J. Am. Chem. Soc. **1988**, *110*, 4412-4414

Conformational Properties of Angiotensin II in Aqueous Solution and in a Lipid Environment: A Fourier Transform Infrared Spectroscopic Investigation[1]

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Abstract: The conformational properties of the octapeptide hormone angiotensin II and those of its truncated analogue, the heptapeptide des Asp[1]-angiotensin II, were investigated by resolution-enhanced Fourier-transform infrared spectroscopy. In aqueous solution, the conformation-sensitive amide I bands of the two peptides are virtually identical. From the position of the amide I bands it is inferred that the majority of the backbone amide groups are in a nonordered conformation, with a fraction of these groups being involved in turn-like structures. Interaction with an acidic phospholipid, dimyristoylphosphatidylglycerol, results in drastic conformational changes of both angiotensin II and des Asp[1]-angiotensin II; however, the conformations of the two peptide analogues in a lipid environment are different. While the lipid-associated angiotensin II adopts a highly ordered secondary structure consisting of hydrogen-bonded β-strands and turns, there is no evidence for β-strands in the truncated peptide. These observations have important implications for the mechanisms of peptide hormone–receptor interactions and, particularly, for the postulated role of membrane lipid phase as a biologically important modifier of the hormone conformation.

The linear octapeptide angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) is a potent natural pressor agent.[2] It has long been recognized that the biological activity of the hormone may be related to its conformation. Accordingly, a great variety of physicochemical and spectroscopic studies were undertaken to gain insight into the conformation in aqueous solution of angiotensin II and of some of its analogues.[3-10] Yet, the picture emerging from these studies is not totally coherent.

Attempts to establish a conformational basis for the physiological action of angiotensin II (as well as that of other peptide hormones) are further complicated by the legitimate question whether the models developed for the free peptides in aqueous solution adequately describe the conformation prevailing at the receptor site. Current hypotheses emphasize the role of lipid affinity and of lipid-induced peptide folding in peptide hormone–receptor interactions.[11-19] However, very little is known so far about the conformational properties of peptide hormones in a lipid environment. This is doubtless related, at least in part, to the difficulties involved in applying spectroscopic techniques to study membrane-associated peptides.

In the present paper, we have used Fourier-transform infrared spectroscopy to establish the conformation of angiotensin II and that of its truncated analogue, the heptapeptide des Asp[1]-angiotensin II. The infrared method, aided by recent developments in band resolution techniques,[20] allows a penetrating insight into the secondary structure of proteins and peptides. Importantly, the method is equally well suited to study peptide folding in solution and in a lipid environment.[19,21-26]

Materials and Methods

Angiotensin II and des Asp[1]-angiotensin II were obtained from Peninsula Laboratories, Inc., Belmont CA. Dimyristoylphosphatidylcholine and dimyristoylphosphatidylglycerol were from Avanti Polar Lipids, Birmingham, AL. Peptide solutions were obtained by dissolving 1 mg of angiotensin II or its analogue in 50 μL of 50 mM MES (2-[*N*-morpholino]ethanesulfonic acid) buffer prepared in deuterium oxide (99.8% purity, MSD Isotopes, St. Louis) and adjusted to p^{2}H 5.6. For preparation of lipid–peptide complexes, a peptide solution in buffer was added to a solid lipid so that the lipid–peptide weight ratio of 4:1 was reached. The mixture was vortexed for approximately 10 min during which time the sample was warmed and cooled repeatedly through the lipid transition temperature.

Samples for infrared spectroscopy were placed between two CaF_2 windows separated by a 50 μm thick Teflon spacer. Infrared spectra were recorded at 30 °C with a Digilab FTS-15 instrument equipped with a high-sensitivity mercury cadmium telluride detector. For each spectrum, 512 interferograms were added and Fourier-transformed to give a resolution of 2 cm^{-1}. The spectra in the 1500 to 1800 cm^{-1} region were

(1) Issued as National Research Council of Canada Publication No. 28181.

