Optical Rotatory Dispersion of Tyrosine Derivatives

Influence on Estimation of Ordered Structures in Proteins

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The study of the optical rotatory dispersion (ORD) of a series of tyrosine derivatives, some of which possessed a structure resembling that of a covalent, intermediate enzyme-substrate compound, revealed that the presence of an ester linkage, such as the one in an acylenzyme, can give rise to a significant, systematic error in the estimate of the contents of ordered structures, *e.g.* α-helices, in the protein. As esters of L-tyrosine specifically are esterified to the active site of several enzymes, such as chymotrypsin, papain and pepsin, the contribution of the ester bond chromophore to the total ORD-changes accompanying formation of intermediate enzyme-substrate complexes and compounds was estimated to ensure a correct interpretation regarding possible conformational changes in the protein.

The optical rotatory dispersion (ORD) has often been used for structural investigations of proteins because this property, with certain reservations,[1-8] permits the identification and estimation of the contents of ordered structures, *e.g.* α-helices. This analysis is complicated by the contribution to the total, optical activity of tyrosine and tryptophane sidechains, of disulfide linkages, and of electronic interactions between peptide bonds in non-helical regions.

One of the applications of ORD was an attempt to detect conformational changes in enzymes accompanying the formation of a covalent enzyme-substrate compound (acylenzyme). The authors concluded that their data indicated the occurrence of such an isomerization and this has later been confirmed by X-ray diffraction studies. The detection of such a conformational change led to the discovery of a labile α-ammonium-carboxylate ionic linkage which controls as well the integrity, and hence the catalytic power, of the active site of chymotrypsin [6,8,10] as the Bohr effect (pH-dependent O_2 affinity) of hemoglobin.[12] The isomerization was due to the motion of the carboxyl sidechain following cleavage of the ionic linkage by deprotonation of the NH_3^+-group. The search for other examples of the operation of such a *"vital-catch"*-regulation requires an analysis of possible alternative causes of ORD-

changes which could lead to erroneous conclusions. One possibility is the optical activity of an ester or an amide bond chromophore.

As the acylenzyme from chymotrypsin and N-acetyl-L-tyrosine ethyl ester (ATEE) is a serine ester of the latter, this specific substrate may be regarded as a model of an acylenzyme. In this work, an estimate of the contribution of the tyrosine ester linkage to the change in ORD-parameters accompanying chymotryptic catalysis is made.

Such data would be necessary for the application of ORD to similar studies of the many other enzymes with serine in the active site. Besides, a comparison of the ORD of related compounds possessing optically active chromophores has general interest.

EXPERIMENTAL

The ORD was recorded at 23° on an automatic spectropolarimeter (FICA Spectropol 1) using a 1 mm quartz cuvette and nitrogen atmosphere. The ORD of the solvent was subtracted from that of the sample solution. Corrections of the ORD for the dispersion of the refractive index were made. The values for water were used.[3]

ATEE and ATA were purchased from Koch & Light, England (AR-grade) AT was prepared by hydrolysis of ATEE or ATA in a pH-stat (Radiometer). T was a Sigma product (Lot. No. 15b — 2210).

RESULTS

The ORD-curves of N-acetyl-L-tyrosine (AT), its ethyl ester (ATEE) and amide (ATA) and of L-tyrosine (T), which are seen in Figs. 1 and 2, show that esterification of the carboxyl group markedly increases the numerical value of the optical rotation (of the presumed, positive Cotton effect) at about 220 nm, whereas formation of the simple amide does not have this effect.

The strong departure from linearity of the Moffitt plots of ATEE, ATA, and AT near the wavelength range of the anomalous dispersion (see Fig. 3) shows that the latter (presumably a Cotton effect) has a significant influence
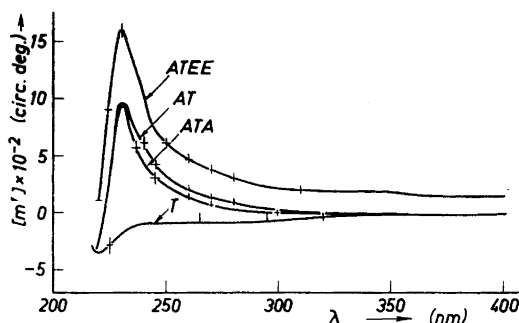

