

Molecular and Immunological Characterization of Arginine Kinase from the Indianmeal Moth, *Plodia interpunctella*, a Novel Cross-Reactive Invertebrate Pan-Allergen¹

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IgE recognition of indoor allergens represents a major cause of allergic asthma in atopic individuals. We found that 52 of 102 patients suffering from allergic symptoms indoors contained IgE Abs against allergens from the Indianmeal moth (*Plodia interpunctella*), a ubiquitous food pest. Using serum IgE from a moth-sensitized patient we screened an expression cDNA library constructed from *P. interpunctella* larvae. cDNAs coding for arginine kinase (EC 2.7.3.3), a 40-kDa enzyme commonly occurring in invertebrates that is involved in the storage of such high-energy phosphate bonds as phosphoarginine, were isolated. Recombinant moth arginine kinase, designated Plo i 1, was expressed in *Escherichia coli* as a histidine-tagged protein with enzymatic activity, and purified to homogeneity by nickel chelate affinity chromatography. Purified recombinant arginine kinase induced specific basophil histamine release and immediate as well as late-phase skin reactions. It reacted with serum IgE from 13 of the 52 (25%) moth-allergic patients and inhibited the binding of allergic patients' IgE to an immunologically related 40-kDa allergen present in house dust mite, cockroach, king prawn, lobster, and mussel. Our results indicate that arginine kinases represent a new class of cross-reactive invertebrate pan-allergens. Recombinant arginine kinase may be used to identify a group of polysensitized indoor allergic patients and for immunotherapy of these individuals. *The Journal of Immunology*, 2001, 167: 5470–5477.

Type I allergic disorders such as rhinoconjunctivitis, atopic dermatitis, and bronchial asthma afflict up to 25% of the population (1). Arthropods represent more than three quarters of all animal species, and some of those which get into close contact with humans are a major allergen source. Whereas the indoor allergens from the house dust mite (2) and cockroach (3–5), and the allergenic venoms from the vespids (6) have been studied in detail, much less is known about allergens from moths.

That moths could be the causative agent of inhalant allergies had been mentioned as early as 1928 by Vaughan (7). Over the years, there have been scattered case reports on bronchial asthma caused by the clothes moth *Tineola bisselliella* (8) and wax moth *Galleria mellonella* (9). Baldo and Panzani (10) and more recently Komase

et al. (11) characterized various insect extracts by IgE immunoblotting and demonstrated several IgE-Ags in the clothes moth or silkworm moth. In studies conducted in Japan, a high proportion of patients with asthma bronchiale (12, 13) or allergic rhinitis (14) were found to react with silkworm moth allergens.

In recent years the Indianmeal moth, *Plodia interpunctella*, has become a widely spread household and stored product pest throughout the United States and Europe. Its larvae feed on dry foodstuffs such as nuts, grains, dried fruit, and chocolate (15). Although it was mentioned as a possible cause of allergies in a review on allergens in mills (16), no detailed studies have been performed whether the Indianmeal moth represents an indoor allergen source. We examined a panel of 102 sera from indoor allergic patients and found a high prevalence of IgE reactivity against Indianmeal moth Ags. One of these IgE-reactive Ags was characterized on the molecular level, and was identified as an arginine kinase by cDNA cloning, demonstration of sequence homology, and enzymatic activity of the recombinant protein. Finally, we demonstrate that this allergen has IgE cross-reactive homologs in several invertebrate species such as mite, cockroach, lobster, king prawn, and mussel.

Materials and Methods

Patients

Sera from the following groups of patients were tested for the presence of IgE Abs against moth allergens: 1) patients with type I allergic symptoms (rhinitis/conjunctivitis, allergic asthma bronchiale) indoors ($n = 90$, patients H1–H90, ages ranging from 17 to 60 years, average age 32 years); 2) patients with type I allergic symptoms indoors as above plus atopic dermatitis ($n = 12$, patients AH1–AH12, ages from 11 to 47 years, average age 28 years); 3) control individuals without type I allergies or atopic dermatitis ($n = 10$, individuals N1–N10, ages from 26 to 35 years, average age 31 years).

The diagnosis of type I allergy was based on case history, skin prick testing, and CAP-RAST (radioallergosorbent test; Pharmacia, Uppsala,

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Sweden) testing using a panel of extracts from indoor (house dust mite, cat dander) and outdoor (birch pollen, grass pollen) allergen sources. The diagnosis of atopic dermatitis was based on the criteria of Hanifin and Rajka (17). The cDNA library was screened with the serum from patient AH11. Skin prick tests were performed in patients AH11 and H60.

