of its absorption extinction coefficient (ε) [13]. Thus, we hypothesize that the far-UV CD signal produced by two 5-cyanotryptophan (Trp_{CN}) chromophores would be larger than that induced by two Trp residues, when other factors (i.e., distance and orientation) are identical or similar. This is because, as indicated in Fig. 1, the extinction coefficient of the π–π^* transition (i.e., the $^1A_1$ to ^1B_b transition) of 5-cyanoindole (the sidechain of Trp_{CN}) in methanol is nearly twice as large as that of indole (the sidechain of Trp). In addition and perhaps more importantly, the far-UV absorption spectrum of 5-cyanoindole (and also Trp_{CN}) is red-shifted by approximately 20 nm from that of indole (and Trp), thereby resulting in a smaller overlap between the Trp_{CN} CD signal and those arising from the protein backbone. Indeed, in many cases, the far-UV CD signals arising from naturally occurring aromatic residues, including Trp, are difficult to discern due to such spectral overlaps [10]. Thus, we believe that these unique spectral features will make the CD couplet arising from a pair of Trp_{CN} residues, a useful probe of protein structures.

To test this notion, we studied a series of Trp_{CN}-containing peptides that can form either an α-helical or a ß-hairpin structure in appropriate solvents. Indeed, the CD spectra of those peptides show strong and distinct Trp_{CN} CD signals that depend on the peptide conformation, thereby providing a direct validation of this idea. As one of the π–π^* transitions of the non-natural amino acid *p*-cyanophenylalanine (Phe_{CN}) is shifted to 240 nm, we also investigated the potential utility of the CD couplet arising from the electronic coupling between Phe_{CN} and Trp_{CN} and between a pair of Phe_{CN} residues. We found that when a Trp_{CN} residue and a Phe_{CN} residue were brought into contact in a ß-hairpin peptide, they produced an intense CD couplet with the long-wavelength component centered at 247 nm. Similarly, two Phe_{CN} residues when placed next to each other in an α-helical peptide, also yielded an observable CD couplet. Thus, taken together, these results indicate that these nitrile-derivatized non-natural aromatic amino acids can be used to probe the formation of protein tertiary structure, via CD measurements. Given the fact that the nitrile stretching vibrations of Trp_{CN} and Phe_{CN} are useful, site-specific infrared (IR) probes of proteins [14–16], we believe that the findings from the current study will further expand the spectroscopic utilities of these non-natural amino acids.

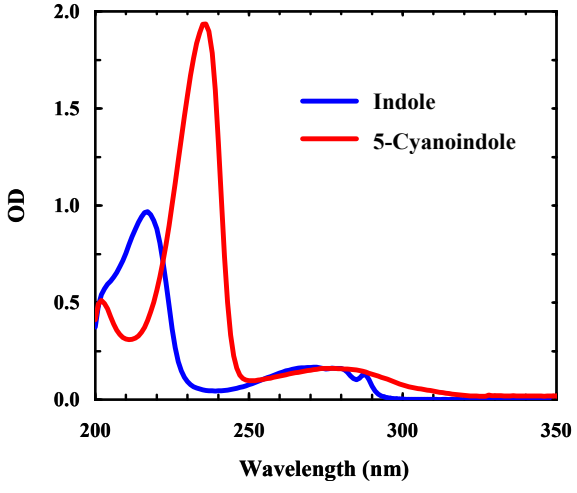

Fig.1. Absorption spectra of indole and 5-cyanoindole in methanol, as indicated. In both cases, the solute concentration was 28 mM, as determined by weight.

Materials and methods

All peptides were synthesized on a microwave-assisted automated Liberty Blue peptide synthesizer (CEM, Matthews, NC, USA) using Fmoc-protected amino acids purchased from Protein Technologies (Tucson, AZ, USA). Peptide purification was achieved by reverse-phase high-performance liquid chromatography (HPLC), and the identity of each peptide was confirmed by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry. All peptide samples were prepared by dissolving lyophilized peptides in either 10 mM sodium phosphate buffer (pH 7.0) or 2,2,2-trifluoroethanol (TFE) or in a mixture of 10 mM sodium phosphate buffer (pH 7.0) and TFE. The peptide concentration was determined optically using the absorbance of the peptide at 280 nm and an $\varepsilon_{280} = 5500\ cm^{-1}\ M^{-1}$ for Trp_{CN} [17] and an $\varepsilon_{280} = 850\ cm^{-1}\ M^{-1}$ for Phe_{CN} were considered [18]. UV–visible (UV–Vis) spectra were collected on a PerkinElmer Lambda 25 UV–Vis spectrometer. All CD data were collected on an Aviv 62 DS spectrometer (Aviv Biomedical, Lakewood, NJ, USA) using a 1-mm sample cuvette.

Results and discussion

To verify whether a pair of Trp_{CN} residues can produce a useful CD couplet, we first studied a Trp to Trp_{CN} mutant of an antimicrobial peptide, CP10A [19]. We then studied three alanine-based peptides, with each containing two differently located Trp_{CN} residues. Next, we studied a Trp to Trp_{CN} mutant of Trpzip5, a β-hairpin designed by Cochran and coworkers [7]. We chose these peptide systems because they all contain at least two Trp residues that have been shown to engage in exciton coupling and, hence, exhibit an exciton CD band. Finally, we investigated the Trp to Trp_{CN} and Phe to Phe_{CN} double mutant of another stable β-hairpin that was designed through "loop optimization" by Anderson and coworkers [20].

CP10A Trp_{CN} mutant

CP10A is an indolicidin-based antimicrobial peptide that contains five Trp residues (sequence: ILAWKWAWWAWRR-NH_2) and adopts an α-helical structure when bound to membranes [19,21]. In addition, a previous study [19] reported the CD spectra of CP10A obtained under different solvent conditions. Therefore, this peptide can serve as a convenient model system to compare the CD signals arising from Trp and Trp_{CN} sidechains. Specifically, all five native Trp residues in CP10A were replaced with Trp_{CN} (hereafter referred to as Cp10A–Trp_{CN}). As shown in Fig. 2, the CD spectrum of