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SOLUTION CONFORMATIONS OF THE PITUITARY OPIOID PEPTIDE DYNORPHIN-(1-13)

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SUMMARY: Circular dichroism spectra have been measured for dynorphin-(1-13) in water and in solutions of sodium dodecyl sulfate and L-α-lysophosphatidylcholine (palmitoyl). Spectra in water have the features expected for a peptide containing little, if any, order. Small changes are brought about by L-α-lysophosphatidylcholine (palmitoyl), but the resulting spectrum retains the characteristics expected for a random coil. In contrast, sodium dodecyl sulfate produces significant changes which are those expected for induction of α helical content. Quantitative analysis of the circular dichroism spectra suggests the conformation changes from about 5% helix in water to 17% helix in sodium dodecyl sulfate. These results from experiment are in excellent agreement with those obtained from our formulation of the configuration partition function. This formulation predicts a change in helical content from 1% to 19%. The ordering influence is felt most strongly by those residues immediately following the enkephalin sequence.

Dynorphin is a potent pituitary opioid peptide whose amino terminal pentapeptide has the amino acid sequence of leucine-enkephalin.[1] The amino terminal tridecapeptide has been shown to be Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys.[1] A fragment with the amino acid sequence of the N-terminal octapeptide has been isolated from porcine hypothalami.[2] Dynorphin-(1-13) and dynorphin-(1-8) exhibit significant opiate activity, with the longer peptide being the more potent. These observations are consistent with the suggestion[1] that the tail of dynorphin-(1-13) is important in receptor binding.

Recently we performed a theoretical study of the conformational changes expected in endogeneous opioid peptides when they interact with acidic lipids,[3] which have been suggested to be active components of the brain opiate receptor.[4-6] That study found that acidic lipids tend to produce order in dynorphin-(1-8) and dynorphin-(1-13). The ordering influence is felt most strongly by those amino acid residues immediately following the

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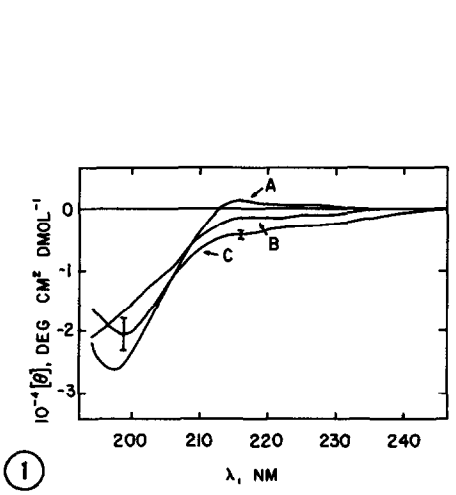
enkephalin sequence. It is stronger in dynorphin-(1-13) than in dynorphin-(1-8). We report here the results of a circular dichroism study of dynorphin-(1-13) which confirm the major conclusions from the theoretical work. The experimental study also shows that anionic lipids are more effective than zwitterionic lipids in producing order in dynorphin-(1-13), as must be the case if assumptions vital to the theoretical work are correct.[7-9]

METHODS

Dynorphin-(1-13), L-α-lysophosphatidylcholine (palmitoyl), and L-α-phosphatidic acid (dipalmitoyl) were obtained from Sigma Chemical Company. Phosphatidyl glycerol was obtained from Calbiochem, and sodium dodecyl sulfate was from Matheson, Coleman, and Bell. Dynorphin-(1-13) solutions investigated experimentally had concentrations in the range 0.0052 - 0.026 mg/ml. They were prepared by dilution of a concentrated stock solution. The concentration of the stock solution was determined by quantitative analysis of the acid hydrolysate using a Beckman 119 amino acid analyzer. Circular dichroism spectra were measured using a Durrum-Jasco J-20 recording spectropolarimeter calibrated with d-10-camphorsulfonic acid.[10] The signal-to-noise ratio was found to be more favorable at 5° than was the case at higher temperatures.

RESULTS AND DISCUSSION

Circular dichroism in water. Figure 1 depicts circular dichroism spectra of dynorphin-(1-13) in water at 5°, 25°, and 54°. Additional spectra (not shown) at 15° and 35° demonstrate that the temperature effect is essentially monotonic. At 5° the dominant feature is a strong negative band near 197 nm with $[\theta]$ of about -26,000 deg cm^2 dmol^{-1}. A much weaker positive band occurs at higher wavelength, $[\theta]_{216}$ being about 1400 deg cm^2 dmol^{-1}. Increasing temperature produces a loss of intensity for the strong negative band and also causes a change in sign for the circular dichroism near 216 nm. The temperature effect on the positive band yields $d[\theta]_{216}/dT$ = -90 deg cm^2 dmol^{-1} K^{-1}, which is comparable with $d[\theta]_{215}/dT$ = -50 deg cm^2 dmol^{-1} K^{-1} for poly(hydroxyethyl-L-glutamine),[11] $d[\theta]_{218}/dT$ = -95 deg cm^2 dmol^{-1} K^{-1} for cationic poly(L-lysine),[12] and an estimate of $d[\theta]_{215}/dT$ of about -100 deg cm^2 dmol^{-1} K^{-1} for random coil poly(L-alanine).[13] The circular dichroism spectra show that dynorphin-(1-13) has little, if any, ordered structure in water.