IgE reactivity of natural moth, mite, and cockroach extracts as well as the purified recombinant arginine kinase

Preparations from two moth species, house dust mite, and cockroach were used to detect specific IgE in patients' sera. Extracts from the Indianmeal moth *P. interpunctella* were obtained by homogenizing 25 late-stage larvae per 1 ml of PBS. Reducing gel loading buffer was added 1/1, samples were denatured for 10 min at 95°C, and debris was removed by centrifugation in a microcentrifuge (5 min, room temperature, 10,000 × g). In the same way, extracts were prepared from commercial preparations from adult Mediterranean flour moth (*Ephestia kuehniella*), house dust mite (*Dermatophagoides pteronyssinus*), and cockroach (*Blattella germanica*) obtained from Allergon Pharmacia (Uppsala, Sweden). The extracts or the purified arginine kinase were electrophoresed on preparative 12.5% SDS-polyacrylamide gels with an approximate protein concentration of 20 µg cm⁻¹ (extracts) or 10 µg cm⁻¹ (purified recombinant arginine kinase) as estimated by Coomassie blue-stained test gels. Proteins were blotted onto nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany), and 5-mm strips were cut from the sheets after the transfer. The nitrocellulose membranes were blocked 2 × 5 min and 1 × 30 min at room temperature with three changes of buffer G (42 mM Na₂HPO₄, 6.4 mM NaH₂PO₄, 0.5% (v/v) Tween 20, 0.5% (w/v) BSA, 0.05% (w/v) Na₂S₂O₃, pH 7.5) and incubated with a 1/10 dilution of patients' sera in buffer G overnight at 4°C. After washing 2 × 5 min and 1 × 30 min in buffer G, bound IgE was detected by overnight incubation at room temperature with ¹²⁵I-labeled anti-IgE Abs (Pharmacia), washing as above, and autoradiography.

Construction and IgE immunoscreening of a cDNA library from P. interpunctella larvae

The insect larvae were grown on oats. One hundred eighty larvae (2.4 g) in the prepupal stage were homogenized in 30 ml of TRIzol reagent (Life Technologies, Frederick, MD), and total RNA was prepared. Poly(A)⁺ RNA was prepared with the Poly(A)Tract system (Promega, Madison, WI). The cDNA library was prepared in the Uni-ZAP system (Stratagene, La Jolla, CA) according to the supplier's protocol. The primary library from 5 µg of poly(A)⁺ RNA contained 3 × 10⁶ clones and was amplified with standard methods.

λ ZAP phages (360,000 in total) were used to infect *Escherichia coli* XL1-Blue (Stratagene) in 24 140-mm petri dishes. Synthesis of recombinant proteins was induced by adding nitrocellulose filters soaked in 10 mM isopropylthio β-D-galactoside. The filters were blocked and probed with the serum from patient AH11 as described above.

Sequence analysis of the IgE-reactive clones

The cDNA-containing plasmids were obtained from the 31 isolated IgE-reactive phages by in vivo excision (18). The DNAs were sequenced using Thermosequase (Amersham Pharmacia Biotech, Piscataway, NJ) and IRD800-labeled primers (MWG Biotech, Ebersberg, Germany) on a LI-COR sequencer (LI-COR, Lincoln, NE).

The deduced protein sequences were compared with sequences deposited in the SwissProt database using the FastA program (19). Clones were aligned with each other and homologous protein sequences with the GAP program from the University of Wisconsin Genetics Computer Group package (Madison, WI) (20). Further protein sequence analysis was performed by software provided at the ExPASy molecular biology server (<http://www.expasy.ch/tools/>) such as NetPhos (21), PROSITE (22), and NetOglyc (23) for the predictions of putative phosphorylation and N- and O-linked glycosylation sites.

Expression and purification of the recombinant moth allergen in E. coli

One full-length cDNA, coding for a 40-kDa protein with end-to-end sequence similarity with arginine kinases, was inserted in two steps between the *Eco*RI and *Xho*I sites of the plasmid pET23⁺ (Novagen, Madison, WI). The ribosome binding site was inserted by oligonucleotide-directed mutagenesis (24) using the oligonucleotide 5'-GGT AGC GGC GTC CAC CAT GGT ATA TCT CCT TCT AGA GGG AAA CCG-3' giving the vector pETAK1. A second mutagenesis with the oligonucleotide 5'-ATC TCA GTG GTG GTG GTG GTG CAG GGA TTT CTC GAT TTT GAT-3' inserted the coding sequence for a hexahistidine tag for purification by nickel chelate affinity chromatography giving plasmid pETHisAK1.

This plasmid was checked by DNA sequencing and transformed into *E. coli* BL21 (DE3) for protein expression. The cells were grown at 37°C to an optical density at 600 nm of 0.8. Recombinant protein synthesis was induced for 3 h by adding isopropylthio β-D-galactoside to a final concentration of 0.4 mM. The cells were pelleted and lysed by 30-min treatment in buffer L (50 mM NaH₂PO₄, 300 mM NaCl, 10 mM imidazole, 1 mg ml⁻¹ (w/v) lysozyme, pH 8). Undissolved material was pelleted by 30-min centrifugation at 2000 × g and 4°C. The recombinant protein was then purified by nickel chelate affinity (25) under native conditions using small spin columns (Qiagen, Hilden, Germany).