Fig. 1. The optical rotatory dispersion curves of ATEE, ATA, AT, and T in 28 % v/v aqueous methanol adjusted to pH 8.00 with base. Room temperature (23°). Light path: 1 mm. Band width: 0.2 nm. Scanning speed 20 nm/min.
ATEE: N-acetyl-L-tyrosine ethyl ester. ATA: N-acetyl-L-tyrosine amide. AT: N-acetyl-L-tyrosine. T: L-tyrosine. The standard deviation from repeated measurements (3 — 5) is indicated by vertical and horizontal lines.
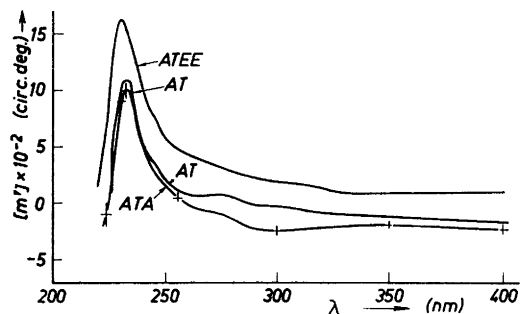
Fig 2. The optical rotatory dispersion curves of ATEE ATA and AT in 0.004 M tris-buffer at pH 8.00 in 28 % v/v aqueous methanol. Conditions as in Fig. 1. Tris: Tris-hydroxymethyl-amino-methane.

on the ORD parameter b_0 and that esterification of the carboxyl group considerably enhances this contribution. As the apparent helical content often is estimated from the b_0-value of the Cotton effect with minimum at 233 nm arising from peptide-peptide interaction, the ester contribution to b_0 represents a systematic error in this estimate. Since the change in apparent helical content upon acylation of the active site is taken as a measure of the isomerization, the ester contribution could also falsely be interpreted as evidence of the occurrence of a conformational change.
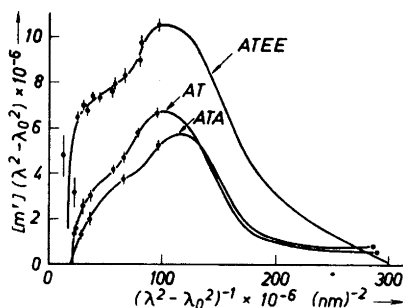

Fig. 3. Moffitt plots of ATEE, ATA, and AT in 28 % v/v aqueous methanol. $\lambda_0 =$ 212 nm. The data from Fig. 1. were used. *Curves:* Best fitting smooth curves between the points.

The apparent helical content of proteins may be estimated by a variety of methods, *e.g.*[1,2] from b_0:

$$\% \text{ helix} = -b_0/630 \qquad (1)$$

where b_0 is a parameter in the Moffitt equation, or from the reduced residue rotation, $[m']$, at 233 nm:

$$\% \text{ helix} = \frac{[m'_{233}] + 1800}{109} \qquad (2)$$

where $[m'_{233}] = [\alpha_{233}] \dfrac{MRW}{100} \dfrac{3}{n^2 + 2}$

$[\alpha_{233}]$ = specific rotation = $100\alpha_{obs}/l \times c$
l = length of cuvette in dm
c = concentration in g/100 ml
α_{obs} = observed rotation in circular degrees
MRW = mean amino acid residue weight

Eqn. (2) was derived from the residue rotation of 100 % and of 0 % (random coil) righthanded α-helix:[2]
$[m'_{233}] = -12\,700$ circ.deg. for 100 % α-helix (R)
$[m'_{233}] = -\;1\,800$ circ.deg. for 100 % random coil.
In this work, the apparent helical content was estimated from eqn. (2).

Table 1. Optical parameters of acyl-enzymes and model compounds.

