INVERSION AND ENHANCEMENT OF THE CIRCULAR DICHROIC SPECTRUM OF FLAVIN ADENINE DINUCLEOTIDE BY THE COMBINATION TO D-AMINO ACID OXIDASE

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The optical rotatory dispersion (ORD) of D-amino acid oxidase (EC 1.4.3.3; pig kidney) has been reported by Aki et al. (1966). The bound coenzyme, flavin adenine dinucleotide (FAD), displays Cotton effects centered about the wavelengths of the corresponding absorption maxima. Measurements of circular dichroic (CD) spectra of the bound FAD clearly indicated the presence of CD absorption bands. In this paper, a marked change of CD spectrum of FAD by the combination to D-amino acid oxidase is reported. This phenomenon can be taken as an evidence of a conformational change of FAD as the result of the binding to the apoenzyme.

EXPERIMENTAL —— D-Amino acid oxidase was crystallized from kig kidney. FAD and flavin mononucleotide (FMN) were obtained from Wakamoto Pharmaceutical Co. Ltd. A_{260}/A_{450} of the FAD was 3.20 in good agreement

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with the reported value (3.27) (Whitby, 1953). Concentration of FAD and FMN were measured using $\epsilon=11.3\times10^3$ (at 450 mμ for free FAD and 460 mμ for the bound FAD) and $\epsilon=12.2\times10^3$ (at 450 mμ), respectively (Whitby, 1953). CD spectra were measured with a JASCO ORD/UV-5 recording spectropolarimeter with a CD attachment. Other details of experiments were the same as reported previously (Aki et al., 1966). All measurements were performed in pH 8.0, 0.1 M pyrophosphate-HCl buffer at 20°-25°C.

RESULTS —— Fig.1 shows the CD spectra of FAD. Free FAD had one negative CD band at 372 mμ (curve 1). Another small negative CD band was observed around 440mμ. FAD bound to D-amino acid oxidase showed a CD spectrum (curve 2) completely different from that of free FAD. The CD band at 372 mμ was inverted and increased its amplitude. $\epsilon_L-\epsilon_R$* at 372 mμ changed from -1.21 to 5.19 following the change from free state to the bound state. Fig.2 shows CD spectrum of FMN. Two negative CD bands were observed at 377 and 450 mμ. When compared with the CD spectrum of free FAD, the intensity of the former decreased, but that of the latter increased. 5'-Adenylic acid showed no CD absorption in the visible region.

DISCUSSION —— Such an inversional change of CD spectrum as observed with FAD has been reported with some steroids (Wellman and Djerassi, 1965) and with ketones having flexible ring structure (Wellman et al., 1965). The inversional changes were observed

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*A_L-A_R and $\epsilon_L-\epsilon_R$ are the difference of the left and right circularly-polarized components in absorbance and molar extinction coefficient, respectively.

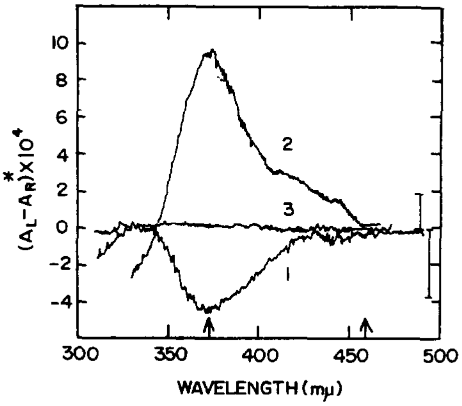

Fig.1 CD spectra of free FAD (curve 1, 3.70×10^{-4}M) and the bound FAD (curve 2, 1.77×10^{-4}M). Curve 3, base line. The bound FAD solution contained 1.55×10^{-4}M D-amino acid oxidase (assuming M.W.=100,000) and 7×10^{-6}M free FAD. The concentration of the latter was determined with the outer solution of the dialysis carried out for the preparation of the solution. Arrows on the abscissa indicate the positions of the peaks in the absorption spectrum of the bound FAD. Vertical bars indicate scales corresponding $\epsilon_L-\epsilon_R=1$ for curves 1 and 2, respectively.

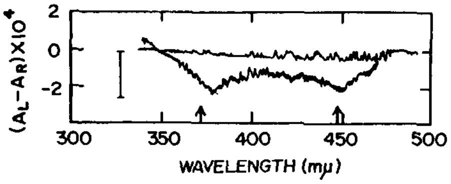

Fig.2 CD spectrum of FMN (2.52×10^{-4}M) together with the base line. Arrows indicate the positions of the peaks in the absorption spectrum of FMN. Meaning of vertical bar is the same as in Fig.1.

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when temperature was changed markedly, for example, from 25° to -192°C, and were attributed to the shift of equilibrium between two or more conformations of the respective compounds. The isoalloxazine ring is rigid, and is not presumed to change the steric structure of ring system. Change of the steric orientation of

17-acetyl group to the steroid ring causes an inversional change of CD spectrum (Wellman and Djerassi, 1965). Interaction between the isoalloxazine and the adenine ring systems of FAD has been suggested from fluorescence quenching (Bessey et al.,1949; Weber, 1950), from hypochroism in absorption spectrum (Whitby, 1953), and from dipole moment and infrared spectrum (Shikita, 1956). The fact that the sum of CD spectra of FMN and 5'-adenylic acid does not give the CD spectrum of FAD supports this concept. The observed inversional change of the CD spectrum of bound FAD without appreciable shift at 372 mμ suggests that the interaction between the two ring systems are preserved, but their mutual steric orientation is markedly changed when FAD is bound to the apoenzyme. The small negative band at 440 mμ seems also to be inverted. Appearance of a new band around 420 mμ suggests formation of a new interaction which was not present in free FAD.

Since the optical activity of FAD is markedly influenced by the state of FAD as described above, following points must be considered when CD spectrum or ORD curve of D-amino acid oxidase is treated quantitatively. 1) Two molecules of FAD can be bound to the enzyme molecule (Miyake et al., 1965). Whether the two molecules of FAD are in the same state or not must be carefully examined. 2) Excess FAD in the buffer solution apparently diminishes the optical activity of the bound FAD.

Recently, Simpson and Vallee (1966) reported ORD of flavin nucleotides. They observed with FAD a negative Cotton effect with a crossover at 375 mμ which corresponds with the negative CD band centered about 372 mμ. Simpson and Vallee (1966) and Wellner (1966) could not find any appreciable Cotton effect with FMN in the visible region. As shown in Fig.2, FMN showed two negative CD bands of almost equal amplitude in near positions. Presumably two Cotton effects due to these bands overlap to give no appreciable anomaly in the wavelength region. Simpson and Vallee (1966) measured ORD of diaphorase which has FAD as coenzyme, and suggested the presence of a positive Cotton effect around 375 mμ.

As illustrated in this paper, measurements of CD spectra of optically active coenzyme both in free and bound states will give more important information concerning interactions between coenzyme and apoenzyme than measurements of ORD.

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