Measurement of the arginine kinase activity of the recombinant allergen

Arginine kinase (Enzyme Commission number: EC 2.7.3.3) activity was measured by determining the rate of formation of ADP. The ADP is converted back to ATP by pyruvate kinase, and the pyruvate formed is reduced to lactate by lactate dehydrogenase. The rate of consumption of NADH in this reaction is measured photometrically (26). Protein concentration was estimated from the optical density at 280 nm and the extinction coefficient calculated from the deduced protein sequence of the recombinant allergen (27). The reaction was performed at 30°C in 1-ml volumes containing 2 mM L-arginine and 4 mM ATP, 50 mM Tris/acetate, 5 mM Mg-acetate, 0.75 mM phosphoenolpyruvate, 0.2 mM NADH, 10 µg ml⁻¹ pyruvate kinase, and 12.5 µg ml⁻¹ lactate dehydrogenase at pH 6.8.

The measured activity was 18.5 U mg⁻¹, which corresponded to a turnover number *k*_{cat} of 12.3 s⁻¹. When either arginine or ATP was omitted from the reaction mixture, no activity was observed.

Histamine release assay

Heparinized blood samples were obtained from the patients H20 and AH11 with IgE-reactivity to arginine kinase, and granulocytes were prepared by dextran sedimentation (28). Cells were resuspended in histamine release buffer and incubated with increasing concentrations of recombinant moth allergen or, for positive control, with anti-IgE mAb E124-2-8 (Immuno- tech, Marseille, France) at 37°C for 30 min. Then cells were sedimented by centrifugation at 4°C and the cell-free supernatants were recovered. Liberated histamine expressed as percentage of total histamine was measured in the cell-free supernatants by radioimmunoassay (Immuno- tech) (28). Triplicate determinations of histamine release by the recombinant moth allergen were conducted.

Skin prick test

After written informed consent was obtained, skin prick tests were performed in two atopic individuals, one with (AH11) and one without (H60) IgE, against the recombinant moth arginine kinase. Purified recombinant allergens (recombinant arginine kinase (this study) and, for control purposes, recombinant birch pollen allergen Bet v 1 (Biomay, Linz, Austria)) were diluted in sterile 0.9% NaCl to five different concentrations: 50 ng µl⁻¹ allergen, 25 ng µl⁻¹, 12.5 ng µl⁻¹, 6.25 ng µl⁻¹, and 3.12 ng µl⁻¹. NaCl (0.9%) and histamine dihydrochloride (Allergopharma, Reinbek, Germany) in a concentration of 1 mg ml⁻¹ were used for negative or positive controls.

IgE immunoblot inhibition experiments

The cross-reactivity of the moth arginine kinase with allergens from mite (*D. pteronyssinus*), cockroach (*B. germanica*), king prawn (*Penaeus monodon*), lobster (*Homarus gammarus*), mussel (*Mytilus edulis*), and cod (*Gadus morhua*) was tested in this experiment. Fresh, uncooked seafood was purchased from a local market, and white meat was prepared. The different samples (1–5 g) were frozen in liquid nitrogen and crushed to a powder in a mortar. Ice-cold H₂O containing 5 mM PMSF was added and Ags were extracted by stirring for 1 h at 4°C. After addition of 1 volume of gel loading buffer, samples were denatured for 10 min at 95°C and insoluble particles were removed by centrifugation. The protein concentration of the extracts was estimated on a Coomassie blue-stained SDS-PAGE gel. Preparative 12.5% gels containing 20 µg cm⁻¹ protein were run and blotted onto nitrocellulose which was cut into strips. The sera from patients AH11, H89, and H32 were diluted 1/10 in buffer G and preincubated in buffer G with or without 10 µg ml⁻¹ recombinant moth arginine kinase overnight at 4°C, and then exposed to the nitrocellulose-blotted extracts from the different species. Bound IgE was detected as described for IgE immunoblotting.

Results

IgE recognition frequency and profile of *P. interpunctella* Ags

To examine the prevalence of IgE recognition and the allergen profile of the Indianmeal moth, we used sera from patients with type I allergic symptoms indoors ($n = 90$, H1–H90, average age 32), sera from patients with type I allergic symptoms and atopic dermatitis in addition ($n = 12$, AH1–AH12, average age 28), and sera from nonallergic individuals ($n = 10$, N1–N10, average age 31) to detect nitrocellulose-blotted IgE-reactive Ags (Figs. 1 and 2, and Table I). Forty-two of 90 indoor allergic patients (47%) and 10 of the indoor allergic patients with atopic dermatitis (83%) had IgE Abs against *P. interpunctella* larval Ags. The m.w. profile of the IgE-binding components varied significantly for the different patients' sera, and a number of Ags of various m.w. were recognized. More than one-third of the sera revealed significant IgE reactivity to high molecular mass components above 70 kDa. None of the nonallergic control individuals exhibited IgE reactivity to the *P. interpunctella* extract. Reactivity of the ^{125}I -labeled anti-human IgE Abs with a component of around 66 kDa was noted in one set of experiments (Fig. 1).