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0002-7863/88/1510-4412$01.50/0 Published 1988 by the American Chemical Society

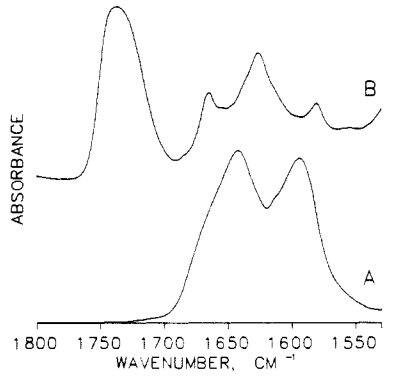

Figure 1. Infrared spectra between 1530 and 1800 cm^{-1} of angiotensin II in aqueous solution (A) and in the presence of dimyristoylphosphatidylglycerol (lipid/peptide weight ratio 4:1) (B). The band between 1720 and 1750 cm^{-1} in the top spectrum represents C=O stretching vibrations of the phospholipid molecules.

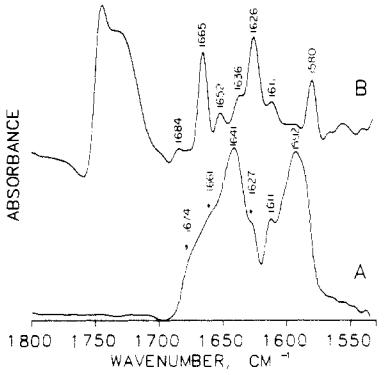

Figure 2. Same spectra as in Figure 1 after band narrowing by Fourier self-deconvolution using a 15-cm^{-1} half-width Lorentzian line and a resolution enhancement factor (K value) of 2.[20] Angiotensin II in aqueous solution (A) and the dimyristoylphosphatidylglycerol-associated peptide (B).

corrected for the weak absorption of the buffer. To eliminate spectral contributions of atmospheric water vapor, the instrument was continuously purged with dry nitrogen. Overlapping infrared bands were resolved by Fourier self-deconvolution procedures.[20]

Results

The relation between specific infrared vibrational modes of proteins or peptides and the secondary structure of the polypeptide backbone is best understood for the so-called amide I bands in the spectral region between approximately 1615 and 1700 cm^{-1}. The conformation-sensitive amide I region of the original infrared spectrum of angiotensin II in ^2H_2O buffer consists of a strong band centered at 1642 cm^{-1} with a shoulder on the high-wavenumber side (Figure 1A). The low-wavenumber side of the amide I band contour overlaps with relatively strong band(s) with a maximum at 1592 cm^{-1}. The overlapping bands that contribute to the absorption in the spectral region of interest can be resolved by using the computational procedure of band narrowing by Fourier self-deconvolution.[20] The resolution-enhanced spectrum of angiotensin II in aqueous solution reveals the presence of four component amide I bands between 1615 and 1700 cm^{-1} (Figure 2A). The major band at 1641 cm^{-1} can be assigned to solvated (and deuterium-exchanged) amide groups of the backbone in a non-ordered conformation.[21,24] The band at 1661 cm^{-1} represents turns.[21,24] The component band at 1674 cm^{-1} lies in the wavenumber range that may contain vibrational modes characteristic of both turns and "in phase" vibrations of peptide C=O groups in β-strands.[21,24,27] The latter assignment is, however, less likely since the presence of a β-sheet structure is usually marked by a second band around 1630 cm^{-1} that is much stronger than the high wavenumber "β-component" above 1670 cm^{-1}.[21,24] The very

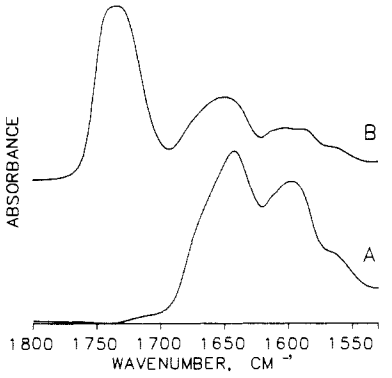

Figure 3. Infrared spectra between 1530 and 1800 cm^{-1} of des Asp[1]-angiotensin II in aqueous solution (A) and in the presence of dimyristoylphosphatidylglycerol (lipid/peptide weight ratio 4:1) (B). The band between 1720 and 1750 cm^{-1} in the top spectrum represents C=O stretching vibrations of the phospholipid molecules.

