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Chiroptical Properties of Selenocystine *

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The circular dichroism (CD) spectra of D- and L-selenocystine were investigated at pH 0.5 and 5.5, and compared with those of their sulfur analogues. At pH 5.5 selenocystine shows CD maxima at 320, 272, 232, 211 and 200 nm. At pH 0.5 the CD spectrum undergoes significant changes, and now exhibits CD maxima at 306, 241, 225 and 207 nm. Two Cotton effects (CE's) of opposite sign at 320 and 272 nm at pH 5.5 collapse at pH 0.5 into a single CE at 306 nm, close to the position of the longest wavelength absorption band in the UV spectrum, and this change is probably linked with an alteration in the diselenide dihedral angle. The CE at 225 nm (*cf.* 220 nm in cystine) is probably associated with the carboxyl $n\rightarrow\pi^*$ transition, and exhibits the correct sign for the absolute configuration of this chiral center. The bands at 320, 272, 232 and 211 nm are therefore considered to be due to optically active transitions of the Se–Se chromophore.

Selenoamino acids first attracted interest in the 1930's when it was found that a cattle disease known as "alkali disease" was due to chronic selenium poisoning. Selenocystine, first synthesized by Fredga,[1–3] plays an important role in selenium-accumulating plants[4] and has been found to have interesting pharmacological effects.[5]

Although a CD study of some selenoamino acids containing one Se atom has appeared,[6] chiroptical data on compounds containing the Se–Se chromophore are scarce. Djerassi *et al.* measured the ORD of L-selenocystine and some other diselenides above 280 nm[7] and later the CD of a cyclic diselenide.[8] On the other hand, the optical rotatory properties of the closely related disulfide group have received considerable interest in recent years,[9] especially because of the importance of the group in molecules of biological interest. The optical activity associated with cystine and cystine residues in proteins is of special interest since it can be used as a probe for studying structural changes in the protein environment about the disulfide groups.[10] However, the difficulties in separating the chiroptical contributions made by aromatic side chains from those made by the disulfide group seriously limit the utility of such studies. Urry *et al.*[11] examined the compound in which the S–S bridge of cystine in the hormonal peptide deaminooxytocin had been replaced[12] with an Se–Se group but the compound still contained a tyrosine residue. The absorption spectra of diselenides are very similar to those of the corresponding disulfides except for a red shift of the characteristic absorption peaks[13–15] and such a replacement therefore should lead to a better band separation in the CD spectrum. However, since the chiroptical behaviour of the Se–Se group even in small molecules is not well understood the correct assignment of the CD bands in peptides containing this chromophore is difficult. This prompted us to investigate the CD spectrum of L-selenocystine (3,3'-diselenobis-L-alanine) *1* as the most approproate diselenide of biological significance and which contains the carboxyl group as the only additional chromophore.

In 0.3 N HCl, where the ionizable groups are expected to be completely protonated,[16] *1* exhibits a CD spectrum (Fig. 1) with apparent maxima at 306, 241, 225 and 207 nm designated as bands A, C, D and E, respectively, (Table 1). The unpolarized UV spectrum shows only one maximum at about 300 nm with strong end absorption below 220 nm. In aqueous solution (pH 5.5–6.0) where *1* exists mainly in its double zwitterionic form, band A undergoes an increase in intensity and a new band (B) appears

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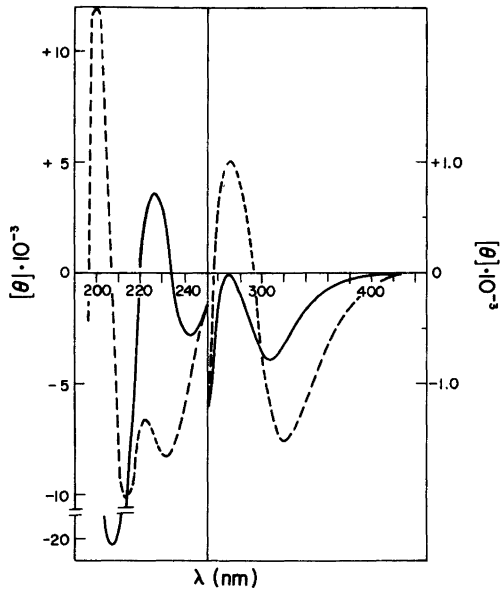

Fig. 1. Circular dichroism of L-selenocystine in water at pH 0.5 (———) and 5.5 (– – –).

around 272 nm. Band C undergoes a blue shift with a concomitant increase in intensity presumably as a result of a decrease of band D. On the other hand band E undergoes an apparent red shift and loses intensity on going from HCl to water as solvent. This effect can be ascribed to band overlap from an oppositely signed strong band (F) around 200 nm.

