Circular Dichroism of Oxytocin and Several Oxytocin Analogues*

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SHERMAN BEYCHOK AND ESTHER BRESLOW

From the Department of Biological Sciences, Columbia University, New York, New York 10027, and the Department of Biochemistry, Cornell University Medical School, New York, New York 10021

SUMMARY

The circular dichroic spectra of oxytocin and four analogues of oxytocin are presented at several pH values in the wavelength interval, 220 to 320 nm. The analogues are 2-isoleucine- and 2-glycine-oxytocin, in which the tyrosine residues in Position 2 have been replaced by isoleucine and glycine, respectively, as well as deamino-oxytocin and deamino-2-isoleucine-oxytocin, in which the α-NH₂ groups of oxytocin and 2-isoleucine-oxytocin, respectively, have been replaced by hydrogen. Measurements of lysine-vasopressin are also reported. All of these compounds show positive circular dichroism bands associated with disulfide absorption near 250 nm, corresponding to disulfide dihedral angles close to 90°. The intensity of this circular dichroism band is sharply diminished in deamino-2-isoleucine-oxytocin, and the band is only barely discernible in deamino-oxytocin. Neutralization of the amino group—when one is present—also diminishes the intensity of the band, but not as dramatically as replacement altogether by hydrogen. At longer wavelengths, near 280 nm, oxytocin and its analogues exhibit a band which, at least in one case, can only be due to disulfide and which, in two other cases, may be contributed to by tyrosine. A large tyrosine band is also observed in oxytocin and lysine-vasopressin at shorter wave lengths, but this band is absent in deamino-oxytocin. These results are discussed in terms of interactions among residues within these peptides and of possible conformational differences among the derivatives examined.

EXPERIMENTAL PROCEDURE

Oxytocin, lysine-vasopressin, and all other analogues were made available for this study by Dr. Vincent du Vigneaud. Deamino-oxytocin samples had been crystallized from water (6); all other samples were in the lyophilized form. The biological activity of oxytocin samples was approximately 500 units of avian vasodepressor activity per mg; deamino-oxytocin activities were 999 to 925 avian vasodepressor units per mg; 2-isoleucine-

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Oxytocin and deamino-2-isoleucine-oxytocin had activities of 48 and 81 avian vasodepressor units per mg, respectively. The lysine-vasopressin was a highly purified synthetic sample which behaved as a single peak during partition chromatography on Sephadex G-25 (7).

For circular dichroism studies at pH 2 the solid material was dissolved in 0.01 N HCl to give a final concentration of approximately 1 mg per ml. For studies at higher pH values, the pH of the initial solution was adjusted by the addition of small portions of NaOH.

Circular dichroism measurements were performed on a modified Jouan Dichrographe. Sensitivity, range, and principle of operation are described elsewhere (5, 8). All measurements were performed at room temperature.

RESULTS

Ultraviolet CD spectra of oxytocin, 2-isoleucine-oxytocin, deamino-2-isoleucine-oxytocin, and deamino-2-isoleucine-vasoressin are shown in Fig. 1, A, B, C, and D, respectively. In acid, oxytocin exhibits a negative band at 280 μm, a positive shoulder near 250 μm, and a large positive band at 225 μm. On neutralization of the α-amino group at pH 7.5, the negative band diminishes in intensity, the shoulder at 250 μm is resolved into a maximum, and the band near 225 μm shifts to a somewhat longer wave length, with a large decrease in maximum ellipticity. At pH 10.6, with ionization of the tyrosine residue, the character of the CD spectrum radically changes: a positive plateau appears between 280 and 290 μm and a large positive band appears at 245 μm, obscuring almost entirely a small shoulder between 250 and 260 μm. Studies of lysine-vasopressin at pH 2 indicate that the main qualitative features of the oxytocin CD spectrum are also present in this analogue, although some quantitative differences between the two peptides are manifest.

Deamino-oxytocin gives a markedly different ultraviolet CD spectrum (Fig. 1C). The very large positive band at 225 μm seen in oxytocin is not present. Instead, the ellipticity is slightly negative in this wave length region. In the region of disulfide absorption, near 250 μm, only a very weak positive band is discerned. The signal to noise ratio for this band is highly unfavorable; several measurements gave the average values shown in the figure, but the uncertainty was sufficiently great that values of zero at 255 μm cannot be excluded.

The major feature of this spectrum is a negative band at 280 μm. At pH 11.5, with the tyrosine residue fully ionized, this band changes sign and shifts several millimicrons to the red. Again, noise prevented secure location of the maximum but it is at a shorter wave length than the spectral maximum at this pH value.