All the sera were also tested with an extract from the commercially available mature flour moths *E. kuehniella* (Allergon Pharmacia) (data not shown, summary in Table I). The results were comparable with those obtained for the Indianmeal moth larval extracts. Thirty-four (38%) from the indoor allergic group without atopic dermatitis and six (50%) from the indoor allergic group with atopic dermatitis showed specific IgE reactivity.

When we tested those sera containing IgE-Abs against Indianmeal moth larval Ags with commercial extracts of house dust mite

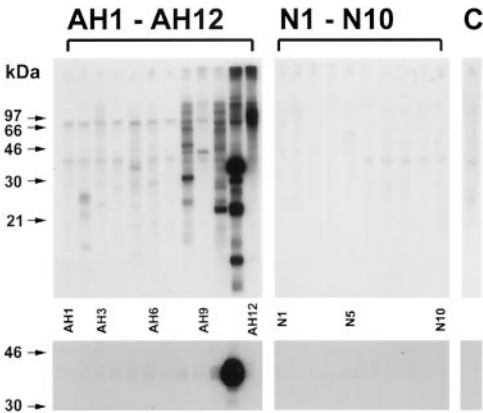


FIGURE 2. Nitrocellulose-blotted Indianmeal moth larval extracts (*top*) and recombinant arginine kinase (*bottom*) as probed with serum IgE from 12 indoor allergic patients with atopic dermatitis in addition (AH1–AH12) and with 10 nonallergic individuals (N1–N10). The positions of molecular mass markers are given at the *left side*. C represents the buffer control without addition of serum.

(*D. pteronyssinus*) and cockroach (*B. germanica*), we found that many of the sera did not cross-react with mite or cockroach Ags. As many as 23% of all moth-allergic patients had IgE reactive with neither mite nor cockroach extracts (Table II).

Identification of arginine kinase as *P. interpunctella* allergen

Thirty-one IgE-reactive clones were isolated from a λ ZAP cDNA library prepared from *P. interpunctella* larvae using the serum of

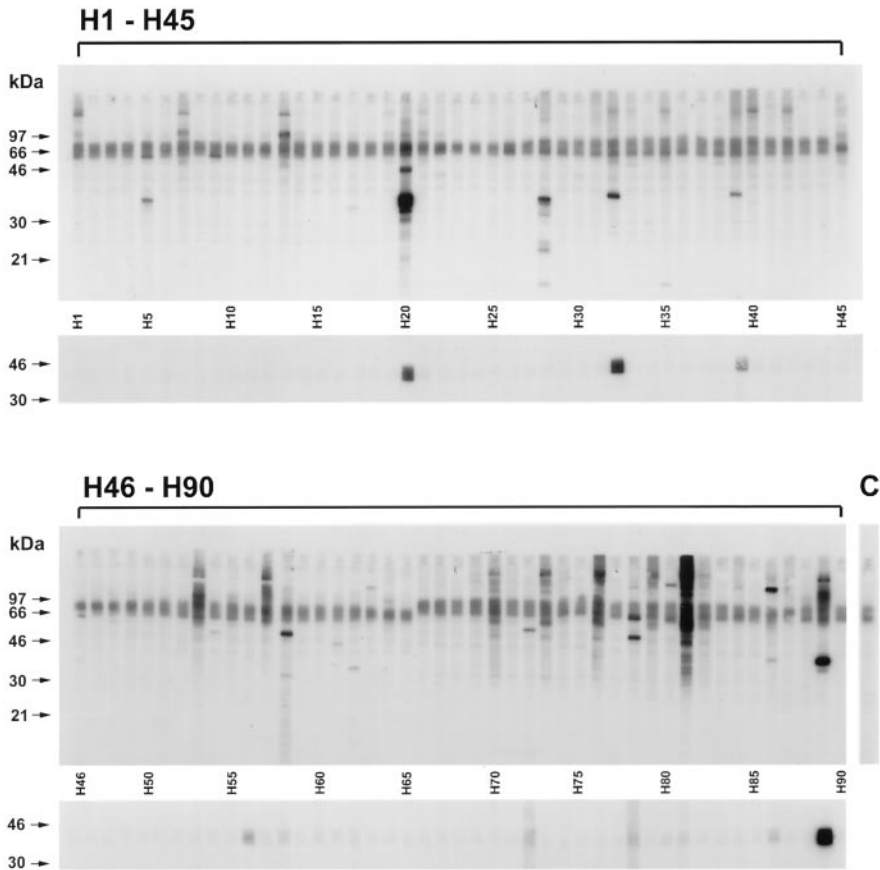


FIGURE 1. Nitrocellulose-blotted Indianmeal moth larval extracts (*top*) and recombinant arginine kinase (*bottom*) as probed with serum IgE from 90 indoor allergic patients (H1–H90). The positions of molecular mass markers are given at the *left side*. C represents the buffer control without addition of serum.