Compound	Conditions	$[m'_{233}] \times 10^{-2}$ circ.deg.	Apparent % helix	Ref.
ATEE	28 % v/v aq. CH_3OH, 23°, pH 8.0	11.1 ± 0.3	26.7 ± 0.6 L	This work
ATA	»	7.3 ± 0.3	23.2 ± 0.6 »	» »
AT	»	6.3 ± 0.3	22.3 ± 0.6 »	» »
T	28 % v/v aq. CH_3OH, 23°	-1.2 ± 0.3	15.4 ± 0.5 »	» »
100 % helix		-127	100 R	2
100 % r.c.		-18	0	2
CT	pH 3.8, 12°	—	14.8 ± 0.4 R	5.8
DIP-CT	» »	—	18.7 ± 0.4 »	5.8
Ac-CT	» »	—	18.7 ± 0.4 »	8
ATEE	28 % v/v aq. CH_3OH, 23°, 4×10^{-3} M tris, pH 8.0	11.1 ± 0.3	26.7 ± 0.6 L	This work
ATA	»	6.0 ± 0.3	22.0 ± 0.6 »	» »
AT	»	6.9 ± 0.3	22.8 ± 0.6 »	» »

ATEE: *N*-Acetyl-L-tyrosine ethyl ester.
ATA: *N*-Acetyl-L-tyrosine amide.
AT: · *N*-Acetyl-L-tyrosine.
T: L-Tyrosine.
r.c.:   Random coil.
L: Lefthanded helix.
R: Righthanded helix.
Tris: Tris-hydroxymethyl aminomethane.
$[m'_{233}]$ Reduced residue rotation at 233 nm.

$$[m'_{233}] = [\alpha_{233}] \frac{MRW}{100} \times \frac{3}{n^2 + 2}$$

circ.deg.: Circular degrees.
MRW: Mean residue weight.
Ac-CT: Monoacetyl-α-chymotrypsin.
CT: α-Chymotrypsin.
DIP-CT: Diisopropylphosphoryl-α-chymotrypsin.
$[\alpha_{233}]$:   Specific rotation at 233 nm.
n: Refractive index.
$\left(\dfrac{3}{n^2+2}\right)_{233}$: 0.7643.

Table 2. Estimated error in optical parameters due to esterification of the active site of the enzyme.[a]

Compound	Error in $[m'_{233}]$ % of corr. value	Error in H'		Comments
		% of corr.value	% H	
Protein with low H'	62			Max.error
Protein with high H'	9			Min. »
DIP-CT		19	4.4	
"Acyl-fold"		47	4.3	Active serine-enzymes (some)

H': Apparent helical content: $H' = \pm([m'_{233}]+1800)/109$, where $+$ applies to lefthanded helices and $-$ applies to righthanded helices

"Acyl-fold": Change in apparent helical content due to esterification of the active serine in chymotrypsin by a substrate. (incl. conformational changes [6]).

[a] Solvent: 28 % v/v aqueous methanol, pH 8.0. Similar results were obtained with a solvent which also contained 0.004 M tris, pH 8.0. The nature of the error is discussed in the text.

Ester correction: $H' = +4.4$ % α-helix (L)/residue
Amide   » $H' = 0.9$ %   »   »
Corrected content of righthanded helix in DIP-CT: 23 %
Ester contribution: 4.4 % "α-helix" (L)
Amide   »   0.9 %   »   »
Corrected "acylfold": 8.3 %   »   (R)
L: Lefthanded.
R: Righthanded.

DISCUSSION

The existence and position of the peptide bond Cotton effect have been established by Schellman and others.[1,2,9] The structural resemblance between peptides and amides leads to the expectation that N-acetyl compounds and esters also have Cotton effects in approximately the same wavelength range. The origin of these Cotton effects is probably an $n-\pi^*$ transition of an electron from the O-atom in the $>C=O$ chromophore.[11] In the case of tyrosine derivatives, the observed anomalous rotatory dispersion may be due to a superposition of a phenolate [1,2] and a $>C=O$ Cotton effect. A comparison between the dispersion curves of T and the N-acetyl-T-derivatives shows that the N-acetyl group plays a major role in the production of the Cotton effect.

In Table 1, the apparent helical content of a number of compounds, including those discussed above, has been calculated as previously described.[1,2,8] A comparison between the values for the enzymes and those for the tyrosine derivatives indicates that the formation of the ester linkage can contribute considerably to the estimate of ordered structures, such as the α-helix. The magnitude of this systematic error has been evaluated in Table 2. Fortunately,

in the case of chymotrypsin, the true conformational change, as measured by b_0 or by the change in apparent helical content, was of sufficient magnitude and of such a direction that an erroneous conclusion was precluded, also in the absence of supporting structural evidence of other nature.[6]

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