

Reactivity of Sequences Encompassing Tyrosine and Arginine Residues of Allergen M from Cod

S. ELSAYED AND J. APOLD

Laboratory of Clinical Biochemistry, and Department of Pediatrics, University of Bergen, Norway

On the basis of the strong amino acid sequence homology of cod Allergen M and carp pI 4.25, a close similarity in their tertiary structure was presumed. The high immunological cross reactivity of fragments TM1 and TM 2 of Allergen M supported this assumption (1).

Studies on the allergenicity of long sequence peptides of Allergen M showed that the sequences 33-44 and 88-96 tentatively incorporated allergenic determinants (2, 3). In the preceding abstract it was demonstrated that modification of either Tyr-30 and Arg-75 decreased the activity of the intact Allergen M. In the case of Arg-modification simultaneous perturbations of the native molecular conformation were encountered, precluding conclusions as to whether Arg-75 is located inside or outside a determinant. In this connection the reactivity of two tryptic peptides encompassing Tyr-30 and Arg-75 were investigated.

Fragment TM 1 with Tyr-30 was modified by either acetyl imidazole or *N*-bromosuccinimide reactions. The acetyl- and dibromodienonetyrosine TM 1 were recovered and tested.

Modification of tyrosine decreased the allergenic reactivity of fragment TM 1 as shown by the direct RAST

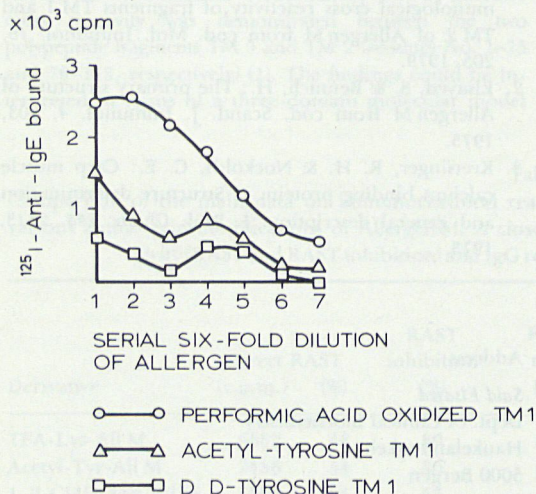


Fig. 1. Direct RAST results obtained by coupling TM 1 and TM 1 modified at Tyr-30 to the allergosorbent. The uptake of ¹²⁵I-anti-IgE is distinctly decreased for the modified derivatives. DD = dibromo-dienone.

results in Fig. 1. The results of RAST inhibition showed a decreased inhibition for either modifications. The antigenicity was similarly diminished by both modifications. In rocket-line immunoelectrophoresis, the end points of deflection of Allergen M line were 0.2, 2.5 and 5 µg of the TM 1 and derivatives, respectively. The findings indicated that Tyr-30 was involved on a sequential basis in an active determinant.

Arginine modification was investigated in a nonadecapeptide (65-83). This peptide was obtained from the intact blocking of guanido-Arg-75 prior to extensive tryptic hydrolysis. The results of RAST and RAST-inhibition were not significantly influenced by blocking Arg-75. Peptide 65-83 also showed *in vivo* allergenic activity using PK test. The wheal areas were 14

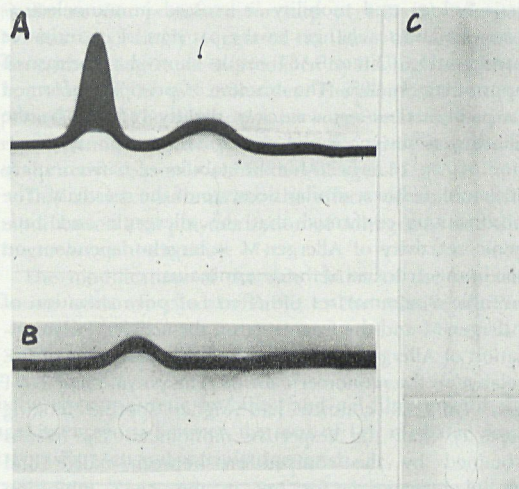


Fig. 2. A: Rocket-line immunoelectrophoresis showing the deflection of an Allergen M line precipitate by 40 µg of extensive tryptic hydrolysate of 1, 2-CHD Allergen M (applied in middle well), and the lack of deflection by the same amount of extensive tryptic hydrolysate of non-blocked Allergen M (right well). (In the left well 2.5 µg of Allergen M were applied). B: The corresponding deflection produced by approximately 1 µg of peptide 65-83 (applied in second well from left; in the other wells inactive extensive tryptic peptides). C: Direct rocket immunoprecipitate formed by approximately 2 µg of peptide 65-83.

