Amino Acid Composition of the Highly Purified Allergen from *Dirofilaria immitis*

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Abstract

The amino acid analysis of the purified allergen from *Dirofilaria immitis* revealed that it had a total number of 98 amino acid residues. The allergen was remarkably rich on glutamic acid (15 residues), lysine (14 residues) and aspartic acid (12 residues). No **half-cystine** was obtained. The ratio of the acidic residues to the basic ones was 1.4. The weak positive charge of the allergen is contributed by the fewer number of basic residues relative to acidic residues.

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Experiments reported in a preceding paper [1] showed that a highly purified allergen derived from a crude aqueous extract of *Dirofilaria immitis* was prepared by a combination of Sephadex G-200 gel filtration and ion-exchange chromatography. The highly purified allergen had a molecular weight of 20,000 and appeared as one band after sodium dodecyl sulfate polyacrylamide gel electrophoresis and one precipitin arc by immunoelectrophoresis. The allergen behaved as a positive charged protein, and was remarkably stable to various kinds of proteases, periodate digestion and physicochemical treatments [2]. The purpose of the present investigation was to determine the amino acid composition of the purified allergen and to know the relationship between the composition and the physico-chemical properties of the allergen. The purified allergen was hydrolyzed in sealed vials, under vacuum, in 6 N HCl for 24, 48 and 72 h at 110°C [3]. After removal of the hydrochloric acid by repeated concentration under reduced pressure, the amino acid content was determined by Hitachi amino acid analyzer. The amino acid composition of the allergen is shown in table I, giving a total number of 98 amino acid residues. The allergen contained all the naturally occurring amino acids except cystine. The allergen was remarkably rich in glutamic acid (15 residues), lysine (14 residues) and aspartic acid (12 residues), regardless of the time of hydrolyzation. According to the present views concerning atopic allergens, N-gly-cosidic linkages between aspartic acids may play a role for allergenic activity [4], and almost all allergens have lysine bonds [5]. The fact that the *Dirofilaria* allergen had such an amount of aspartic acid, as well as lysine residues, was interesting in understanding these common features among allergens.

Total number of the basic, neutral, and acidic residues was 20, 51 and 27, respectively. The net charge of a protein depends on the ratio of acidic to basic amino acid residues, and this can be estimated when the amino acid composition is known. As already reported by us [1], the allergen from *D. immitis* was positively charged, and it is contributed by the fewer number of basic

residues relative to acidic residues in the amino acid composition of the *Dirofilaria* allergen. The *Dirofilaria* allergen was also reported to be remarkably stable to chemical denaturation. The stability of the allergen, which is a common trait among the naturally occurring allergens, depends on the fact that the allergen contained no half-cystine at all. The *Dirofilaria* allergen contained a few residues of histidine, proline, tyrosine and phenylalanine. The limited number of arginine (4 residues) and methionine (4 residues) should prove to be valuable cleavage sites in future primary structure study of the purified *Dirofilaria* allergen.

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Table I. Amino acid composition of purified allergen from *D. immitis*. Amino acid residues were calculated for a molecular weight of 20,000

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