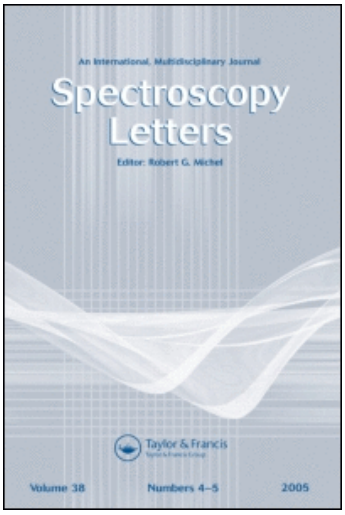

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Model Studies of the Cotton Effects of Derivatives Containing β-Homophenylalanine

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MODEL STUDIES OF THE COTTON EFFECTS OF DERIVATIVES CONTAINING β-HOMOPHENYLALANINE

Key words: β-homoamino acids, circular dichroism, peptides

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Abstract

The CD spectra of simple derivatives, di- and tripeptides containing phenylalanine and β-homophenylalanine (β-Hph)** at their C-terminals were studied. The reverse sign contribution of the aromatic ^1L_a transition of β-homophenylalanine in comparison of phenylalanine was found. The implication of this hitherto undescribed fact is discussed in relation to a diagnostic value of the CD measurements in spectral region 200-240 nm.

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** The three letter symbol accepted for homoamino acids (1) was used; β-denotes the position of an amino group.

INTRODUCTION

The CD method is considered to be very convenient in application to the conformational analysis of peptides and proteins in solution (2-5). The main source of information is the region of the spectrum between 200 and 230 nm covering the forbidden n-π^* peptide, the weakly allowed ^1L_a phenylalanine, tyrosine and tryptophane transitions. Owing to the symmetry properties of the transition dipole moments of ^1L_a band of tyrosine and phenylalanine (the sign of the rotational strength is independent of χ_2) the signs of the Cotton effect in the model compounds have been calculated (6). The tendency for the ^1L_a band rotational strength to give a positive Cotton effect over the energetically allowed backbone conformations was found (7-9).

The extensive experimental studies on the structure of the highly ordered proteins have shown the domination of the coupled n-π^* peptide over the aromatic transitions which result in strong, negative Cotton effect in the region of 200-230 nm (5). As opposed to proteins the molecules of the short peptides i.e. di- and tripeptides are considered to be characterized by a high degree of conformational freedom. In the solutions they are represented as an assembly of energetically allowed conformations being in the state of dynamic equilibrium. This specific situation implicates weakness of the mutual interaction of the peptide chromophores which, in turn, results in the reduction of the rotational strength of the forbidden n-π^* peptide transition and domination of the aromatic contribution (8-10).

The linear tetrapeptides which exhibit a tendency to create the β-turn structure, usually, show pronounced negative values of the Cotton effects in the region of 200-230 nm although the aromatic contribution is still positive (8).

Thus, in general, the negative Cotton effect appearing between 200-230 nm in the CD spectra of the oligopeptides containing natural, aromatic α-amino acids points unequivocally toward the ordered structures of the molecules and the domination of the rotational strength of the coupled peptide oscillator.

Among the analogues of the biologically active peptides studied there exists a group of compounds which include L-β--homoamino acids in their molecules i.e. in place of -NHCH(R)CO-, -NHCH(R)CH_2CO- residue is substituted (11-14). The question arises as to whether the above picture concerning the contribution of the ^1L_a aromatic transition is transformale to the interpretation of the CD spectra of the peptides containing β-homoamino acids. The present work will partially answer the question.

MATERIALS AND METHODS

N-protected amino acid, di- and tripeptides were synthesized in our laboratory (15). t-Butyloxycarbonyl group as a transient protection was used. Benzyl esters were used as the C-terminal protection. We also chose the benzyl group as the side chain protection of imidazole ring of histidine. The peptides were synthesized by classical method, in solution using the DCCI/HOBT system. The final deprotection was

performed by acidolysis reaction with 4N HCl/dioxane followed by catalytic hydrogenation. Some difficulties in the removing of N^{im}-benzyl group were noticed; this process has to be carefully investigated by the tlc method. After purification by means of column chromatography the chemical homogeneity of compounds was checked by mass spectrometry, amino acids ratio in the acid hydrolysates and tlc method.

