# THE STRUCTURE OF BRADYKININ - A PLASMA KININ FROM OX BLOOD

D.F. Elliott, G.P. Lewis and E.W. Horton

National Institute for Medical Research, Mill Hill, London, N.W. 7.

Received July 5, 1960

In a previous communication it was stated that bradykinin yielded on acid hydrolysis the amino acids serine, glycine, proline, phenylalanine and arginine in the molar proportions 1:1:2:2:2 and contained a single N - terminal residue of arginine (Elliott, Lewis and Horton, 1960a). In a later communication the sequence Arg.Pro.Pro.Gly.Phe.Ser.Phe.Arg. was proposed for bradykinin (Elliott, Lewis and Horton, 1960b). In the light of synthetic work carried out by Dr. Boissonnas and his collaborators (Boissonnas, Guttmann, Jaquenoud, Konzett and Stürmer, 1960) and communicated to the authors before publication, the above structure for bradykinin was clearly incorrect. Discussions between Dr. Boissonnas and ourselves led to the view that bradykinin contained three residues of proline and that its structure was Arg.Pro.Pro.Gly.Phe.Ser.Pro.Phe.Arg. Further degradative work has confirmed that this structure is the correct one.

Edman degradation of bradykinin.

Bradykinin (0.5 mg.) was degraded by the Edman method using the technique adopted for hypertensin (Elliott and Peart, 1957) whereby the phenylthiohydantoins obtained at each stage were converted into amino acids by reductive hydrolysis with hydriodic acid. Under these conditions a synthetic sample of

the PTH-derivative of serine was converted into alanine. The amino acids were identified by paper chromatography in pyridine:water (4:1). As expected, the first stage of the degradation gave no amino acid because arginine gives a PTH-derivative which cannot be extracted from aqueous solution. The sequence of the next four amino acids was found to be Pro.Pro.Gly.Phe., consequently the sequence of the first five residues of bradykinin was Arg.Pro.Pro.Gly.Phe. It was decided not to continue beyond this point owing to the accumulation of artefacts which made chromatographic identification progressively more difficult.

Action of chymotrypsin on bradykinin.

Bradykinin (0.5 mg.) was dissolved in 2 ml. of 0.05M ammonium bicarbonate:ammonium acetate buffer, pH 7.5, the solution was mixed with 0.15 ml. of a solution of chymotrypsin in the same buffer containing 2 mg. of the enzyme per ml. and was incubated for 18 hr. at 37°. After addition of 0.1 ml. of glacial acetic acid the solution was evaporated to dryness in a vacuum desiccator at room temperature. The residue had no action upon isolated smooth muscle preparations. Electrophoresis, on Whatman No. 1 paper, of a small portion of the residue in 2M-acetic acid using a voltage gradient of 25V/cm., gave the results shown in Fig. 1. Bands 2, 3 and 4, could be

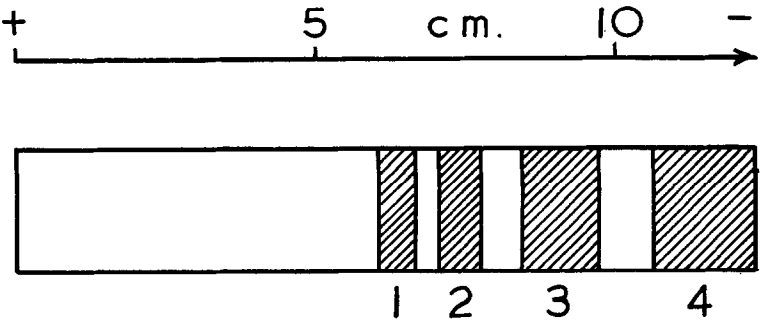


Fig. 1. Electrophoresis of a chymotryptic digest of bradykinin.