The CD spectrum of 2-isoleucine-oxytocin (Fig. 1B) shows a well resolved band at 250 μm. In addition, a smaller, negative band is observed at 280 μm. Neutralization of the amino group leads to a reduction in the size of the 250 μm band and a still greater reduction in the intensity of the negative band.

In 2-glycine-oxytocin also (not shown) there is a long wave length shoulder near 280 μm as well as a well resolved 250 μm band, but in this compound the long wave length band is positive. Neutralization of the amino group brings about a reduction of the intensity of both bands, but the effect is much smaller than in 2-isoleucine-oxytocin. Furthermore, the shorter wave length band is red shifted at pH 7.18 by almost 10 μm.

The last derivative of this series we have examined is deamino-2-isoleucine-oxytocin. Again, there is a long wave length, positive “tail” on the main band at 250 μm, indicating one or more small bands in addition to that at 250 μm. There is no noticeable effect on this spectrum when the pH is raised to 7.

2-Isoleucine-oxytocin and deamino-2-isoleucine-oxytocin show similar ultraviolet absorption spectra in the wave length interval, 240 to 320 μm. In the spectra of each compound there is a well marked shoulder near 250 μm, the intensity and position of which allow an unequivocal assignment to the disulfide. Neutraliza-
tion of the α-amino group in 2-isoleucine-oxytocin leads to a slight red shift in the position of the shoulder, and the spectrum becomes almost indistinguishable from that of deamino-2-isoleucine-oxytocin.

DISCUSSION

In oxytocin itself and in four of the five analogues we have examined, a characteristic feature of the CD spectrum in the wave length interval, 310 to 215 mp, is a positive band or shoulder centered near 250 mp. That this band arises from a disulfide electronic transition may be inferred from its presence in 2-isoleucine-oxytocin, 2-glycine-oxytocin, and deamino-2-isoleucine-oxytocin, in which compounds the disulfide is the only chromophore absorbing in this spectral region. The presence of a shoulder near 250 mp in the absorption spectra of these compounds adds security to the assignment, since all open chain disulfides exhibit a weak absorption band near 250 mp (9, 10). Furthermore, l-cystine, various derivatives of l-cystine, and oxidized glutathione all exhibit bands of comparable magnitude in the CD spectra near 250 mp, in various states of ionization (8). In these compounds, however, the 250 mp band is always negative, in contrast to what is observed with oxytocin and its analogues.

Optical activity in a disulfide may arise from inherent dis-symmetry (a disulfide normally exists in a screw configuration and the mirror images are not superimposable), from asymmetric perturbation, or from both. If the dominant contribution to an optically active absorption band is from inherent dis-symmetry, then the band is signed according to the screw configuration (11). If the dominant contribution is from an external perturbation, then the situation may be more complex. It will be shown elsewhere that the optical activity of disulfides due to external perturbation (perturbed C₄ symmetry) follows a quadrant rule. Rotation around the disulfide bond (change in screw configuration) which leaves the externally perturbing group unaltered relative to a fixed coordinate system would change the sign of the optically active band. In a small molecule, however, this is not very likely to occur. Accordingly, there may be no simple relationship between screw sense and the sign of the CD band.

The main disulfide band in all these compounds except deamino-oxytocin is near 250 mp. Studies of various cyclic and noncyclic disulfides have indicated that the absorption maximum of this long wave length band varies with the dihedral angle. An angle of 90° is associated with an absorption maximum between 243 and 250 mp (9, 10). Thus it may safely be assumed that in the compounds reported here the dihedral angle is close to 90° and that this angle does not vary significantly when the α-amino group is neutralized.

The intensity of the 250 mp CD band, however, does depend on the state of ionization of the amino group. In oxytocin, 2-isoleucine-oxytocin, and 2-glycine-oxytocin, the magnitude of the band diminishes when the α-amino group is neutralized. The effect is greatest in 2-isoleucine-oxytocin. In oxytocin, the diminution of the band may be only apparent in that it is contributed to by the much larger band at 225 mp, which also decreases substantially consequent to neutralization of the amino group.

The available experimental facts do not allow us to decide whether the sensitivity of the 250 mp band to amino charge is a direct result of a difference in external perturbation of the disulfide transition or a change in the conformation involving the disulfide bond. Replacement of the α-amino group by hydrogen has a profound effect on this band, diminishing it in deamino-2-isoleucine-oxytocin and virtually or actually abolishing it in deamino-oxytocin. In the presence of the amino group, replacement of tyrosine by isoleucine has only a minor effect on the 250 mp band. Although it may be that deamino-oxytocin is somewhat different in conformation from deamino-2-isoleucine-oxytocin, the most pronounced changes appear to be associated with replacement of the amino group by hydrogen.

