



## Expression and evaluation of IgE-binding capacity of recombinant Pacific mackerel parvalbumin

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**Background:** Parvalbumin is the major and cross-reactive allergen in fish. Sufficient amounts of IgE-reactive recombinant fish parvalbumin are needed for diagnosis and immunotherapy of fish allergy.

**Methods:** A DNA fragment corresponding to parvalbumin of the Pacific mackerel *Scomber japonicus* was synthesized and cloned into the expression vector pGEX-6p-3 to produce glutathione S-transferase (GST)-fusion parvalbumin in *Escherichia coli*. The GST-free recombinant parvalbumin was purified using the RediPack GST Purification Module (Amersham Pharmacia Biotech, Buckinghamshire, UK). Parvalbumins of seven species of fish (Japanese eel, horse mackerel, red sea bream, Pacific mackerel, skipjack, bigeye tuna and Japanese flounder) were purified by gel filtration and reverse-phase HPLC. The IgE-binding capacity was examined by ELISA and antigenic cross-reactivity by inhibition ELISA.

**Results:** The GST-free recombinant Pacific mackerel parvalbumin was obtained in an electrophoretically pure state. Data from ELISA and inhibition ELISA revealed that the recombinant parvalbumin contains most of the IgE-binding epitopes of the natural counterpart. In addition, the recombinant parvalbumin inhibited the IgE reactivities of the pooled patient serum to parvalbumins purified from six species of fish in almost the same magnitude as the natural Pacific mackerel parvalbumin.

**Conclusions:** Because the recombinant Pacific mackerel parvalbumin bearing the IgE-binding capacity of the natural counterpart is cross-reactive with various fish parvalbumins, it can be a useful tool for the diagnosis and immunotherapy of fish allergy.

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