CD spectra were recorded in TFE on Jasco J-20 spectropolarimeter. The results were expressed in molar ellipticity [deg $cm^2 dmol^{-1}$].

RESULTS

In this paper we present chiroptical properties of the N-protected amino acids, and peptides (1-10) with the C--terminal phenylalanine or β-homophenylalanine, in the region of 200-230 nm. The CD spectra were recorded in TFE solution (Fig.1,A,B,C) as this solvent was most frequently used in the conformational studies of short peptides, known as a supporting pseudocyclic structures such as γ- or β-turns (16).

For reason known the CD spectra of the compounds studied should demonstrate domination of aromatic ^1L_a over $n-\pi^*$ peptide transition or, at least, equal contribution of both to the overall Cotton effect. We were therefore able to observe directly the differences in the sign of the Cotton effect of the ^1L_a band of the peptides with phenylalanine or β-homophenylalanine incorporated.

ZβHph, 1; ZPhe, 2; BocβHph, 3; BocPhe, 4. Both pairs of protected amino acids demonstrate opposite signs of the Catton effect (Fig.1A) in the range of 214-216 nm; positive in the case of Phe-compounds and negative if β-Hph is incorporated into the molecule. Owing to a symmetry forbidden $n-\pi^*$ urethane transition, the aromatic ^1L_a transition of the side chains and the protected function would mainly participate in the overall Cotton effect in this region of the spectra.

AlaPhe, 5; AlaβHph, 6. The Cotton effects of these two peptides show opposite signs in the 200-230 nm range (Fig.1B). It is positive for AlaPhe and negative in the case of AlaβHph. The position of the extrema of both effects are specific to aromatic transition (17).

GlyAlaPhe, 7; GlyAlaβHph, 8. The CD spectrum GlyAlaPhe shows two comparable, Cotton effects of the opposite signs (Fig.1B). There is minimum at 220 nm and maximum at 210 nm and the curve intersects the zero-ellipticity around 218 nm. The CD pattern of the second tripeptide GlyAlaβHph, resembles the AlaβHph spectrum at first. The minimum appears at 215 nm and characterizes the position of the aromatic band but is much deeper as compared with the dipeptide. The shape of curve shows distinct asymmetry; it raises very sharply in the shorter wavelength part of the spectrum and broadens on the side of the longer wavelength.

ProβHph, 9; HisProβHph, 10. These two peptides are analogues of the C-terminal di- and tripeptides of angiotensin II. The

