Circular Dichroism of the Tyrocidines and Gramicidin S-A*

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SUMMARY

The cyclic decapeptides gramicidin S-A and tyrocidines A, B, and C provide excellent models for the testing of current notions on the circular dichroism of complex polypeptides. Experimental data utilizing both the naturally occurring peptides and, for the first time, hydrogenated derivatives, are presented. Significant contributions of the aromatic residues to the spectra are noted in the 200 to 230 nm region. Aggregation of the tyrocidines is shown markedly to affect their spectra. Finally, the results are discussed in terms of the mode of aggregation of the tyrocidines.

Much interest is currently centered upon gramicidin S-A and the tyrocidines, cyclic decapeptides of related primary structure (Fig. 1). From both energetic (1) considerations and conformational studies by nuclear magnetic resonance, it is likely that these peptides possess discrete and definable conformations (2). Furthermore, studies of the solution properties of the tyrocidines indicate that extensive aggregation occurs in aqueous solution (3-5). Thus, these peptides provide a unique set of models for understanding some of the factors responsible for the tertiary and quaternary structures of proteins. It would seem reasonable to conclude that, unless one can understand the relationship between physical measurements and conformation in the case of these relatively simple molecules, it is less likely that such can be done in the case of proteins. Certainly, this argument applies to circular dichroism. It is the purpose of this paper both to provide data for the testing of current theories relating circular dichroism to conformation and to point out some of the difficulties involved. This is particularly relevant in view of the wide use of the technique for studying conformation and recent theoretical advances (6). In the course of this investigation, data relevant to the aggregation phenomena were also obtained.

MATERIALS AND METHODS

The peptides used were purified by countercurrent distribution in a 1000-tube machine (3). Chromatography on Sephadex G-25, followed by ultraviolet absorption and fluorometric analysis, was used to assess the purity of the fractions. By proper choice of cuts from the distribution train, fractions of virtually complete purity were obtained. Since the question of the contribution of aromatic side chains to dichroic spectra is of considerable current interest, hydrogenated derivatives of all four peptides were prepared as described previously (4). Since gramicidin S-A has been shown to be relatively rigid and unaffected by changes in solvent or temperature, it is unlikely that hydrogenation affects backbone conformation (2, 7-9). After hydrogenation, no indications of the aromatic chromophors of tyrosine, phenylalanine, or tryptophan were found in either the ultraviolet absorption or the fluorescence emission spectra. Amino acid analyses showed only trace amounts of phenylalanine in the hydrogenated peptides and were used for determining their concentrations. All circular dichroism measurements were performed on a Cary model 60 instrument. Results are expressed in terms of mean residue ellipticity, i.e. one-tenth the molar ellipticity for a decapeptide, and in units of degrees cm² per decimole.

RESULTS AND DISCUSSION

At present, there is disagreement as to the number of minima in the dichroic spectrum of gramicidin S-A (7, 10). As can be seen from Fig. 2, our data clearly reveal two minima at about 207…
and 215 μm, in good agreement with the data of Quadrifoglio and Urry (7). Closer examination of data to the contrary (10) indicates that two minima may well be present. Aromatic amino acids, whose spectra are poorly understood, are known to contribute to this region (11). To determine if the bands observed were due to aromatic side chains, the hydrogenated derivative was used. The spectra of all hydrogenated derivatives, due to solubility problems, were taken in methanol. The results (Fig. 3) show that the same bands are present after hydrogenation, although with altered intensities and shapes. Therefore, while the two minima are probably manifestations of backbone conformation, their appearance is influenced by the presence of the aromatics via direct interactions or simply through spectral overlap. The occurrence of this type of spectrum is significant because of its strong resemblance to that considered typical of the α helix. However, nuclear magnetic resonance studies have ruled out a conformation for gramicidin S-A containing α helical stretches (2). Therefore, it appears that naturally occurring backbone conformations other than the α helix may give rise to the same type of dichroic spectra. The implications in connection with helicity determinations by circular dichroism are clear, especially for proteins or protein fragments known to contain cyclic structures such as those mediated by disulfide bridges.

Despite the similarities in primary structure, the solution properties of the tyrocidines differ greatly from those of gramicidin S-A (5). Whereas gramicidin S-A shows no tendency for self-association, the tyrocidines associate readily to form extensive aggregates and under suitable conditions may even undergo gelation (3). All three tyrocidines show similar association properties and exhibit heteroaggregation. It has been shown that the tyrocidines are monomeric at or below 0.002% concentration in aqueous solution (3). Spectra taken of solutions in the absence of salt are shown in Fig. 4. Except for the effects on band intensities, the spectra are comparable to those taken in methanol, a solvent which dissociates the aggregates (3). All of the tyrocidines exhibit minima at about 205 and 215 μm. In addition, tyrocidine B shows a shoulder in the 230 μm region. With tyrocidine C a well defined peak is found in the same region. Inspection of Fig. 5 reveals that both peak and shoulder disappear upon hydrogenation. Therefore, this band is at least partially due to an aromatic residue. Since tyrocidine A, which contains no tryptophan, also has no 230 μm band, we identify the chromophore as the indole ring of tryptophan, which is known to have optically active absorption bands in this region (11). Since the two large minima do not disappear upon hydrogenation, it is again clear that they are not due to aromatics and probably result from some aspect of the conformation of the peptide backbone.