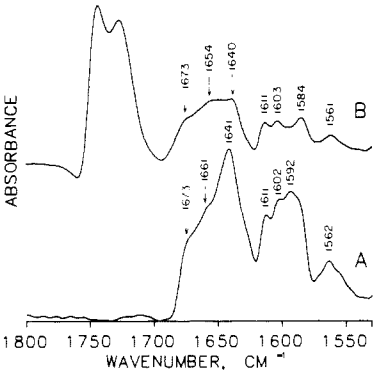

Figure 4. Same spectra as in Figure 3 after band narrowing by Fourier self-deconvolution using a 15-cm^{-1} half-width Lorentzian line and a resolution enhancement factor (K value) of 2.[20] Des Asp[1]-angiotensin II in aqueous solution (A) and the dimyristoylphosphatidylglycerol-associated peptide (B).

low intensity of the "β-band" at 1627 cm^{-1} in the spectrum of aqueous angiotensin II suggests that the probability of a β-sheet structure is very low. The bands at wavenumbers below 1615 cm^{-1} are outside the amide I region and represent vibrational modes due to end carboxyl groups (strong band at 1592 cm^{-1})[28,29] and due to various side chains.[30]

The overall shape of the infrared spectrum of angiotensin II in the amide I region is not affected by the incubation of the peptide with dimyristoylphosphatidylcholine (spectrum not shown for brevity). On the other hand, drastic changes are observed upon interaction of angiotensin II with dimyristoylphosphatidylglycerol (DMPG) (see spectra in Figures 1B and 2B). The dominant features of the spectrum of DMPG-associated angiotensin II[31] are bands at 1626 and 1665 cm^{-1} that represent β-strands and turns, respectively.[21,24,27] The bands at 1636 and 1684 cm^{-1} are also characteristic of amide groups in β-strands. The remaining weak feature at 1652 cm^{-1} may indicate residual α-helices,[21,27] type II β-turns,[27] or some other less typical structures (see the discussion below of the 1654-cm^{-1} band in the spectrum of DMPG-associated des [1]Asp-angiotensin II). Binding of angiotensin II to DMPG is also accompanied by a drastic drop in the intensity of the band at 1592 cm^{-1} due to end carboxyl groups.

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(31) After separation of DMPG-peptide liposomes from the bulk aqueous phase by filtration on an Amicon filter (30000 molecular weight cutoff) no infrared signal due to the peptide could be detected in the lipid-free filtrate. This indicates that at the lipid to peptide ratio used in this study, virtually all angiotensin II or des Asp[1]-angiotensin II present in the system was associated with phosphatidylglycerol.

While the environmental sensitivity of this band is by itself interesting and may be of considerable diagnostic value, it is of no direct relevance to the main topic of this study.

The infrared spectrum in the amide I region of an aqueous solution of the heptapeptide analogue des Asp^1-angiotensin II (Figures 3A and 4A) is virtually identical with that of angiotensin II (Figures 1A and 2A). However, the effect of phosphatidylglycerol on the conformation of the truncated peptide is markedly different from that observed with the native hormone. The broad amide I band contour of DMPG-bound des Asp^1-antiotensin II (Figure 3B) can be resolved into three major components: at 1640, 1654, and 1673 cm^{-1} (Figure 4B). While the assignment of the 1640- and 1673-cm^{-1} bands is identical with that discussed for the aqueous forms of the peptides (non-ordered conformers and turns, respectively), the origin of the band at 1654 cm^{-1} is not fully clear and its unambiguous interpretation would require normal mode calculations of the vibrational frequencies of the peptide. We ascribe this band tentatively to type II β-turns[27] or certain atypical nonperiodic structures; however, an alternative assignment could be α-helix.[21,27] The circular dichroism spectrum of the lipid-associated peptide does not corroborate an α-helix alternative, though a detailed interpretation of the CD spectrum is hampered by light scattering on membrane fragments (unpublished results). Previous circular dichroism studies with angiotensin analogues in organic solvents also argue against any tendency of the peptide to fold into α-helical structures.[32]