Table I. Patients' demographic data, symptoms, and IgE-immunoblot results for moth Ags^a

	Indoor Allergic Patients Without Atopic Dermatitis	Indoor Allergic Patients With Atopic Dermatitis	All Indoor Allergic Patients
Patients			
Number of patients	90	12	102
Age range	17–60	11–47	11–60
Average age	32	28	31
Female:male ratio	38:52	6:6	44:58
Symptoms			
Rhinitis	82 (91%)	8 (67%)	90 (88%)
Conjunctivitis	82 (91%)	10 (83%)	92 (90%)
Asthma bronchiale	25 (28%)	4 (33%)	29 (28%)
IgE reactivity to nitrocellulose-blotted			
Indianmeal moth larvae	42 (47%)	10 (83%)	52 (51%)
Recombinant arginine kinase	10 (11%)	3 (25%)	13 (13%)
Flour moths	34 (38%)	6 (50%)	40 (39%)

^a Summary of patients' demographic data, symptoms, and IgE-immunoblot results for two different moth allergen extracts and the recombinant moth arginine kinase. Percentages refer to the total number of individuals tested in each column.

patient AH11. Although the clones obtained in the first screening experiment had different insert sizes, all of them were derived from the same cDNA. Two more screening experiments using another three patients' sera identified three more *P. interpunctella* IgE-binding Ags (B. Hayek, unpublished data). The longest cDNA clone coded for a polypeptide of 39.9 kDa including an initiator methionine (Fig. 3). The untranslated regions were 24 bp at the 5'-end and 195 bp at the 3'-end upstream of the poly(A) tail. Comparison with the databases showed an end-to-end similarity of the deduced amino acid sequence with arginine kinases from various arthropod species. The closest homologs of the moth enzyme were arginine kinases from the grasshopper (*Schistocerca americanus*) (29) and the honeybee (*Apis mellifera*) (30), with 86 and 85% amino acid sequence identity, respectively (Fig. 3). Fig. 3 shows that there was also a very high degree of sequence identity (82%) with the enzyme from lobster (*H. gammarus*) (31). Even an arginine kinase from a protozoan, *Trypanosoma cruzi* (32), had 70% identical residues.

The moth arginine kinase has a predicted isoelectric point of 6.24. The NetPhos program identified seven high-probability phosphorylation sites, underlined in Fig. 3 (Ser²⁰, Ser⁴⁴, Ser¹⁵⁶, Ser²⁸¹, Thr³³³, Tyr⁷⁴, Tyr¹³³, and Tyr¹⁴⁴). All except Ser²⁰ were conserved in all three species. No O-glycosylation sites were identified, but there was one possible N-glycosylation site at Asn²¹⁴ and an actinin-type actin-binding site (residues Glu²¹³ to Asn²²², marked in Fig. 3). In this context it is noteworthy that scallop muscle arginine kinase AK1 binds to filamentous actin, leading to noncompetitive inhibition of the enzyme (33). Recently, the crystal structure of arginine kinase from the horseshoe crab (*Limulus polyphemus*) has been reported (34). All the residues putatively interacting with the substrates (Arg¹²⁶, Glu²²⁵, Arg²²⁹, Cys²⁷¹, Thr²⁷³, Arg²⁸⁰, Arg³⁰⁹, and Glu³¹⁴) are also present in the corre-

sponding positions in the moth, honeybee, and lobster arginine kinases (double-underlined in Fig. 3).

Purification of recombinant enzymatically active *P. interpunctella* arginine kinase

The pET23⁺-derived expression plasmid pETHisAK1 was constructed, and recombinant *P. interpunctella* arginine kinase was expressed and purified as shown in Fig. 4. The induced cultures produced the recombinant protein as a major, soluble protein which could be purified under native conditions to high purity by a single step of nickel chelate affinity chromatography. The yield was around 5 mg of purified protein per 1000 ml of *E. coli* culture. A standard coupled assay for arginine kinase activity was performed using the recombinant enzyme, and an activity of 18.5 U/mg of protein was measured, corresponding to a turnover number of 12.3 molecules per second.

Recombinant *P. interpunctella* arginine kinase binds to allergic patients' IgE and specifically induces histamine release and skin reactions

All the sera from the patients and control individuals were tested for specific IgE against the nitrocellulose-blotted recombinant arginine kinase. Ten of the 90 house dust allergic patients and three of 12 patients with atopic dermatitis and indoor allergy, but none of the nonallergic individuals, had IgE Abs to recombinant arginine kinase. This corresponded to 13% of all patients and 25% of the patients who were IgE-reactive with moth larval extract (Figs. 1 and 2, and Table I).

