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Address:

T. P. King
The Rockefeller University
New York, NY 10021
U.S.A.

Effect of Polymerization and Modification on the Immunochemical Reactivity of Cod Allergen M

S. ELSAYED AND J. APOLD

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

Allergen M belongs to a large group of fish muscle calcium binding proteins (parvalbumins), characterized by their ability to bind 2 mol Ca^{2+} /mol protein. The primary structure of Allergen M as well as several other members of parvalbumins has been established (2). The two Ca^{2+} -coordinations as well as the internal salt bridge between Arg-75 and Glu-81 are essential for the stability of the molecular conformation (3). An extensive cross reactivity was demonstrated between the two polypeptide fragments TM 1 and TM 2 (residues No. 1–75 and 76–113, respectively) (1). The findings could be interpreted in terms of a three-domain molecular model

similar to that postulated for carp pI 4.25 (3). Allergen M was accordingly suggested to have an analogous domains arrangement as predicted by the high sequence homology of the two molecules. The amino acid inter-periodicity of the three domains suggested that the immunochemical reactivity must be effected by repetitions of the active sequence within the Allergen M.

This abstract summarizes the effects of amino-acid modification and polymerization of Allergen M and the fragments on the activity.

The modification of the side chains of the selected amino acids are cited in Table 1. The relative elution

Table 1

Comparison of the main data on immunochemical reactivity, electrophoretic mobility, and gel filtration for the various amino acid modifications of Allergen M. A close agreement exists between the tests of IgE mediated reactivity (RAST and RAST inhibition) and IgG reactivity (Farr test and precipitation tests)

Derivative	Direct RAST (c.p.m.)	(%)	RAST inhibition (%)	Farr test (%)	Max. immune- PPT. (Turbid- ity, 340 nm)	Electro- phoretic mobility (mm/30 min)	Relative elution volume
TFA-Lys-All M	6682	48	30	61	0.42	19	0.41
Acetyl-Tyr-All M	7486	54	30	62	0.47	20	0.29
1, 2-CHD-Arg-All M	8865	64	57	51	0.52	12	0.38
EDTA-All M	9756	71	n.d.	n.d.	0.66	13	0.31
Oxindole-Tyr-All M	10 773	78	74	50	0.73	12	0.28
-Amidine-All M	11 357	83	63	n.d.	0.74	16	0.28
Native All M	13 741	100	80	31	0.73	10	0.28

Table 2

Pertinent data on the immunochemical reactivity of poly-Allergen M, poly-TM 1, and poly-TM 2 as compared to the monomeric forms

Allergen	Direct RAST (c.p.m.)	(%)	RAST inhibition (%)	Farr test (%)	Max. immune-PPT. (Turbidity, 340 nm)
Allergen M	8571	100	80	16	0.73
Poly-Allergen	5554	64	38	60	0.34
TM 1	4220	49	34	23	0.25
Poly-TM 1	5345	62	17	83	0.37
TM 2	2506	29	14	92	0.14
Poly-TM 2	5411	63	29	69	0.20

volume was used as criterion for changes of the native conformation expressed by the molecular size. The relative elution volume of the tyrosine or tryptophan modified All M and amidine-Allergen M were not significantly altered. The trifluoroacetyl (TFA) Lys and cyclohexane-dione (CHD-) Arg derivatives showed decreased ratios. The molecular size of EDTA-Allergen M, with no calcium ligands, was slightly increased. Direct RAST results were comparatively reduced by all the side-chain modifications. RAST inhibition showed similar tendencies. The treatment with EDTA- and the release of calcium led to an altered precipitate morphology and mobility in crossed immunoelectrophoresis and to changes in the patterns of quantitative precipitation. Direct RAST results showed a decrease of approximately 30%. The amount of precipitate formed in precipitation test was only slightly reduced by the changes of the molecular conformation. The modification of Arg-75, crucial for the stability of native tertiary structure, led to a similar decrease of the reactivity. The observations confirmed that the allergenic and antigenic reactivity of Allergen M is largely dependent on the linear structure of the determinants.

Table 2 summarizes the effects of polymerization of Allergen M and the fragments on the activity. Polymerization of Allergen M led to a diminished binding of IgE relative to the monomeric forms. The polymers of TM 1 and TM 2 in contrast had higher specific binding capacity than the respective monomers. The results obtained by the competition between native and modified materials for IgE in the RAST inhibition experiments generally agreed with these findings. Similarly the polymerization of Allergen M and TM 1 reduced the antigenicity relative to the monomeric forms.

In contrast, the cross linking of fragment TM 2 to a trimeric molecule distinctly enhanced the immunochemical reactivity in all test systems. The trimeric TM 2 molecule has approximately the same molecular

size as Allergen M and consists of an analogous number of domains. In addition it has an extra Ca^{2+} coordination sequence.

Rabbits immunized with poly-Allergen M had distinctly lower antibody titer against Allergen M than those immunized with the native Allergen M (a ratio of 1:20). Poly TM 2 gave no detectable antibody response. The decrease of the immunogenicity of the polymerized derivatives indicated that polymerized allergens are not generally valid in immunotherapy.

Parallel decrease of reactivities of chemically modified derivatives was found in tests using IgE and IgG antibodies. This suggests the presence of sequence homologies between the binding sites of Allergen M molecule for both antibody populations.

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Address:

Said Elsayed
Dept. of Clinical Biochemistry
Haukeland sykehus
5000 Bergen
Norway

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