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Water molecules can control the side-chain rotamer distribution of an aryl peptide in a nonpolar environment

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With computer-controlled circular dichroism (CD) spectrophotometry it is possible to obtain difference CD spectra which result from small perturbations to the environment of a chiral molecule. In the experiments described here a dry iso-octane solution of cyclobis-*N*-methyl-L-phenylalanine (c-(NMe-L-Phe)$_2$) has been perturbed by exposure to water vapor. The resulting difference spectrum shows that water coordination to c-(NMe-L-Phe)$_2$ eliminates negative ellipticity in the 244 nm region, while it simultaneously creates positive CD intensity in the 212 nm region. These two features of the difference spectrum plus related features of other direct spectra imply that water coordinated with p-orbital unpaired electrons of the carbonyl interferes sterically with the $\chi = 180°$ side-chain rotamer. It can be expected that in this way hydrogen bonding of any species to backbone carbonyls can control the rotamer distribution of aromatic side-chains, if one of the rotamers occludes unpaired electrons of the carbonyl. Such control may offer an on-off switch for electron transport through proteins.

1. Introduction

Experiments using NMR spectroscopy to determine the side-chain rotamer population of model peptides containing monodeuterated β-carbons have shown that the relative rotamer populations depend on the polarity of the solvents [1]. Often the rotamer lying nearest the carbonyl decreases in population frequency as the polarity of the solvent increases [1]. For one phenylalanine model compound the decrease in extended form near the carbonyl is very marked in water [1]. There are two ways in which water can affect the rotamer population of aryl peptides. First, as a surrounding atmosphere, water will promote association of hydrophobic parts of a peptide, minimizing surfaces which cannot interact with the solvent via hydrogen bonds. Second, as a molecule coordinating with a peptide carbonyl, water might interfere sterically with rotamer occupation of a particular site near the carbonyl. The second possibility is the focus of this study. In these experiments water is introduced into a nonpolar solution of cyclobis-*N*-methyl-L-phenylalanine (c-(NMe-L-Phe)$_2$). This addition perturbs the CD spectrum sufficiently such that spectral changes due to coordination of water with the carbonyl can be identified. These changes can be attributed to alteration of the side-chain rotamer distribution.

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2. Materials and methods

c-(NMe-L-Phe)$_2$ was essentially of crystallographic purity [2]. A solution of c-(NMe-L-Phe)$_2$ (0.0011 mg/ml in spectral grade iso-octane) was introduced into the cell, and the cell plus its contents were warmed gently to achieve dryness.

The cell was immediately capped, and the cap was wrapped with parafilm. The cell with solution was then placed in the sample block and allowed to equilibrate for 75 min. A general CD spectrum was then obtained of the 200–290 nm region (fig. 1). The cell was not disturbed before taking the difference spectra 18 h later.

In preparation for obtaining the difference spectra the computerized Cary 61 CD spectrophotometer was warmed up for 2 h while the sample block was continually perfused by a Haake temperature controller. Observations about three times per h showed that the temperature remained at $24.8 \pm 0.2°$ C for the entire experiment. The spectrum of c-(NMe-L-Phe)$_2$ in dry iso-octane took about 6 h for the accumulation of 100 scans. Points were taken every 0.5 nm, and each point was a boxcar average of signals over approx. 1 s.

Water was introduced by uncapping the cell and exposing the solution to water vapor saturated air at 37° C. The cell was again capped and sealed, replaced in the still-running Cary 61, and allowed to reach equilibrium for 2 h, a time probably sufficient for near thermal equilibrium [3]. Then spectra were gathered and averaged for another 6 h. The first spectrum, stored in the computer, was subtracted from the second to give the CD difference spectrum (fig. 2).