The allergenic activity of recombinant arginine kinase was demonstrated by histamine release assay using basophils from the two

Table II. IgE-immunoblot results of Indianmeal moth-reactive sera tested with mite and/or cockroach Ags^a

	Indoor Allergic Patients Without Atopic Dermatitis	Indoor Allergic Patients With Atopic Dermatitis	All Indoor Allergic Patients
Number of patients	90	12	102
Indianmeal moth-reactive patients	42	10	52
Negative with mite extract	22 (52%)	2 (20%)	24 (46%)
Negative with cockroach extract	18 (43%)	3 (30%)	21 (40%)
Negative with mite and cockroach extracts	10 (24%)	2 (20%)	12 (23%)

^a Patients' sera containing IgE against Indianmeal moth larval Ags were tested for IgE against house dust mite and cockroach extracts. The numbers of sera with IgE against moth Ags but without IgE against mite or cockroach Ags or both are displayed. The percentages refer to the total number of moth-reactive sera in each group.

FIGURE 3. cDNA and deduced protein sequence of arginine kinase from the Indianmeal moth *P. interpunctella* (Pi). The amino acid sequences from the honey bee *A. mellifera* (Am) and lobster *H. gammarus* (Hg) arginine kinases were aligned to the sequence from the Indianmeal moth, with dots representing identical residues and dashes representing gaps. The putatively phosphorylated Ser, Thr, and Tyr residues as well as the predicted actinin-type actin-binding site were single-underlined. The residues predicted to interact with the substrate were double-underlined. The nucleotide and amino acid sequences are available from the GenBank/EBI databases under the accession number AJ315030.

1	TCAAGTGTGAGAAAGCAGCAGCA	Pi
25	ATGGTGGACGCCGTACCTTGAGAAATTGGAGGCTGGCTTCAGCAAGCTT---GCCGCCTCCGACTCAAAGTCGCTGCTG	Pi
1	M V D A A T L E K L E A G F S K L - A A S D S K S L L	Pi
1	. . . Q . V . D . . . T . . . S S	Am
1	. A . . . I A . . . E . . . K . . . E . . . T . C . . .	Hg
103	AAGAAATACCTCACCAGGGAGGTATTGATGCTCTCAAGAACAGAGACCTCATTGGTCAACTCTCCTGGATTCTATC	Pi
27	K K Y L T R E V F D A L K N K K T S F G S T L L D S I	Pi
27	. . . S K D . . . Q . . . T D C .	Am
28	. . . S K D I . . . S . . . A L . A V .	Hg
184	CAGTCAGGTGTTGAGAACTTACATTCCGGTGTGGAATTATGCCCCAGATGCTGAGGCATATGATGATTGTCAGACTTG	Pi
54	Q S G V E N L H S G V G I Y A P D A E A Y V V F A D L	Pi
54	. . . I . . . D . . . S T L	Am
55	 D K A . D F . . . S	Hg
265	TTCGACCCCATCATTGAAGATTACCAATGGCTTCAAGAAACGACAAGCACCTCCCAAGAACTGGGGAGATGTTGAG	Pi
81	F D P I I E D Y H N G F K K T D K H P P K N W G D V E	Pi
81	 G Q A . D F . . . S	Am
82	 K Q A . D F . . . S	Hg
346	ACCCTCGGAAGTGGATCTGCTGGTGAATTGTTGTCTCCACCCGTGTCGCTCGCTCCATGGAAGGCTACCCA	Pi
108	T L G N L D P A G E F V V S T R V R C G R S M E G Y	Pi
108	S N . . . I L	Am
109	K F I . V . . . E . . . T . . . I	Hg
427	TTCAACCCCTGCTTAACAGAGGCCAATACAAGAAATGGAAGAGAAAGTCTCTCCACTCTCCGGCTCGAGGGTGAA	Pi
135	F N P C L T E A Q Y K E M E E K V S S T L S G L E G E	Pi
135	. .	Am
136	. .	Hg
508	CTGAAAGGCACCTTTTCCCACTCACAGGCTATGCAAGGAGACTCAACAACAGTGATTGATGACACTTCTGTTCAG	Pi
162	L K G T F F P L T G M S K E T Q Q Q L I D D H F L F K	Pi
162	 Y K	Am
163	. . . S Y T . . . V . . . K	Hg
589	GAGGGTGATCGTTCTCCAGGCCGCTAACGCTTGCCGCTTCTGGCCCTCCGGTCTGGCATCTTACCACATGAGAACAAG	Pi
189	E G D R F L Q A A N A C R F W P S G R G I Y H N E N K	Pi
189	 T D D . . .	Am
190	 Y . . . A D . . .	Hg
670	ACTTCTCGTGTGTCATGAGGAGGACCACTCCGCTGATCTCCATGCAAAATGGGCGCGACCTGAAGCAGGTGTAC	Pi
216	T F L V W C N E E D H L R L I S M Q M G G D L K Q V Y	Pi
216	. G . . .	Am
217	. G . . .	Hg
751	AAGAGGCTGGTGAGGGGAGTGAACGACATCGCGAAGAGGATCCCATCTCGCACAACGAGCGGCTGGGCTTCTGACTTTC	Pi
243	K R L V R G V N D I A K R I P F S H N E R L G F L T F	Pi
243	R H A . . . E . . . L L D . . .	Am
244	R S A . . . E . . . V D . . .	Hg
832	TGCCCCACCAACCTGGGCACACCGGTGCGCGCATCGGTGCACATCAAGCTGCCCAAGTGGCGCGCCAGAGCCCAAGCTG	Pi
270	C P T N L G T T V R A S V H I K L P K L A A D K A K L	Pi
270	. N R .	Am
271	. N R E .	Hg
913	GAGGAGTGGCCAGCAAGTACCACCTGCAGGTGCGCGGCCCGCGCGGACACAGGAGCCGAGGCGCGGCTCTACGAC	Pi
297	E E V A S K Y H L Q V R G T R G E H T E A E G G V Y D	Pi
297	. . I . . G . F N I . .	Am
298	. . . A . F S I . .	Hg
994	ATCTCCAACAAGAGGCGCATGGGACTCACCGAGTACGAAGCCGTCAAGGAGATGTACGAGCGCATCGTGAAGTATGATCAA	Pi
324	I S N K R R M G L T E Y E A V K E M Y D G I A E L I K	Pi
324	 L Q H G V Y D	Am
325	 F Q Q L . .	Hg
1075	ATCGAGAAATCCCTGTAAGATGTTAACGATCTCGCGCTATCAGTATTTTTGTATTATTATCGTTCACATAAGTATT	Pi
351	I E K S L *	Pi
351	L . . E L	Am
352	. . . E M	Hg
1156	GGATGTGAAGGGGCGAGGGCGACACTAGTCAGCGGCCTTGAGCGGGGCGGGCACGCGGGCGGCCACTATACTGTTTCGT	Pi
1237	AAAAGTATTGTCTATAAGGAAATGGAATAAAGACAGCTAGCGTTAAGACAAAAA	Pi