revealed by the Sakaguchi reagent (Jepson and Smith, 1953), or by ninhydrin, whereas band 1 was stained only by ninhydrin. The enzyme:substrate molar ratio in this experiment was 1:40; at a ratio of 1:100 bands 2 and 4 formed the main products and bands 1 and 3 were scarcely visible. This showed that one bond was hydrolysed more rapidly than the other by the enzyme. The main bulk of the chymotryptic digest was then submitted to electrophoresis as before and the products isolated by a technique previously described (Elliott and Peart, 1957). It was deduced that band 4 was arginine on the basis of the chromatographic behaviour of the substance itself and of its dinitrophenyl derivative; band 2 yielded on hydrolysis all five amino acids present in bradykinin. These two substances were clearly the products of fission of the first bond in bradykinin by chymotrypsin. When band 2 was submitted to the action of carboxypeptidase at 37° and pH 8 phenylalanine was liberated, thus showing that the C - terminal sequence of bradykinin was Phe.Arg. Hydrolysis of band 3 yielded arginine, proline, glycine and phenylalanine which were estimated as their dinitrophenyl derivatives by the method of Elliott, Lewis and Horton (1960b) and found to be in the molar proportions 1:2:1:1. Band 3 was evidently one of the products of fission of the second bond in bradykinin by chymotrypsin and its amino acid composition was consistent with the sequence Arg.Pro.Pro.Gly.Phe. deduced from the results of the Edman degradation on bradykinin. The N - terminal residue of band 1 was found to be serine by the fluorodinitrobenzene technique. Hydrolysis of band 1 and chromatography of the hydrolysate in butanol/acetic acid revealed the presence of serine, phenylalanine and a smaller amount of proline, but in view of the fact that two proline residues had already been placed it

was assumed that the presence of proline was due to contamination of band 1 by self-digestion products of the enzyme. Band 1 was therefore assigned the structure Ser.Phe. (Elliott, Lewis and Horton, 1960b), but as a result of the information given to us by Dr. Boissonnas it has now been examined more closely. After separation from a chymotryptic digest of 0.5 mg. of bradykinin by electrophoresis as already described, band 1 was further purified by chromatography on Whatman No. 1 filter paper in n-butanol:acetic acid:water (63:27:10).

Two equal portions of the product were hydrolysed with 6N-HCl for 16 hr. and 48 hr. respectively and the products subjected to chromatography in butanol/acetic acid. The yields of the amino acids in the hydrolysates were roughly estimated by comparison of the size and intensity of the spots with those of standard amounts of serine, proline and phenylalanine on the same chromatogram. The yield of proline from both the 16 hr. and the 48 hr. hydrolysates of band 1 was about 50% of the theoretical amount. Evidently destruction of proline took place at some stage before complete hydrolysis, but these results leave no doubt that band 1 has the structure Ser.Pro.Phe and not Ser.Phe as originally thought. The presence of proline in this position is also consistent with the fact that carboxypeptidase had no further action on the octapeptide (band 2) or its dinitrophenyl derivative after it had liberated phenylalanine. This was the case even at an enzyme:substrate molar ratio of 1:10.

## Conclusions

Bradykinin has the structure Arg.Pro.Pro.Gly.Phe.Ser.Pro.Phe.Arg and not Arg.Pro.Pro.Gly.Phe.Ser.Phe.Arg as originally proposed (Elliott, Lewis and Horton, 1960b). Destruction during acid hydrolysis probably accounts for the incorrect result obtained

for the proline content of bradykinin.

## REFERENCES

Boissonnas, R.A., Guttmann, St., Jaquenoud, P-A., Konzett, H. and Stürmer, E. Experientia. (In press).

Elliott, D.F., Lewis, G.P. and Horton, E.W. Biochem. J. 74, 15P (1960a).

Elliott, D.F., Lewis, G.P. and Horton, E.W. Biochem. J. (In press). (1960b).

Elliott, D.F. and Peart, W.S. Biochem. J. 65, 246 (1957).