THE PRIMARY SEQUENCE OF PHOSPHOLIPASE-A FROM BEE VENOM

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We recently reported [1] the purification and characterization of phospholipase-A from the venom of the common European honey bee (*Apis mellifica*). We now wish to report the primary (peptide) sequence of the enzyme.

Most experiments were carried out using reduced and carboxymethylated enzyme. A short digestion with trypsin gave 18 peptides, cleavage being observed at 6 arginine, 9 lysine, 1 tyrosine and 1 phenylalanine residues. The peptides were separated on Sephadex G-25 (superfine) and then by paper electrophoresis at pH 6.5 or pH 3.5 in solvent cooled tanks. Paper chromatography was used as an additional step where necessary. The amino acid sequence of the peptides was determined by the Dansyl-Edman procedure [2].

Similar experiments were carried out using the peptides derived from digestion with chymotrypsin, pepsin and elastase. Some of the peptides obtained by tryptic digestion of enzyme which had been reduced, carboxymethylated and maleylated were also examined. The *C*-terminal sequence was identified as X-Lys·Tyr-COOH by treatment with carboxypeptidase-A where X was not liberated by the enzyme. The *N*-terminal sequence was readily found by using the Edman procedure on the native enzyme.

The results of these experiments lead to the sequence shown in table 1.

The positions of cleavage produced by trypsin and chymotrypsin are shown by the symbols T and C respectively. All overlaps were satisfactorily established except for positions Tyr(76)–Phe(77). This particular peptide bond was cleaved by all the enzymes used and under all experimental conditions examined. It is, therefore formally possible that the sequence given is incomplete in that a fragment lying between residues (76) and (77) has escaped detection. That this is not the case was established by examining *all* the peptides produced by separate digestion with chymotrypsin, trypsin and pepsin. Furthermore, the stoichiometric amino acid composition calculated from the amino acid analysis [1] and from the sequence given in table 1 are in close agreement (A and B respectively).

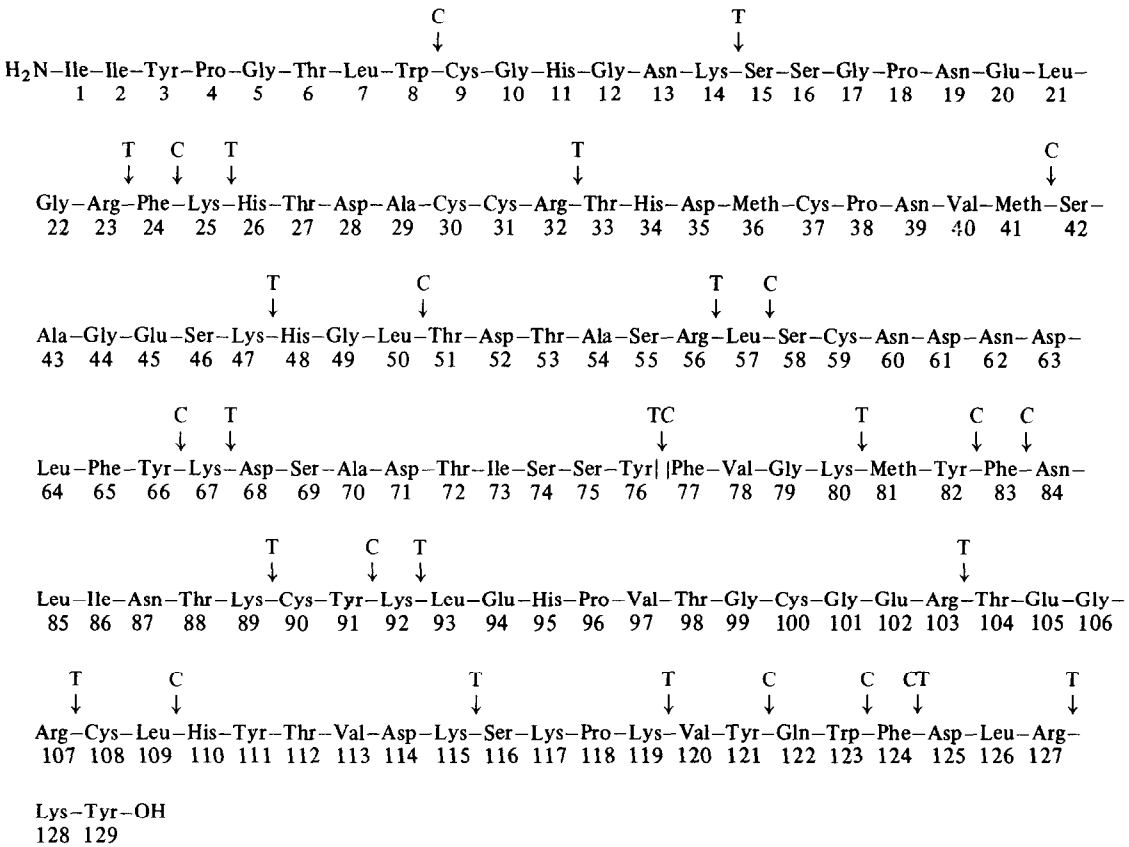
A Asx_{16} Thr_{11} Ser_{10} Glx_6 Pro_5 Gly_{11} Ala_4 Val_5 $½Cys_8$ Met_3 Ile_4 Leu_9 Tyr_8 Phe_5 Lys_{12} His_6 Arg_6 Trp_2

B Asx_{16} Thr_{10} Ser_{10} Glx_6 Pro_5 Gly_{11} Ala_4 Val_5 $½Cys_8$ Met_3 Ile_4 Leu_9 Tyr_8 Phe_5 Lys_{11} His_6 Arg_6 Trp_2

The molecular weight of the enzyme was reported by us [1], from ultracentrifugation measurements, as 20,000. However, the peptide sequence leads to a value of the molecular weight of 14,629. The discrepancy

Table 1

Proposed sequence of phospholipase-A from bee venom.



is due to the fact that the enzyme contains covalently bound carbohydrate. For example, the *N*-terminal peptide Ile(1)–Lys(14) contains glucosamine, mannose, galactose and fucose. The effect of this is that the molecular weight calculated from the amino acid sequence is too low whereas that calculated from the ultracentrifugation data is too high since the presence of carbohydrate lowers the partial specific volume.

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References

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