Discussion

Several different conformations have been previously suggested for the structure of angiotensin II in aqueous solution.[3-10,34] The present data clearly do not support the early models proposing α-helix[3] or totally random coil[4] conformations for the aqueous form of the peptide. On the other hand, infrared spectra indicate that while the majority of the backbone amide groups adopt an apparently "non-ordered" conformation, a fraction of these groups is involved in specific intramolecular hydrogen bonds. The pattern of this hydrogen bonding is compatible with turn-like structures. The picture emerging from our infrared study is thus consistent with the view[6,10] that in aqueous solution angiotensin II adopts a partly folded conformation, stabilized by one or more intramolecular hydrogen bonds.

The main objective of the present study was to establish the effect of lipid binding on the conformation of angiotensin II. Numerous observations point to the role of lipids in the receptor binding of many peptide hormones.[11-19] It has been hypothesized that the lipid phase of a target cell membrane may facilitate the accumulation of the hormone near the receptor site and, even more importantly, induce the correct folding of the peptide prior to its interaction with the receptor.[13,15,16] Infrared experiments give clear evidence that while there is very little interaction between angiotensin II and the zwitterionic lipid phosphatidylcholine, association with the negatively charged phosphatidylglycerol results in a drastic conformational change of the peptide. In the environment of an acidic phospholipid angiotensin II adopts a highly ordered secondary structure, the major elements of which are β-strands and turns. The β-sheet structure has been previously deduced also from circular dichroism spectra of angiotensin II in organic solvents.[32]

The most common conformation adopted in a lipid environment by biologically active peptides is believed to be the α-helix.[13,18,33] Probably the best known example of a peptide that behaves differently and that folds in a hydrophobic membrane environment into an antiparallel β-sheet structure is the adrenocorticotropin-(1-10)-decapeptide ($ACTH_{1-10}$).[13] However, the β-sheet conformation of $ACTH_{1-10}$ could be observed only when the peptide was forced into a dry phosphatidylcholine membrane,[13] and this structure most likely represents aggregated patches of the solid peptide. The system that appears to resemble more closely the situation observed with angiotensin II is the recently studied atrial natriuretic peptide atriopeptin III.[26] The transition of both atriopeptin III and angiotensin II from a random conformation to an ordered β-structure occurs upon spontaneous peptide association with lipid membranes. Moreover, the requirement of both peptides for acidic phospholipids clearly indicates the crucial role of surface electrostatic interactions between the basic amino acid residues and the negatively charged lipid head groups.

While the present results show that the interaction with an acidic phospholipid has a profound effect on the folding of angiotensin II, they do not prove that the β-sheet structure observed in a lipid environment is indeed the "active" conformation of the peptide. One approach to gain insight into the conformational basis for the hormone action involves studies with different peptide analogues. In this respect, it is notable that des Asp^1-angiotensin II, the hormone analogue that has comparable receptor affinity and retains many of the biological activities of angiotensin II,[34,35] also undergoes a conformational transition in the presence of phosphatidylglycerol membranes. Somewhat unexpectedly, however, the conformations adopted in a lipid environment by the two peptide analogues are markedly different. Particularly, there is no evidence for β-strands in the lipid-bound des Asp^1-angiotensin II. The above observations do not undermine the conceptually attractive and stimulating hypothesis of a lipid phase acting as a biologically important modifier of the peptide hormone conformation.[13-17] They do, however, indicate that the relationship between the conformation induced by non-specific interaction with phospholipids and the specific receptor binding of the peptides may be very complex. As suggested recently by Schwyzer,[36,37] the actual role of hormone–membrane interaction may be not only to induce the correct peptide folding but also, and maybe even more importantly, to facilitate the accumulation of the peptide in a correct membrane compartment. Studies with a large number of angiotensin analogues are now planned to address in more detail the fundamental problem of the intricate relationship between the lipid affinity, membrane location, and lipid-induced folding of peptide hormones and their biological activity.

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