More extensive examination of model compound behavior will be required before it can be decided whether the observed difference resides in a different preference for the two screw isomers. Such studies are in progress.

Optical Activity near 280 mp—The position of the 280 mp band in oxytocin, lysine-vasopressin, and deamino-oxytocin and its response to pH suggest that it might be due to tyrosine, inasmuch as these analogues contain tyrosine residues in Position 2. However, this band is also present in 2-isoleucine-oxytocin, and a positive band near 275 mp is present in deamino-2-isoleucine-oxytocin and in 2-glycine-oxytocin. In these latter compounds, at least, no tyrosine is present and so the band must originate elsewhere. The only possible contributor to this band in 2-isoleucine-oxytocin and deamino-2-isoleucine-oxytocin is the disulfide bond; examples of optically active disulfide transitions at this wave length are known (8). It is therefore reasonable to assume that the disulfide contributes to this transition in oxytocin, vasopressin, and deamino-oxytocin as well, although a possible additional contribution to this band by tyrosine in these analogues cannot be ruled out.

The 280 mp band is markedly sensitive to pH. Both in oxytocin and in 2-isoleucine-oxytocin the negativity of the band is diminished when the pH is increased from 2 to 7.5. The only ionization occurring within this pH region is that of the α-NH₂ group, which has a pKₐ of 6.3 (12). This pH effect therefore indicates a sensitivity of the disulfide transition to the charge on the neighboring α-NH₂ group. Tyrosine ionization in oxytocin derivatives occurs with a pKₐ of 9.7 (12). At pH 10.6 the band near 280 mp has become positive in both oxytocin and deamino-oxytocin. This effect could result from the sensitivity of the disulfide transition to the charge on the tyrosine residue or from the optical activity of the ionized tyrosine residue.

Optical Activity near 225 mp—The positive band near 225 mp at pH 2 which is found in oxytocin (and in vasopressin) is most readily attributed to a tyrosine transition. It is not observed in any of the analogues which do not contain a tyrosine residue, and it occurs at a wave length associated with an optically active tyrosine transition in model systems (5). Moreover, the effect of pH on the wave length of the transition and the absolute magnitude at different pH values is similar to that observed in model tyrosine compounds (5). Peculiarly, this positive band is not observed in deamino-oxytocin. However, the molar ellipticity is 10,000° per decimole more positive at 220 mp than that in deamino-2-isoleucine-oxytocin. The analysis of this region of the spectrum is complicated by the fact that the peptide bond might generate bands here, and even the disulfide-peptide interaction might be responsible for some of the observed intensity. For example, oxidized glutathione shows a large positive band at 225 mp in acid (molar ellipticity, 5,000) which is
reduced by more than one-half upon neutralization of the amino group and is red shifted by approximately 5 m.

*Other Implications as to Conformation*-One of the striking features of these data is the extent to which the α-NH₂ group influences the optical activity of the disulfide group and, possibly, of the tyrosine residue. The only peptides studied with qualitatively similar CD patterns are oxytocin and vasopressin, each of which contains an α-NH₂ group and a tyrosine residue in identical positions. One possible interpretation of these data is that direct bonding can occur between the α-NH₂ group and tyrosine and that this bonding influences both optical activity and conformation. However, this is unlikely. The pKₐ of that tyrosine residue in oxytocin is 9.7 (12) and is unchanged in deamino-oxytocin (12). The pKₐ of the α-NH₂ group is 6.3 in oxytocin (12) and it is 6.5 in 2-phenylalanine-oxytocin (12). Although the amino pK value may seem low, it is in fact essentially normal for peptides in which cystine occupies the NH₂ terminus (13). Thus, both the α-NH₂ and the tyrosine pK values are normal in oxytocin derivatives and each is essentially unaffected by the other. It is unlikely that these relations would hold in the event of any bonding between the two.

With the exception of the influence of the amino group on both optical and biological activity, the present data do not reveal any obvious relationship between optical activity and biological activity. Oxytocin and vasopressin have very different biological activities but at least qualitatively similar CD patterns. 2-Isoleucine-oxytocin is biologically active, but significantly less so than oxytocin; however, the near ultraviolet disulfide band transitions in 2-isoleucine-oxytocin are very similar to those in oxytocin. Extension of these studies to other analogues will be necessary to help clarify any relationships between conformation and biological activity.

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Sherman Beychok and Esther Breslow


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