sensitized patients H20 and AH11 (Fig. 5, A and B). In both patients, the recombinant allergen induced a dose-dependent histamine release with a maximum between 1 and 10 ng ml⁻¹ and a

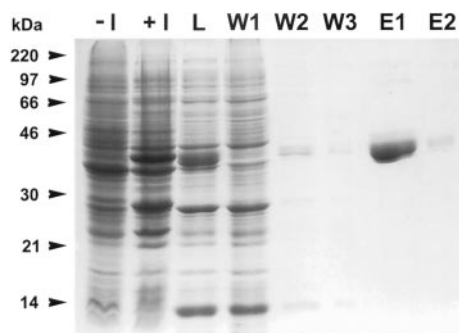


FIGURE 4. Coomassie-stained gel showing the purification of the recombinant arginine kinase. Lanes: -I, whole *E. coli* cells before induction; +I, whole cells after induction; L, lysate from induced cells; W1, W2, W3, three wash fractions from nickel chelate columns; E1, first elution step from column; E2, second elution step. The positions of molecular mass markers are given at the left side.

significant release with as low as 10 pg ml⁻¹ allergen for patient H20 and 100 pg ml⁻¹ for patient AH11.

Skin prick testing with recombinant arginine kinase induced typical flare and wheal reactions in a sensitized patient AH11 but not in patient H60, who had IgE-Abs only to Bet v 1 (Fig. 6). Recombinant Bet v 1 induced skin reactions in both patients having specific IgE Abs. After 24 h patient AH11 developed papules within the marked borders of the previous immediate reactions to the moth arginine kinase and to Bet v 1, being indicative of a late-phase reaction (not shown). No reactions in either patient were induced with physiological NaCl alone.

Arginine kinases from the Indianmeal moth, mite, cockroach, king prawn, lobster, and mussel share IgE epitopes

The moth arginine kinase displayed high sequence similarity with homologs from other species (Fig. 3). To investigate whether cockroach (*B. germanica*), mite (*D. pteronyssinus*), king prawn (*P. monodon*), lobster (*H. gammarus*), mussels (*M. edulis*) and cod (*Gadus morhua*), a vertebrate, contained allergens cross-reactive with moth arginine kinase, IgE immunoblot inhibition experiments were performed. Preincubation of sera from patients (AH11, H89, and H32) containing specific IgE against the recombinant moth

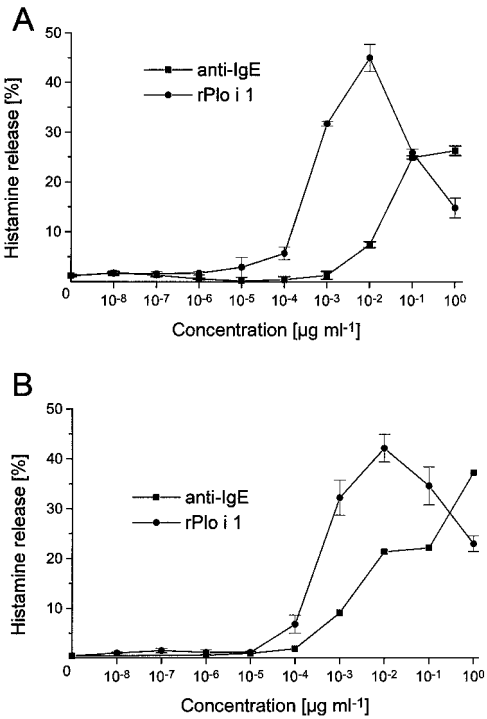


FIGURE 5. Recombinant moth arginine kinase induces histamine release from basophils of two sensitized patients H20 (A) and AH11 (B). Basophils were incubated with various concentrations of moth allergen Plo i 1 or, as a positive control, with anti-IgE Abs as indicated on the x-axis. Ag-specific histamine release (circles) or anti-IgE induced histamine release (squares) are represented as percentage of total histamine on the y-axis.