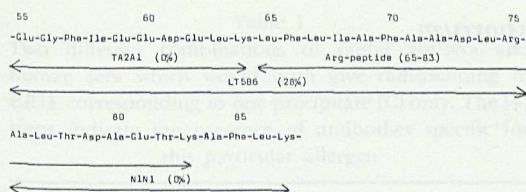


Fig. 3. The amino acid sequence of the 55-87 region, deduced from the known primary structure of Allergen M. The isolated tryptic hydrolysis peptides covering this region are underlined with their respective RAST inhibition values in brackets.

and 16 mm² for Arg-blocked and non-blocked peptides, respectively. Peptide 65-83 distinctly deflected Allergen M line precipitate in rocket-line immunoelectrophoresis (Fig. 2).

Considering the tertiary structure model of parvalbumins, this peptide is strategically located within the native polypeptide chain as:

1. It forms the junction between fragment TM 1 and fragment TM 2.
2. It covers the (Arg-75/Glu-81) salt bridge crucial for the Ca²⁺ binding function and is consequently essential for the maintenance of the molecular conformation.
3. It includes the axis relating the two analogous domains CD and EF (4).

In preceding publications three separate segments overlapping the peptide 65-83 were examined, as cited in Fig. 3. The limited tryptic hydrolysis peptide LT5B6 (55-75) was active in RAST inhibition tests (28% inhibition). The two other peptides TA2A1 (55-64) and N1N1 (76-87) were non-reactive. Integrating the data of the immunochemical reactivity of these peptides and the present results of Arg-75 modification justified the conclusion that the activity of peptides 65-83 was linked to the region 65-74.

This region partially forms the axis relating the assumed helices D and E of Allergen M. The corresponding peptide joining helices B and C (33-44) was investigated and tentatively shown to be reactive (3). The striking homology (five amino acid residues out of seven) between the two segments 35-41 and 67-73, not

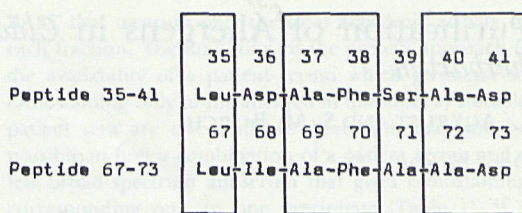


Fig. 4. Intramolecular homology of the peptides 35-41 and 67-73, showing identical residues at five of the seven positions. One of the two positions that differ (Ser/Ala) are accounted for by only a single base substitution in its triple codon.

systematically lineated in the phylogenetic homology of parvalbumins, provides further evidence as to the repetitive nature of the allergenic determinants (Fig. 4).

REFERENCES

1. Apold, J. & Elsayed, S.: Characterization of the immunological cross reactivity of fragments TM 1 and TM 2 of Allergen M from cod. *Mol. Immunol.* 16, 205, 1979.
2. Elsayed, S. & Apold, J.: The allergenic structure of Allergen M from cod. II. Allergenicity of the limited tryptic hydrolysis peptides of fragment TM 2. *Int. Arch. Allergy Appl. Immunol.* 54, 171, 1977.
3. Elsayed, S., Apold, J., Aas, K. & Bennich, H.: The allergenic structure of Allergen M from cod. I. Tryptic peptides of fragment TM 1. *Int. Arch. Allergy Appl. Immunol.* 52, 59, 1976.
4. Kretsinger, R. H. & Nockolds, C. E.: Carp muscle calcium binding protein. II-Structure determination and general description. *J. Biol. Chem.* 248, 2313, 1973.

Address:

Said Elsayed

Dept. of Clinical Biochemistry

Haukeland sykehus

5000 Bergen

Norway

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