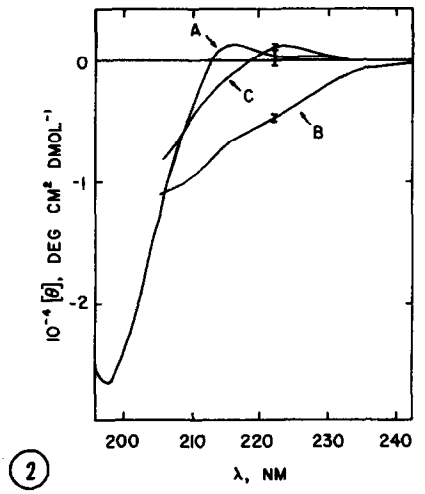


Fig. 1. Circular dichroism of dynorphin-(1-13) in water at A, 5°; B, 25°; C, 54°. The pH is 6.7 at 25°.

Fig. 2. Circular dichroism of dynorphin-(1-13) at 5° in A, water; B, 3.6 x 10^{-3} molar sodium dodecyl sulfate; C, 8 x 10^{-5} molar L-α-lysophosphatidylcholine (palmitoyl).

Anionic detergents and anionic lipids. Figure 2 shows that dodecyl sulfate produces a significant change in the circular dichroism of dynorphin-(1-13). Development of negative ellipticity above 208 nm is to be expected if sodium dodecyl sulfate induces α helix formation. The helical content can be estimated from the measured ellipticity at 222 nm. For this purpose we use 1400 and -36,000 deg cm^2 dmol^{-1}, respectively, for the disordered and completely helical conformations. The former is the experimental result for poly(hydroxyethyl-L-glutamine) in water at this temperature,[11] and the latter is widely used for completely helical polypeptides.[14,15] The experimental ellipticity for dynorphin-(1-13) then yields a helical content of 17% in dodecyl sulfate (the same calculation yields a helical content of only 5% when the ellipticity used is that measured in water). When our matrix calculation[3,8,9] is performed for the amino acid sequence of dynorphin-(1-13), computed helical contents are 1% in water and 19% in dodecyl sulfate. Consequently the measured circular dichroism spectra show the features predicted from theory. Computed helix probability profiles are depicted in

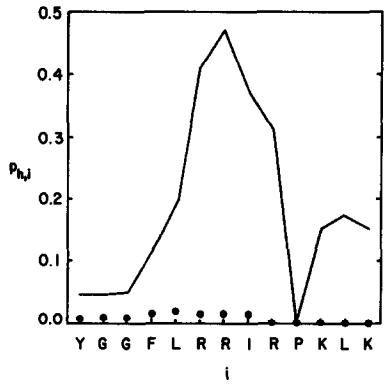


Fig. 3. Computed helix probability profiles for dynorphin-(1-13) in water (dotted line) and in the presence of sodium dodecyl sulfate (solid line). The one letter code used is F = Phe, G = Gly, I = Ile, K = Lys, L = Leu, P = Pro, R = Arg, Y = Tyr.

Figure 3. These profiles differ slightly from those reported earlier[3] because recently determined parameters for the isoleucyl residue[16] were used in the calculation.

Several attempts to measure circular dichroism spectra of dynorphin-(1-13) in the presence of anionic lipids were unsuccessful. A suspension or precipitate formed upon addition of L-α-phosphatidic acid (dipalmitoyl) or phosphatidyl glycerol to dynorphin-(1-13). While the nature of any conformational change remains uncharacterized, it is at least clear that dynorphin-(1-13) interacts with these anionic lipids.

Zwitterionic lipids. The circular dichroism of dynorphin-(1-13) is affected somewhat by L-α-lysophosphatidylcholine (Figure 2). However, the changes seen cannot arise from an increase in helical content, as is immediately apparent from behavior of the circular dichroism near 222 nm. In fact, the spectrum produced by the lysolecithin retains the essential features associated with a random coil conformation. The three circular dichroism spectra reported in Figure 2 emphasize the importance of the polar head group for the induction of order in dynorphin-(1-13). Helix formation is seen only when the polar head group is anionic.

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