Raising the concentration to 0.02% in the presence of 0.01 to 0.02 M NaCl causes aggregation of the tyrocidines (3). From Fig. 4 the effects stemming from such aggregation are seen to be of two types. In tyrocidine C, and probably in tyrocidine B, there is an inversion of the 230 μm band. In all three tyrocidines, 1 The primary changes on substituting methanol as solvent seem to be in band intensities, although small shifts in band position cannot be ruled out. Since this same effect is found with many peptides of differing structures, it is probably due to a general solvent effect on the transition involved (12).

The dissociating effect of methanol is also shown by both the nuclear magnetic resonance and the dialysis experiments of Stern, Gibbons, and Craig (13).

![Fig. 2. The circular dichroism spectrum of gramicidin S-A (GSA) in water.](http://www.jbc.org/)

![Fig. 3. The circular dichroism spectra of hydrogenated and unmodified gramicidin S-A (GSA) in methanol.](http://www.jbc.org/)
vent from H₂O to methanol or trimethylphosphate had no such effects, indicating that a "nonaqueous" environment within the aggregates is not the cause of these changes. Although it is possible that some type of conformational change occurs on aggregation, this cannot be rigorously inferred since aggregation itself could lead to new juxtapositions of chromophores and, therefore, quite possibly to changes in the spectra. It seems unlikely that tryptophan-tryptophan interactions are the cause of the band inversion, as indicated by the following "mixing" experiment. Tyrocidine C was mixed with a 3-fold excess of tyrocidine A under conditions favoring aggregation. A spectrum was taken. Then the mixture was heated to 70°, the temperature quenched to 15°, and another spectrum taken. In both cases, the 230 nm band inversion occurred and was of virtually the same magnitude as observed in tyrocidine C alone. Under these conditions, extensive heteroassociation should occur and greatly diminish tryptophan-tryptophan interactions (3). Since heteroassociation had no effect on the inversion, it is unlikely that such interactions are involved. No time dependence was detected in any of these experiments.

The solutions showed no signs of turbidity. This is not surprising since the particles are probably spherical and of molecular weight less than 20,000 under the conditions used (3). Increasing the salt concentration from 0.01 to 0.02 M, which increases the molecular weight many times, has no further effect on the spectra. Consequently, the results do not arise from a "particulate" system, and it is unlikely that the effects observed are due to scattering or absorption flattening (14).

The above findings should allow more profitable use of the tyrocidines and gramicidin S-A for structure-spectra relationships in circular dichroism. It is clear that the aromatic side chain chromophores have considerable effects on band shapes and intensities and therefore cannot be neglected in attempts to calculate the spectra of the unmodified peptides from theory. Furthermore, the experiments with the aggregated tyrocidines indicate that the low wave length band of tryptophan is exceptionally sensitive to changes in its electronic environment, a fact thus far appreciated only for its 280 nm band (11). Fortunately, the simple hydrogenation technique used here obviates some of the difficulties involving the aromatics and allows a clearer estimate to be made of the contribution of the peptide backbone to the spectra. However, these data do not allow us to state precisely what feature of the peptide backbone gives rise to the two large minima shown by all of the peptides. We can only observe that, although the spectra of the hydrogenated tyrocidines and grami-

Other results bearing on this point have recently been brought to our attention. Bihára and Frič (Proceedings of the Ninth European Peptide Symposium, Orsay, France) have shown that the circular dichroic spectra of synthetic cyclodipeptides and cyclohexapeptides, in the 210 to 280 nm region, are greatly influenced by the presence of aromatic side chains. Phenylalanine residues gave substantial positive contributions, a finding consistent with our results for gramicidin S-A.
cidin S-A are not identical, there are strong similarities with respect to both the number of bands and their relative intensities. This, along with the observation that the gramicidin S-A bands have almost twice the intensity of those of the tyrocidines, suggests that loci of very similar conformations are involved and that the former molecule has twice as many as the latter. Such an interpretation is appealing in view of the double occurrence of the common amino acid sequence in gramicidin S-A. The Phe-Pro segment has been specifically suggested (9). Nonlinear hydrogen bonds, which are thought to contribute strongly to the $n \rightarrow \pi^*$ transition, appear to be present, at least in the case of gramicidin S-A.

The fact that the spectra of the three hydrogenated tyrocidines are almost identical suggests a common backbone conformation. Since the tyrocidines differ in several amino acids, yet readily form homo- and heteropolymers, it is reasonable to assume that the aggregation process is largely a function of that conformation and the resulting orientation of the side chains. The side chain substitutions occurring in tyrocidines A, B, and C do not seem to prevent the aggregation. Such a conclusion is supported by the failure of all side chain modifications thus far attempted to have a significant effect (15). Furthermore, if the aggregation process were dependent on a common backbone conformation, it would be expected that the arrangements of monomers in the aggregates are quite similar in all three cases. Direct evidence for this can be obtained from Fig. 4, where it can be seen that the qualitative changes in the spectra upon aggregation are in agreement. The "mixing" experiment is perhaps even more convincing. Inversion of the 230 nm band, which must be a result of the arrangement of the monomers in the aggregate, occurs regardless of the types of monomers constituting that aggregate.

The lack of specificity with regard to the side chains makes the elucidation of the aggregation phenomena of fundamental importance. Some general factor is likely to be involved. Since the only evident similarity between the various side chains, both the natural ones and their modifications, seems to be their hydrophobic character, it is tempting to invoke a role for hydrophobic interactions, as previously hypothesized by this laboratory (4). Further investigations of this possibility are in progress.

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