The 2-Nitrophenylsulphenyl Chromophoric Derivative of the α-Amino-group as a Circular Dichroism Probe for the β-Structure in Oligo-tyrosine Peptides

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A comparison of circular dichroism (c.d.) spectra has revealed that the randomly coiled lower oligomers of the Nps[L-Tyr(Bzl)]$_n$Gly-OPEG-M [n = 3—8, Nps = 2-nitrophenylsulphenyl, Bzl = $PhCH_2$, OPEG-M = poly(ethylene glycol monomethyl ether)] peptide series have a positive Cotton effect at 350—400 nm, associated with the 2-nitrophenylsulphenylamino-chromophore, while the β-forming higher oligomers have a negative Cotton effect in that region of their spectra; this suggests the use of the Nps group at the *N*-terminus as a c.d. probe for β-structure in oligo-Tyr-peptides.

In the last 20 years the circular dichroism (c.d.) technique has been widely employed in investigating the conformations adopted by peptides in solution. However, most peptides contain cystine and/or aromatic amino-acid residues. The contribution of disulphide and aromatic chromophores to the c.d. spectra below 250 nm, where the peptide chromophores absorb, is poorly understood.[1] Therefore, corrections to the spectra in order to obtain the backbone contributions cannot be made with confidence. In addition, information on the conformational preferences of peptides, which can be extracted from the Cotton effects in the 250—300 nm region, is also extremely limited, mainly due to the fact that in general more than one Cys (disulphide), Trp, Tyr, or Phe residue occur together in the same sequence.

The above considerations demonstrate the usefulness of chromophoric derivatives, absorbing above 300 nm,[2] as c.d. probes for the ordered structures of peptides. It is noteworthy that this approach would also allow a c.d. analysis of the conformational properties of peptides to be carried out not only in the solvents generally employed (water, alcohols, and halogenated alcohols) but also in the solvents used in peptide synthesis (halogenated hydrocarbons, cyclic ethers, amides, and sulphoxides), which are not transparent in the region of absorption of peptide chromophores.

In this communication we describe the application of a chromophoric derivative as a c.d. probe for β-structure formation in oligo-Tyr-peptides. For this study we took advantage of a project on synthetic (-L-Tyr-)$_n$ oligopeptide carriers prepared *via* protection of the α-amino group with the 2-nitrophenylsulphenyl (Nps) moiety.[3] Specifically, the series examined was Nps[L-Tyr(Bzl)]$_n$Gly-O-C(Me)$_2$-CH_2-OPEG-M [where n = 3—8, Bzl = $PhCH_2$, and OPEG-M = poly(ethylene glycol monomethyl ether)].

The i.r. absorption technique was used to obtain *independent* spectroscopic information on the conformations[4] adopted by the [L-Tyr(Bzl)]$_n$ peptides. In the three solvents tested (methylene chloride, dioxan, and 2,2,2-trifluoroethanol) the lower oligomers (n = 3,4) exist invariably in a disordered conformation, while the higher oligomers (n = 7,8) adopt a well developed β-structure.[3] The peptides of intermediate size (n = 5,6) only partially assume the ordered structure, the amount of which is solvent-dependent. Most importantly, the effect of the Nps group on the conformational preferences of the [L-Tyr(Bzl)]$_n$ peptides was shown to be negligible.

The c.d. spectra of the Nps-protected [L-Tyr(Bzl)]$_n$ peptides in methylene chloride above 300 nm are shown in Figure 1. A dramatic variation is seen upon increasing the chain length. The sign of the c.d. maximum at 370—390 nm changes from *positive* (for the lower oligomers) to *negative* (for higher oligomers). A qualitatively analogous phenomenon is visible in the spectra recorded in 2,2,2-trifluoroethanol and dioxan. A comparison of the i.r. absorption results with these findings strongly supports the view that the Nps group, when present at the α-amino-group, could represent a useful c.d. probe for

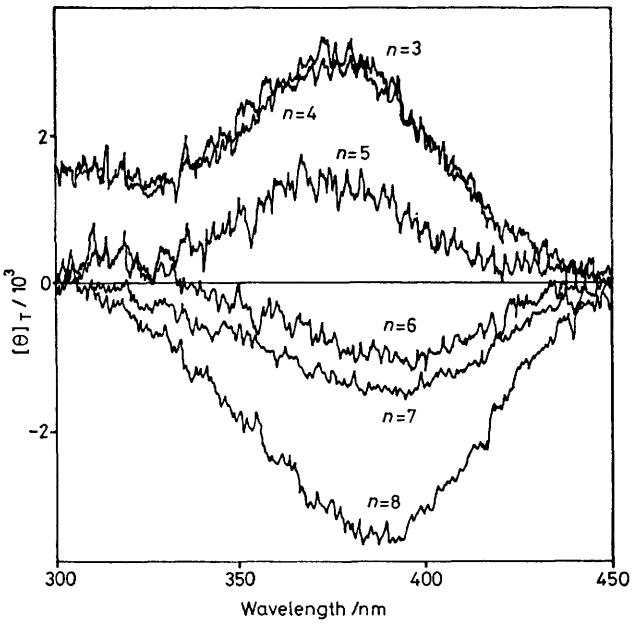

Figure 1. Original c.d. spectra (total ellipticity *vs.* wavelength) between 300—450 nm of the Nps[L-Tyr(Bzl)]$_n$Gly-O-C(Me)$_2$-CH$_2$-OPEG-M (n = 3—8) peptides in methylene chloride solution (concentration 2 mg/ml). The instrument used was a Cary model 61 dichrometer equipped with a Jasco model DP-501 N data processor. The temperature at which the spectra were recorded was 20 °C.

detecting the onset of the β-structure in Tyr-containing peptides. If the c.d. curves of the 'borderline' peptides (n = 5,6) are compared in the three solvents, it is found that the most extensive β-structure formation is promoted by 2,2,2-trifluoroethanol, while the least extensive is seen in dioxan.

Interestingly, in *N*,*N*-dimethylformamide, a solvent that competes effectively with the peptide–peptide N–H . . . O=C hydrogen bonds characterizing a β-structure,[5] the c.d. maximum above 300 nm is positive for all the peptides. Hence, this finding indirectly supports our interpretation that the sign inversion of the c.d. band of the nitroaromatic chromophore shown in Figure 1 is due to β-structure formation.

There are several reports in the literature indicating that the 2-nitrophenylsulphenylamino-chromophore exhibits more than one optically active band above 300 nm.[2,6,7] However, a complete understanding of the transitions characterizing the nitroaromatic chromophores in that spectral region has not yet been achieved.[8,9] Therefore, only a tentative explanation of the phenomenon illustrated in Figure 1 can be proposed. It is conceivable that the onset of the ordered association of the peptide chains in the β-structure would favour an interaction of the 2-nitrophenylsulphenylamino-chromophores, which in turn, would be responsible for the observed inversion of the sign of the Cotton effect at 370—390 nm.

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