Importance of Amino Acid Side Groups for Biologic Activity of Angiotensin II

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Before one can prepare antagonists or antimetabolites for any physiologically active compound, it is necessary to gain some idea of the functional or necessary groups for biologic activity. With the smaller molecules, possessing fewer functional groups, the number of analogs is small as compared to those of larger polypeptide molecules with structures made more complex by their spatial arrangement or molecular conformation.

Our studies to determine the important side groups of angiotensin were begun by synthesizing analogs of aspartyl-1-isoleucyl-5-angiotensin II, each of which would lack only 1 amino acid side group. This synthesis was accomplished by substituting alanine, the smallest optically active amino acid, for each of the amino acids of angiotensin, 1 at a time. The biologic activity of the analogs, as determined in experiments by Khairallah, was compared for both pressor and oxytocic activity against the parent compound, angiotensin II. Schwyzer and coworkers have likewise prepared angiotensin analogs, and the results obtained by both groups are presented here.

In figure 1 are shown the points of substitution in the angiotensin II molecule.

The importance of the various functional groups is discussed below starting with substitutions for aspartic acid and proceeding toward the C-terminus of angiotensin:

Position 1: N-p-nitrobenzoyl- and succinyl-1-angiotensin II both possess high pressor activity. Polymers of high molecular weight made by uniting the amino group of angiotensin to a synthetic polymer or to a naturally occurring protein likewise are quite active.

In addition to the lack of importance of the N-terminal amino group, this evidence suggests that the angiotensin action is on a membrane surface.

The asparaginyl and aspartyl compounds have identical activities, while arginyl-1-angiotensin is somewhat depressed but still quite active. It is evident that the acidic carboxyl group is not necessary for biologic activity. Even the heptapeptide without aspartic acid retains about 30 per cent of the pressor activity.

Position 2: Since the hexapeptide (octapeptide minus amino acids 1 and 2) has slight pressor activity, it appears that position 2 is of little importance. Substantiating this conclusion is the fact that the nitroarginyl-2 and ornithyl-2 peptides retain considerable pressor activity.

Position 3: Replacement of valine with leucine had no effect on biologic activity.

Position 4: Substitution of tyrosine in the hexapeptide with either phenylalanine or alanine results in a complete loss of activity. Schwyzer stated (personal communication) that the phenylalanyl-4-angiotensin retains some pressor activity. Nevertheless, the phenolic hydroxyl is possibly involved in the combination between angiotensin and its receptor site.

Position 5: Valyl-5- and isoleucyl-5-angiotensins have been isolated from bovine and horse (hog) blood, respectively. They appear to have equal biologic activity. This is to be expected since both amino acids have branching on the β carbon atom. Substitution of leucine in this position, however, considerably reduces the vasopressor activity of this polypeptide.

Position 6: No substitutions have been made in this position. Paiva and Paiva re-
port that angiotensin with its imidazole destroyed by photo-oxidation is almost completely inactive.\(^5\)

Position 7: Alanyl-7-angiotensin retains about 1 per cent of the pressor activity of the parent compound.\(^1\) Proline, an amino acid with a secondary amino group, cannot contribute to hydrogen bonding when it is in a peptide chain. It is likely that the alanyl-7-peptide has a different conformation from that of prolyl-7-angiotensin, which may account for the low biologic activity.

Position 8: The C-terminal amino acid seems to be the most important in angiotensins. The heptapeptide formed by removal of phenylalanine with carboxypeptidase is completely inactive. The complete lack of activity of alanyl-8-angiotensin shows the importance of the phenyl side residue in that position. That the free carboxyl group is necessary was shown by the very low activity of the octapeptide phenylalanine ester and amide.\(^3\) The very low activity of the decapptide also suggests that a free carboxyl group is necessary on position 8.

Halverson et al.\(^6\) report that angiotensin decapptide is as active as the octapeptide in bloodless preparations of the toad. They suggest that it is not necessary to postulate hydrolysis of the decapptide to the octapeptide for pressor activity to result. It is possible, however, that an enzyme, contained intracellularly or absorbed on the surface of isolated muscle preparations, might convert angiotensin I to II. The degree of this conversion to angiotensin II was measured by the increase in oxytocic activity during the incubation of the decapptide with homogenates of various tissues. As has been shown,\(^2\) angiotensin octapeptide and the heptapeptide, Arg-Val-Tyr-Ileu-His-Pro-Phe, are the only peptides which could arise from angiotensin I that show significant oxytocic activity. Both of the peptides arise from the loss of the 2 amino acids from the C-terminus of angiotensin I. Therefore, the measurement of the increase in oxytocic activity is an index of conversion.

Homogenates of heart, liver, aorta, and ileum convert the decapptide to its active form, while a homogenate of uterus does not. This observation may explain the lack of activity of angiotensin I on uterine muscle.

There is a difference, however, between the enzymes of those tissues which do carry out the conversion. Diisopropylfluorophosphate does not inhibit the plasma-converting enzyme or the converting enzyme of liver. It does inhibit that of aorta and, to a lesser extent, that obtained from heart.

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**Figure 1**

MINIMUM STRUCTURAL REQUIREMENTS OF ANGIOTENSIN II FOR BIOLOGICAL ACTIVITY

BOXES INDICATE POINTS OF SUBSTITUTION
SOLID LINE INDICATES AREAS NECESSARY FOR BIOLOGICAL ACTIVITY
BROKEN LINE ENCLOSURES HAVE MINOR OR NO SIGNIFICANCE
ARROWS INDICATE GROUPS WHICH REQUIRE FURTHER STUDY BEFORE THEIR ROLES CAN BE DEFINED
BIOLOGIC ACTIVITY OF ANGIOTENSIN II

This suggests the possibility that plasma-converting enzyme is synthesized in the liver. Prolonged incubation of these tissue homogenates with angiotensin completely destroys all biologic activity.

Both urea and arginine in high concentrations inhibit the oxytocic activity of angiotensin almost completely. The inhibition is reversible. We have suggested that the reagents are changing the conformation of angiotensin and thereby reducing its biologic activity.

From these data, it appears that both amino acid sequence and peptide conformation are necessary for biologic activity. The amino acid side groups undoubtedly must exist in a spatial relation to one another in order to react with a specific receptor site. Discovering the important "reactive sites" of angiotensin is a long, tedious task but a necessary one if we are to understand the mechanism by which it acts.

**Summary**

The requirements for biologic activity of angiotensin II may be summarized from available data as follows: (1) a free C-terminal carboxyl group, (2) a phenyl group as a side group of amino acid number 8, (3) a phenolic group on amino acid number 4, (4) proline in position 7, (5) possibly a definite degree of spatial order, (6) at least 6 amino acids from the C-terminus, (7) possibly the imidazole of histidine in position 6.

**References**

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