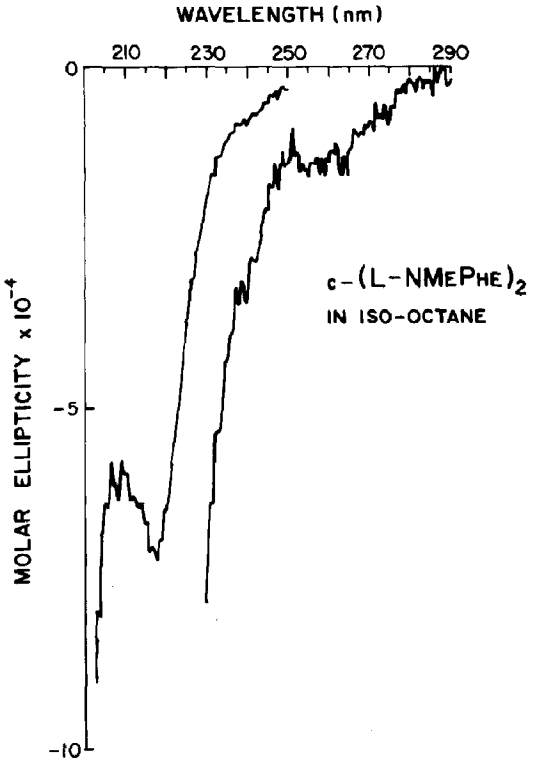

Fig. 1. Tracing of computer graph of CD spectrum of c-(NMe-L-Phe)$_2$ in dry iso-octane at 24.8° C (0.0011 mg/ml). The longer wavelength region is expanded by a factor of 5.

3. Results

The CD spectrum of the 200–280 nm region in iso-octane is completely negative (fig. 1). Addition of water vapor removes negative CD from the 244 nm region and adds positive CD to the 211–217 nm region of the spectrum (fig. 2). Other possibilities, for example, addition of positive CD to the 244 nm region and subtraction of negative CD from the 211–217 nm region, can be excluded by assignment of the 237–247 nm region to the n-π^* transition. In fig. 1 the vibronically allowed ^1L_b band weak intensity is evident from 250 to 285 nm. The ^1L_b band does not usually extend much to the blue of 250 nm in phenylalanine and is not solvent-sensitive. Therefore, the ^1L_b is not a candidate for the 237–247 nm shoulder (fig. 1) or the 244 nm difference band (fig. 2). The 237–247 nm shoulder is also not aromatic ^1L_a intensity because that transition usually takes place at 206 nm [4] and is also solvent insensitive. Likewise the peptide π-π^* is too far toward the blue (below 205 nm) to produce 237–247 nm CD [5]. The peptide n-π^*, however, appears at wavelengths longer than 225 nm in nonpolar solvent [5] and is solvent-sensitive, showing up at 212 nm in water [5]. The 237–247 nm shoulder can therefore be ascribed to the n-π^* transition and the 244 nm peak of the difference spectrum can be assigned to n-π^* intensity which disappears on coordination of water with the peptide carbonyl. This does not necessarily mean that the center of the n-π^* absorption band in nonpolar solvent is at 244 nm, a wavelength toward the red end of prior observations in nonpolar solvents [5]. Difference spectra can displace the observed peak from the actual peak center by as much as almost 50% of the

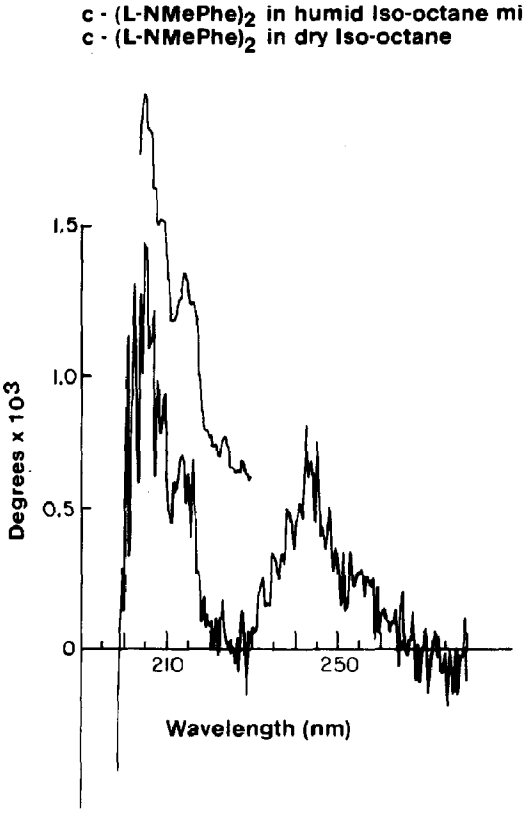

Fig. 2. Difference CD spectrum showing the results of subtracting a spectrum taken in iso-octane with moisture from a spectrum in dry iso-octane. A three-point running average of the data is displayed above the original data in order to clarify the 211–217 nm shoulder. The shoulder between 211 and 217 nm is probably of n-π^* origin, while the sharp pointed peak below 210 nm is likely to be due to the effects of small diketopiperazine angle changes on the peptide exciton or to conformer distribution effects on the B bands of the aromatic chromophore.

bandwidth [6], and therefore the actual center of the n-π^* band might be as much as 10 nm to the blue end of 244 nm in a region where the n-π^* transition has been observed.

Given the assignment of the 237–244 nm shoulder and the 244 nm difference peak to the n-π^* transition, new intensity would be expected in the 212 nm region. This is reflected in the 211–217 nm positive shoulder of the difference spectrum.

