ABSTRACT. The interpretation of the circular dichroism (CD) spectra of proteins to date requires additional secondary structural information of the proteins to be analyzed, (e.g. X-ray or NMR data.) Therefore, these methods are inappropriate for a CD database whose secondary structures are unknown, as in the case of the membrane proteins. The Convex Constraint Analysis algorithm (Perczel, A., Tusnády, G., Hollósi, M. and Fasman, G. D., 1991b, *Protein Engineering*, 4, 669-679), operates on a collection of CD spectral data to extract the common spectral components with their spectral weights. The linear combinations of these derived "pure" CD curves can reconstruct the original data set with great accuracy. For a membrane protein data set, the five component spectra so obtained from the deconvolution, consisted of two different types of $\alpha$ helices (the $\alpha$ helix in the soluble domain and the $\alpha_T$ helix, for the transmembrane $\alpha$ helix), a $\beta$ pleated sheet, a class C-like spectrum related to $\beta$ turns and a spectrum correlated with the unordered conformation. The deconvoluted CD spectrum for the $\alpha_T$ helix was characterized by a positive red-shifted band in the range 195 to 200 nm (+95,000 deg cm$^2$ dmol$^{-1}$), with the intensity of the negative band at 208 nm being slightly less negative than that of the 222 nm band (-50,000 and -60,000 deg cm$^2$ dmol$^{-1}$, respectively) in comparison with the regular $\alpha$ helix, with a positive band at 190 nm and two negative bands at 208 and 222 nm with magnitudes of +70,000, -30,000 and -30,000 deg cm$^2$ dmol$^{-1}$, respectively.

Introduction
Conformational studies of membrane proteins lag far behind that of soluble proteins mainly due to the difficulties associated with crystallization of membrane proteins for X-ray diffraction studies, and to the restricted movement of the proteins embedded in the membrane for NMR studies (Kühnbrandt, 1988; Smith and Griffin, 1988). X-ray crystallography is still the only routine method for determining the three dimensional structures of biological macromolecules at high resolution (Kühnbrandt, 1988) which can be utilized for the comparison of the structures determined by circular dichroism (CD) deconvolution. Three dimensional structures of five membrane protein complexes have been determined. These are: the bacterial photosynthetic reaction centers of *Rhodobacter viridis* (Rb. viridis) (Deisenhofer et al., 1984, 1985; Deisenhofer and Michel, 1989) and of *Rh. sphaeroides* (Chang et al., 1986; Allen et al., 1986, 1987a,b; Feher et al., 1989), porin from *Rb. capsulatus* (Weiss et al., 1991; Kreusch et al., 1991), photoactive yellow protein (PYP) from the purple photoautotrophic bacterium, *Ectothiorhodospila halophilla* (McRee et al., 1989), and bacteriorhodopsin from *Halobacterium*...
halobium (Henderson et al., 1990). While electron microscopy (EM) has provided the overall shape of a protein including the number of helix strands, detailed information of secondary structure is not likely to be easily obtained from EM. Therefore, there is not an adequate conformational database to evaluate the deconvolution results, as was in the case of the soluble proteins (Perczel et al., 1992b).

Circular dichroism spectroscopy has been recognized for its utility and simplicity in operations for conformational studies, even though interpreting the data has not been simple and has been a major controversy concerning the technique. The CD spectrum of each secondary conformation may be obtained on the basis of the CD spectrum obtained either from model polypeptides (Holzwarth and Doty, 1965; Sarkar and Doty, 1966; Greenfield and Fasman, 1969; Woody, 1974; Brahms and Brahms, 1980; Hollósi et al., 1987a, b; Perczel et al., 1991a, 1992a; Perczel and Fasman, 1992), or extracted from a set of protein CD spectra, whose X-ray data is available (Saxena and Wetlaufer, 1971; Chen et al., 1974; Chang et al., 1978; Bolotina et al., 1981; Hennessey and Johnson, 1981; Provencher and Glöckner, 1981).

On the basis of the results of these investigations, the CD spectrum of the α helix consists of a positive peak around 190 to 195 nm, with magnitudes of about 60,000 to 80,000 deg cm$^{-2}$ dmol$^{-1}$ (π-π* transition) and two negative peaks at 208 (π-π* transition) and 222 nm (n-π* transition), with the magnitude of -36,000 ± 3,000 deg cm$^{-2}$ dmol$^{-1}$ (Tinoco et al., 1963; Holzwarth and Doty, 1965; Woody and Tinoco, 1967; Greenfield and Fasman, 1969; Saxena and Wetlaufer, 1971; Chen et al., 1974). The CD spectrum of a β sheet has the π-π* transition around 195 to 200 nm, has a magnitude of 30,000 to 50,000 deg cm$^{-2}$ dmol$^{-1}$ and the n-π* transition at about 215 to 220 nm, with a magnitude of -10,000 to -20,000 deg cm$^{-2}$ dmol$^{-1}$ (Sarkar and Doty, 1966; Greenfield and Fasman, 1969; Saxena and Wetlaufer, 1971), while the spectra generally correlated with the random conformation has a large negative band around 200 nm, with the magnitude of -20,000 deg cm$^{-2}$ dmol$^{-1}$, with a small positive peak or a shoulder with a small negative value at 220 nm (Greenfield and Fasman, 1969; Fulmer, 1979; Yang et al., 1986). For reverse turns, three classes of CD spectra for the different types of turns were theoretically calculated by Woody (1974). The most abundant type (type I or III) of β turn showed class C CD spectrum (Chou and Fasman, 1977; Hollósi et al., 1987a, b) which resembles the spectrum of the α helix, with smaller magnitudes, while the type II turns yielded a class B spectrum with a maximum below 200 nm and a minimum above 220 nm (Hollósi et al., 1987a, b; Perczel et al., 1991a, 1992a; Perczel and Fasman, 1992).

However, the CD spectrum of the β turn derived from 18 protein CD spectra by Chang et al. (1978) was a mirror image of the CD spectrum for an α helix.

The CD spectra of membrane proteins often exhibit various degrees of distortions in shapes, intensities and/or positions of the CD bands, and shifts in cross-over points (Urry and Long, 1980). Such distortions are recognized as the optical artifacts of differential light scattering and differential absorption flattening (Duysens, 1956; Gordon and Holzwarth, 1971; Urry and Long, 1980; Mao and Wallace, 1984; Glaeser and Jap, 1985). The extent of the flattening is a function of the size of the particles and the concentration of the chromophores within a particle (Mao and Wallace, 1984).

The differential light scattering and differential absorption flattening shown in CD spectroscopy (Duysens, 1956; Gordon and Holzwarth, 1971; Urry and Long, 1980; Mao and Wallace, 1984; Glaeser and Jap, 1985) increase the uncertainties of the measured CD spectrum for membrane proteins. However, to avoid the uncertainties arising from the light scattering and absorption flattening, the circular dichroism spectra of the membrane proteins reported herein were measured in detergent-solubilized forms, with a single exception of the purple