Band D most probably has its origin in the carboxyl $n \rightarrow \pi^*$ transition. As found for all other L-amino acids [17,18] the band is positive and ionization of the carboxyl group decreases the intensity. The red shift of the CD band relative to its position in other amino acids may result from electronic interactions between the carboxyl $n \rightarrow \pi^*$ transition and selenium transitions of similar energy or more probably from band overlap and resulting mutual cancellation with the intense negative band E. It should be noted that L-cystine also gives a positive Cotton effect, albeit of higher intensity, in the 220 nm region [19,20] assigned to the carboxyl $n \rightarrow \pi^*$ transition. The only chromophore in *1*, apart from the carboxyl group, having electronic transitions in the spectral region above 200 nm is the Se–Se group. Therefore we consider bands A, B, C and E to have their principal origin in transitions within the Se–Se chromophore. In cystine the transitions due to the S–S chromophore give a broad negative CD band in the 250 nm region and a strong negative band around 190 nm.[18–20]

Table 1. Circular dichroism of L-selenocystine.[a]

Band	λ_{max}, nm ([θ]) 0.3 N HCl	H_2O
A	306 (−783)	320 (−1524)
B		272 (+1050)
C	241 (−2920)	232 (−8358)
D	225 (+3630)	
E	207 (−20280)	211 (−10290)
F		200 (+11940)

[a] D-Selenocystine gave enantiomeric CD data agreeing within ±5%.

Diselenides like disulfides [21] are inherently chiral and can exist in two helical conformations. In the absence of additional chiral centers the two conformers are present in equal amounts and no optical activity can be detected.[21] In molecules possessing a C–Se–Se–C group in the vicinity of one or more chiral centers, an unequal distribution of the M (left-handed helical screw sense) or P (right-handed helical screw sense) [22] chiral C–Se–Se–C groups may exist.[21] This will be the case if the diselenide moiety is incorporated into a ring system when strong intrinsic Cotton effects are observed [8,23] due to the restricted rotation around the Se–Se bond. However, in acyclic compounds as in cystine [24] the two helical conformations should be present in equilibrium without a large excess of either and the rotatory contribution due to inherent dissymmetry within the diselenide group is smaller. In this case, vicinal contributions from the chiral centers outside the Se–Se moiety must also be taken into account when interpreting observed optical activity.

The dihedral angle (ϕ) between the two planes formed by the two selenium atoms with each of the adjacent atoms of an aliphatic strain-free diselenide was predicted [25] to be about 90° and this was confirmed by experiment.[26] Deviations from this value should be small due to the size of the selenium atoms, which makes severe interactions between substituents less probable because of increased distances between them.[14]

Two angle-dependent transitions are believed to be responsible for the 250 nm absorption band of disulfides. According to the Bergson-Linderberg-Michl disulfide theory [27,28] the two transitions become degenerate for dihedral angles $\phi = \pm 90°$, but

have different energies for $\phi \neq \pm 90°$. Theoretically, for chiral disulfides with $\phi \neq 90$, 180 and 0° two Cotton effects of opposite sign, positioned symmetrically around 250 nm and (in the absence of other major perturbations) of equal magnitude should result[28] and have actually been observed both for $\phi < 90°$[29] and $\phi > 90°$.[30] In compounds with $\phi = \pm 90°$ degeneracy would superpose the two Cotton effects and virtually abolish optical activity due to inherent disulfide chirality.[28] These considerations on chiral disulfides may be extrapolated to chiral diselenides, which can be expected to show analogous behaviour.[14,31] (*R,R*)-1,2-Diselenane-3,6-dicarboxylic acid with a dihedral angle of 56°[14] indeed shows two Cotton effects of opposite sign centered around 310 nm,[8,23] the wavelength of the first UV absorption maximum of a diselenide with $\phi \simeq \pm 90°$.[13-15]