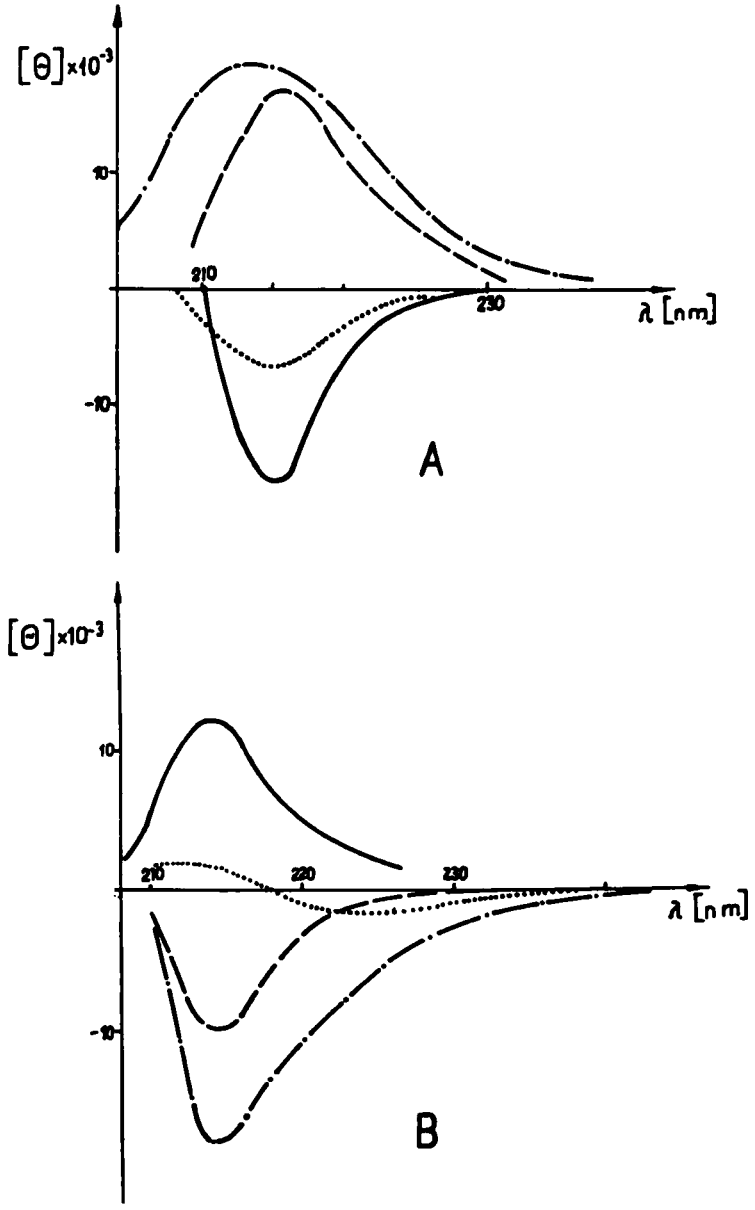

Fig.1. CD spectra of A: BocβHph, 3, ...; ZβHph, 1, ——; ZPhe, 2, ----; BocPhe, 4, -·-·-·-·-; B: AlaPhe, 5, ——; AlaβHph, 6, ----; GlyAlaPhe, 7, ...; GlyAlaβHph, 8, -·-·-·-·-; C: ProβHph, 9, ——, HisProβHph, ----; measured in TFE.

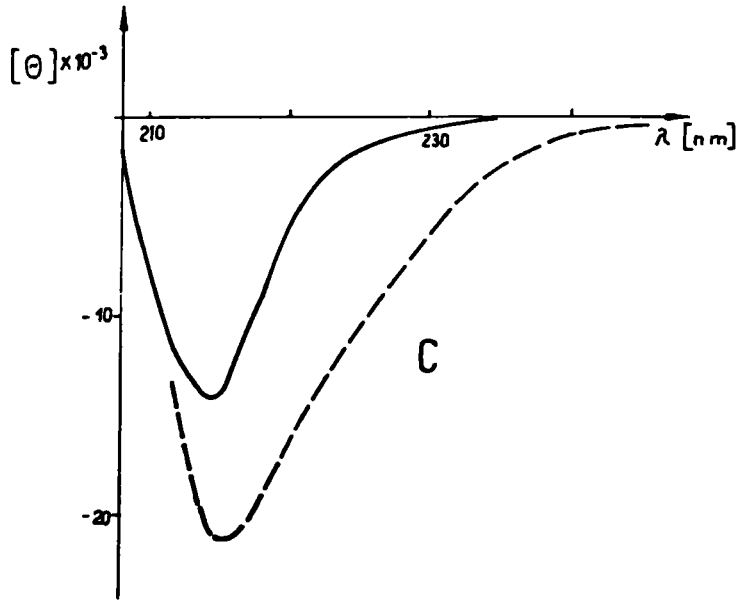

Fig. 1 continued

published CD spectra of Pro-Phe and HisProPhe (18) do not show any deviations from the rule; the ellipticity of ^1L_a bands of Phe are positive and dominate this spectral region. Substitution of βHph in place of Phe in both compounds changes the CD picture entirely; ProβHph as well as HisProβHph show negative Cotton effects between 200 and 230 nm (Fig.1C). The dipeptide is characterized by a narrow, symmetrical band centered around 215 nm. The position of the tripeptide CD minimum appears at the same wavelength as the dipeptide, but there is a visible lack of symmetry. The band

shows a steeper feature at its shorter wavelength and, as was observed in the case of GlyAlaβHph, quite pronounced Cotton effect is still observable above 220 nm.