4. Discussion

The n-orbital responsible for the n-π^* transition of peptides has been successfully approximated by some version of the nonbonded p-orbital on the carbonyl oxygen [7,8]. It is the rotation of electron density from p- to π^*-orbital which gives the n-π^* transition its large CD-to-absorption ratio. Thus, it can be inferred that water molecules affect the n-π^* CD by coordinating with the nonbonded p-electrons of the carbonyl, and it is those hydrogen bonds linking water to the oxygen p-orbital which are responsible for the appearance of the 211–217 nm CD and the disappearance of 244 nm CD in water vapor exposed iso-octane (fig. 2).

Both statistical investigations of peptide hydrogen bonds in crystals [9,10] and quantum-mechanically based energy studies [11] show that the hydrogen donating O-H bond must be very nearly in the peptide plane for hydrogen bonding to the carbonyl to occur. A water molecule in one of the peptide planes of c-(NMe-L-Phe)$_2$ cannot easily approach the carbonyl from the N-methyl side. When the side-chains are pseudoaxial, the approach to the opposite side of the carbonyl is relatively unhindered irrespective of the side-chain rotamer distribution (fig. 3a). In this case, coordination of a water molecule with the p-orbital lone pair would leave the backbone conformation and rotamer distribution unaltered. The blue shift of n-π^* CD would be observed, but the 211–217 nm CD would be negative, not positive (fig. 2). Thus, the observed change in sign of n-π^* CD (fig. 2) suggests that the side-chains are pseudoequatorial and that the approaching water molecule is competing with the $\chi = 180°$ rotamer for space beside the carbonyl (fig. 3b). The possibility that water coordination is creating pseudoaxial from pseudoequatorial side-chain molecules may be discounted, because in fig. 2 there is no evidence of the strong ^1L_b exciton which appears in the pseudoaxial case (as seen in the water spectrum in fig. 4).

The area of the 212– 213 nm shoulder amounts to approx. 20% of the area of the 244 nm peak. This reduction in intensity provides additional evidence that water molecules coordinating with the peptide are altering the side-chain conformer

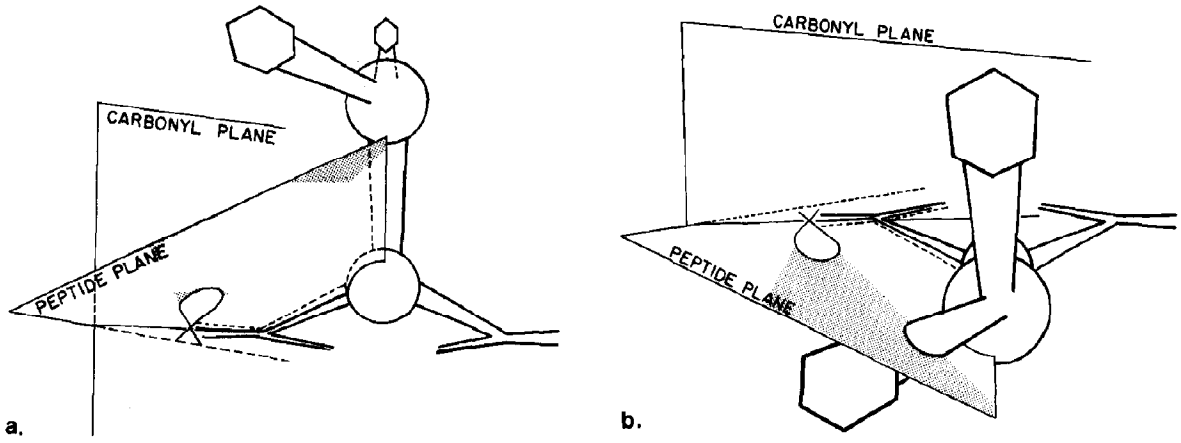

Fig. 3. Exaggerated perspective views of c-(NMe-L-Phe)$_2$. (a) Pseudoaxial side-chains, $\beta < 0$. The stippled area represents the direction of approach of a water O-H to the p-orbital which would result in hydrogen bonding that could change the CD spectrum. The phenyl side-chain is portrayed at both $\chi = 60$ and $180°$. (b) Pseudoequatorial side-chains. The stippled area again represents the direction of approach of water O-H to the carbonyl p-orbital. Interference of the $\chi = 180°$ rotamer with the water approach is clear.

