Analysis of Cotton-Effect Distribution of Optical Rotatory Dispersion for Polypeptides in Solution

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Summary. From analysis of optical rotatory dispersion in ultra-violet and visible regions (not in far ultra-violet region), true net rotational strength, symmetrical center and apparent width of circular dichroism band were obtained for helical, random and intermediate polypeptides in aqueous solutions or organic solvents. For helical and random poly-L-glutamic acid in aqueous solutions, these characteristic values agree pretty well with the theoretical results calculated from circular dichroism measurements in far ultra-violet region. Helical polypeptides in non-aromatic organic solvents seem to have the similar distribution of Cotton-effects with those for poly-L-glutamic acid, however for helical polypeptides in aromatic solvents, the broader distribution and the red shift of symmetrical center were expected. Also poly-L-alanine in dichloroacetic acid was suggested to have intermediate form by the present analysis.

Introduction

During the past decade, optical rotatory dispersion (ORD) has become a useful method for characterizing the conformation of proteins and polypeptides. The general expression for ORD in regions distant from the optically active absorption bands is [10]:

\[ [m'] = \sum_{i} \frac{a_i \lambda_i^2}{\lambda^2 - \lambda_i^2} \]  

where \([m']\) is the reduced mean residue rotation, \(\lambda_i\) the wavelength of the i-th optically active absorption band, and \(a_i\) a parameter proportional to the rotational strength \(R_i\) of the band. Visible ORD is of course influenced by the Cotton-effects of the optically active absorption band, which are usually located in the ultraviolet region.

In 1956, Moffitt [5] proposed that the amide absorption bands of helical polypeptides are split into two components, and then to illustrate visible ORD, only two Drude-terms were used:

\[ [m'] = \frac{a_1 \lambda_1^2}{\lambda^2 - \lambda_1^2} + \frac{a_2 \lambda_2^2}{\lambda^2 - \lambda_2^2} \]  

Later, many authors used the various types of two-term Drude equation. Schechter and Blout [7] have recently proposed a four-term Drude equation, two for the
helices and other two for the random coiled form, however it has been modified into
a two-term Drude equation, in the case of use.

On the other hand, recent circular dichroism (CD) study reveals two over-
lapping negative dichroic band at 209 m\(\mu\) and 222 m\(\mu\) and one positive band at
191 m\(\mu\) for the helical polypeptides [4]. Therefore the visible ORD of helical
polypeptides should be described:

\[
[m'] = \frac{a_{191} \lambda_{191}^2}{\lambda^2 - \lambda_{191}^2} + \frac{a_{209} \lambda_{209}^2}{\lambda^2 - \lambda_{209}^2} + \frac{a_{222} \lambda_{222}^2}{\lambda^2 - \lambda_{222}^2} + \sum_i \frac{a_i \lambda_i^2}{\lambda^2 - \lambda_i^2}.
\] (3)

Here the last term summarized all the background rotations arising from the
Cotton-effects beyond the currently measurable range of wavelength.

Eqs. (2) and (3) can be transformed, by use of Taylor’s series expansion, into
the well known Moffitt-Yang equation [6]:

\[
[m'] = \frac{a_0 \lambda_0^2}{\lambda^2 - \lambda_0^2} + \frac{b_0 \lambda_0^4}{(\lambda^2 - \lambda_0^2)^2}.
\] (4)

Because \(\lambda_0 = 212\) m\(\mu\) is widely accepted, Moffitt-Yang equation uses only two
parameters \(a_0\) and \(b_0\). And the \(b_0\) seems to best meet the requirement for estimation
of helical contents in proteins and polypeptides with random coiled parts. Although
the true distribution of Cotton-effects cannot be estimated only from the value of
\(a_0\) and \(b_0\), Eq. (4) is very simple, but very useful empirical expression to investigate
the conformations of proteins and polypeptides in solutions.

However, the true net rotational strength, the true position of center of CD
bands, and the distribution width of bands have to be analysed in some cases from
visible ORD-data. For instance, CD and ORD Cotton-effect measurements can not
be achieved for poly-\(\gamma\)-benzyl-L-glutamate and other polypeptides in aromatic
solvents, although they have the helical \(b_0\) values in such solvents. And distribu-
tions of effective Cotton-effects seem to be different, probably to some extent,
from those for such polypeptides in helix-forming non-aromatic solvents [8].

In the present study, the general formulae for ORD in regions distant from
the optically active absorption bands are discussed considering the distribution of
the effective Cotton-effects, and estimations of net rotational strengths, centers
and distribution widths of Cotton-effects are achieved for the helical polypeptides
in aromatic solvents and so on, of which CD and Cotton-effects of ORD have not
yet been observed.

Theoreticals

The general expression for ORD in regions distant from the effective optically
active absorption bands is Eq. (1), which can be transformed into the following
Eq. (5) by Taylor’s series expansion:

\[
[m'] = \sum_i a_i \left( \frac{\lambda_i}{\lambda} \right)^2 + \left( \frac{\lambda_i}{\lambda} \right)^4 + \left( \frac{\lambda_i}{\lambda} \right)^6 + \ldots
\] (5)

Introducing the wavelength \(\lambda_0\) in Cotton-effect region, such as \(\lambda_i = \lambda_0 + \delta_i\)
\((\delta_i/\lambda_0 \ll 1)\), so \([m']\) can be expressed in power series of \(\delta_i/\lambda_0\):

\[
[m'] = A_0 \frac{\lambda_0^2}{\lambda^2 - \lambda_0^2} + A_1 \frac{2 \lambda^2 \lambda_0^2}{(\lambda^2 - \lambda_0^2)^2} + A_2 \frac{\lambda^2 \lambda_0^4 (\lambda^2 + 3 \lambda_0^2)}{(\lambda^2 - \lambda_0^2)^3} + \ldots
\]

\[
A_0 = \sum_i a_i \quad A_1 = \sum_i \frac{a_i \delta_i}{\lambda_0} \quad A_2 = \sum_i \frac{a_i \delta_i^2}{\lambda_0^2}.
\] (6)