DISCUSSION

Undertaking the comparative CD studies of the model compounds containing phenylalanine and β-homophenylalanine of this same chirality, we started from the simplest examples such as the urethane protected amino acids, di- and tripeptides. Both aromatic amino acids were first, placed in the C-terminal position of the di- and tripeptides. The high degree of conformational freedom and basically lack of any significant intramolecular amide-amide interactions results in the domination of the ^1L_a aromatic transition over the strongly forbidden $n-\pi^*$ peptide transition in the region of 200-230 nm. This enables direct observation of the differences in the Cotton effects exerted by a phenyl ring of phenylalanine and β-homophenylalanine.

All the compounds differ in the sign of the molecular ellipticity in the waverange under consideration, depending upon whether phenylalanine or β-homophenylalanine is incorporated into the molecule. Phe-compounds show, as predicted (1,19) a positive sign of the Cotton effects of the ^1L_a band, while a negative values are noticed in βHph--compounds. This is not, however, demonstrated uniformly going from the N-protected amino acids, through the di- to the tripeptides. The position of the observed, very pronounced Cotton effects within the first two types of

compounds in the range of ca. 214 nm, indicates the explicit domination of the aromatic transition.

In the case of tripeptides one can not observe such a sharp border, apparently the domination of aromatic transition is not so obvious as formerly. GlyAlaPhe CD spectrum shows the positive blue and negative red part of the Cotton effect in the 200-230 nm range. As stated in the article, the CD curve intersects zero-ellipticity at 218 nm as a result of the substraction of two opposite and comparable Cotton effects; the positive-aromatic and negative peptide transition. We suspect the creation of γ--turn in TFE to be responsible for the negative Cotton effect of the interacting peptide chromophores* (3a).

The CD spectra of GlyAlaβHph and HisProβHph show a high values of the negative molecular ellipticity of the Cotton effect located between 200 and 230 nm. The position of the minima points, undoubtedly, towards the domination of the aromatic transitions in both cases. The negative "tails" above 220 nm results from the addition of the negative Cotton effects exerted by the coupled peptide chromophores; introduction of β-homophenylalanine in the C-terminal position should not disturb the creation of γ-turn. The elevated amplitude of the negative Cotton effect of

* AlaPheGly CD spectrum, for example, does not show any minimum around 220-230 nm. Theoretically, phenylalanine placed in the central position prevents the creation of a pseudocyclic structure.

HisProβHpH as compared with GlyAlaβHph may result from an intrinsic tendency of proline placed in the central position to strengthen the γ-turn pseudocyclic structure (3b). Thus, in the cases of tripeptides containing β-homophenylalanine reinforcement rather than cancellation of the negative chiral effects take place, in contrast to a peptide with C-terminal phenylalanine.

We think that the source of differences in the observed Cotton effects exerted by the phenyl ring of both amino acids may be related to a different fractional population of χ_1-rotamers* in the peptides studied which in turn, can evoke an opposite chirality of the interacting π-π^* aromatic and n-π^* peptide transitions (8). On the other hand the substitution of CH_2COOH in place of COOH group can contribute itself to the change of the sign of the Cotton effect of an aromatic chromophore while the χ_1-rotamer population is preserved. The studies on this subject will be presented in the near feature.

A similar problem may exist in the case of the peptides built up of another aromatic β-homoamino acids. We have noticed such differences in the preliminary work on tyrosine/β-homotyrosine-peptides - this particularly concerns the ^1L_b band.

Although it is difficult to predict the chiral "perturbations" upon the introduction of an aromatic β-homo-

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* Owing to the symmetry of ^1L_a transition dipole moment this band is not sensitive to χ_2 changes.

amino acids into the higher molecular peptides instead of parent α-amino acids, the present paper should warn against overestimation of the tendency to create pseudocyclic structures judging from the sign and value of the molar ellipticity betveen 200-230 nm in the CD spectra.

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   b) His same position, p.30.

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