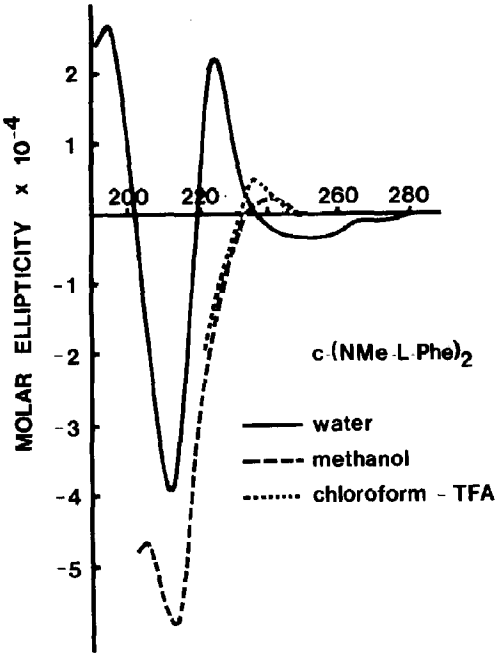

Fig. 4. CD spectra of c-(NMe-L-Phe)$_2$ in water, methanol, and chloroform/trifluoroacetic acid (9:1). The small asymmetric bands at the red end of the spectra in less polar solvents are 11 nm or more toward the red end of the positive exciton peak in water. The spectrum in methanol shows definite indication of the second negative maximum at about 200 nm [12,13]. Data reproduced with permission of the Journal of the American Chemical Society.

frequencies. Since the partially dipole allowed ^1L_a transition is that transition most nearly degenerate with the n-π^*, it would be expected to contribute heavily to the n-π^* CD through the μ-m mechanism [8]. Should the conformer frequencies be unaltered by the coordination of water with the p-orbital electrons, the new n-π^* CD at 212–213 nm would be much more intense, because the two bands are so nearly degenerate. This situation corresponds to what would be expected of pseudoaxial side-chains, since, as mentioned above, the approaching water molecule should disturb the side-chain rotamer distribution only slightly. The reduction in intensity can only be accomplished if the side-chain distribution is altered so that some of the time the side-chain is either much more distant or in a position where the ^1L_a cannot interact with the n-π^* of the carbonyl for geometric reasons.

More support for the hypothesis that hydrogen-bonding species interfere with the $\chi = 180°$ rotamer is available from spectra of c-(NMe-L-Phe)$_2$ in hydrogen-bonding nonaqueous environments [12,13]. A small positive asymmetric band appears at about 240 nm in methanol and 235 nm in chloroform/trifluoroacetic acid (9:1) (fig. 4). This band appears to be the long-wavelength tail of CD due to the n-π' of hydrogen-bonded

peptides. It is not due to a population of the diketopiperazine exhibiting a ^1L_a exciton such as that seen in water (fig. 4). In water the exciton demonstrates its positive long-wavelength peak at 215 nm, but its rotatory strength is zero at 230 nm, and the spectrum is negative at longer wavelengths. The ^1L_a in phenylalanine is relatively insensitive to solvent, particularly when the solvent change under consideration is water to methanol, and therefore there is no reason to expect the exciton to have shifted more than 15 nm toward the red. Further, in the methanol and chloroform/trifluoroacetic acid spectra (fig. 4) there is no evidence of the negative exciton lobe at shorter wavelengths. Thus, it is very likely that the small positive asymmetric bands in methanol and chloroform/trifluoroacetic acid are the tails of positive n-π^* bands which are overwhelmed by negative ^1L_a CD as the wavelength decreases. This implies that the two phenyl groups cannot reach one another. The side-chains are therefore pseudoequatorial, and the weak positive n-π^* is the normal n-π^* for c-(NMe-L-Phe)$_2$ in mildly hydrogen-bonding solvents. The fact that the n-π^* can be positive while the side-chains are pseudoequatorial does not contradict the previous analysis of aliphatic diketopiperazine spectra [14], rather it corroborates the inference that the ^1L_a is close enough in energy to the n-π^* that contributions to the n-π^* due to the rotamer distribution will generally override the backbone contributions.

The CD difference spectrum (fig. 2) and the CD spectra in hydrogen-bonding nonaqueous solvents (fig. 4) provide a consistent body of data which indicates that the diketopiperazine side-chains are pseudoequatorial ($\beta > 0$), and that hydrogen bonding alters the rotamer frequency of the aromatic side-chain of c-(NMe-L-Phe)$_2$ so that the amount of time that a phenyl group spends near the carbonyl is diminished. If aromatic side-chains are indeed involved in electron transport through proteins [15,16], the ability of carbonyl lone pair hydrogen bonding to control the rotamer distribution may effectively provide an on-off switch for electron flow.

Acknowledgements

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