The CD spectrum of *1* in the zwitterionic form exhibits two Cotton effects (bands A and B) of opposite sign positioned almost symmetrically around the longest wavelength absorption band at 300 nm ($\varepsilon = 320$). This suggests a separation of the two transitions within the absorption band and hence a small deviation of the diselenide dihedral angle from the conformation of minimum local energy ($\phi \simeq \pm 90°$). In its dication form *1* displays only one CD band in the 300 nm region centered close to the UV absorption maximum at 298 nm ($\varepsilon = 290$) which suggests a smaller deviation of the diselenide dihedral angle from its normal value.

A slight broadening of the long-wavelength UV absorption band of *1* on raising the pH from 0.5 to 5.5 is in agreement with this interpretation. The long-wavelength negative CD band of cystine shows characteristics similar to those of band A of *1*. Thus in cystine the band is found at longer wavelength (252 – 257 nm) than the shoulder in the electronic absorption spectrum and undergoes a red shift with concomitant increase in intensity on going from dilute HCl to water as solvent.[20,32,33] Although no CD band corresponding to band B of *1* has ever been observed for cystine, Kahn,[34] having resolved the experimental CD of cystine in its zwitterionic form into individual bands, suggested the presence of a hidden CD band at 240 – 245 nm (possibly negative).

Whether the rotatory strength of the first absorption band in open-chain disulfides like cystine is developed primarily from inherent chirality of the disulfide chromophore or from vicinal perturbations is still a matter of debate.[24] For *1* in its dication form the rotational contribution due to inherent chirality of the Se–Se group is, according to theory,[28] expected to be small and the rotatory strength of the 306 nm Cotton effect will contain substantial contributions from the chiral centers outside the diselenide group. However, at dihedral angles deviating from the normal value, as seems to be the case for *1* in its zwitterionic form, the rotational contribution due to diselenide chirality should predominate over that due to vicinal perturbations. It may be significant that the CD spectrum of *1* in water (pH 5.5) shows striking resemblance (both in sign and relative positions of the peaks) to the spectrum postulated by Neubert and Carmack[35] for an M chiral disulfide with a dihedral angle close to 90°. Neubert and Carmack's spectrum[35] contains contributions from six transitions leading to a negative band at ~250 nm (*cf.* band A, Table 1), a positive at ~225 nm (*cf.* band B), at least two negative bands at 210 – 190 nm (*cf.* bands C and E) and a positive band below 190 nm (*cf.* band F). These similarities suggest that *1* in its zwitterionic form prefers M chirality in solution.

EXPERIMENTAL

CD measurements were made on a Jasco J-500A spectropolarimeter or a Roussel-Jouan Dichrograph II at 20 °C and at concentrations of 0.03 – 0.3 %. Three separate CD curves were obtained for each enantiomer and were averaged.

L-*Selenocystine*, obtained from optically pure L-Se-benzyl-*N*-acetylselenocysteine[36] [*anilide*, m.p. 169 – 171 °C, $[\alpha]_D^{20}$ –2.9° (*c.* 1.07, CH_3COOH)] by hydrolysis to L-Se-benzylselenocysteine,[37] $[\alpha]_D^{20}$ +39.4° (*c.* 1.04, 1 N NaOH), reductive debenzylation,[36] and air oxidation to the diselenide,[38] had $[\alpha]_D^{25}$ –184° (*c.* 0.575, 0.1 N HCl). Lit.[3] $[\alpha]_D^{25}$ –183.3° (*c.* 1.02, 0.1 N HCl).

D-*Selenocystine*, obtained simiarly from D-Se-benzyl-*N*-acetylselenocysteine,[36] m.p. 150 – 151°, $[\alpha]_D^{20}$ 18.8° (*c.* 1.00, CH_3COOH), by hydrolysis to D-Se-benzylselenocysteine,[37] $[\alpha]_D^{20}$ –39.2° (*c.* 1.00, 1 N NaOH), reductive debenzylation,[36] and air oxidation to the diselenide,[38] had $[\alpha]_D^{20}$ +169° (*c.* 0.5, 0.1 N HCl). Lit.[3] $[\alpha]_D^{25}$ +170.0° (*c.* 2.03, 0.5 N HCl).

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