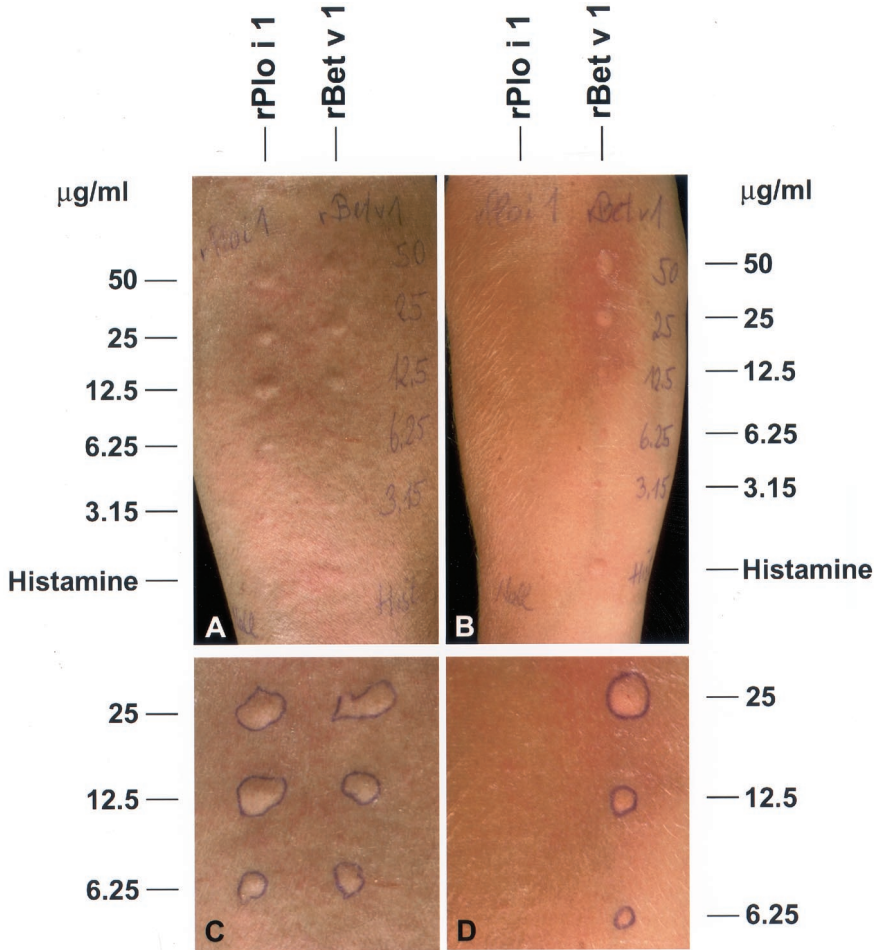
arginine kinase inhibited IgE binding to a 40-kDa allergen in cockroach, house dust mite, lobster, king prawn, and mussel, but not in cod extracts (Fig. 7). IgE reactivity to the 40-kDa protein was blocked completely after preincubation with moth arginine kinase in the cockroach, house dust mite, and mussel extracts. In lobster and king prawn strong IgE binding was observed to the 40-kDa component, which was blocked only partially by recombinant moth arginine kinase. No inhibition of IgE binding to cod fish allergens by the allergen was observed (Fig. 7).

Discussion

In this study we identify moths, in particular Indianmeal moths, as a relevant source of indoor allergens. Of 102 sera from indoor allergic patients, 51% had IgE-Abs to nitrocellulose-blotted Indianmeal moth extract. Testing with sera from a subgroup of 12 indoor allergic patients with atopic dermatitis revealed 83% IgE-reactivity. As many as 23% of the moth-allergic patients displayed IgE only to moth but not to house dust mite or cockroach extracts. The molecular mass profiles of the moth allergens showed significant variation, but a high molecular mass complex above 70-kDa was recognized by more than a third of the tested patients.

So far, moth allergens have not been characterized on the molecular level. We therefore constructed a moth cDNA library and used the serum from a moth-sensitized patient to identify IgE-reactive clones. A set of overlapping cDNA clones all coded for a 40-kDa Ag with high end-to-end similarity with arginine kinases from various invertebrate species. This is probably due to a very strong IgE reactivity of the patient to this Ag and, in addition, to a high level of expression of arginine kinase in the moth larvae.

FIGURE 6. Induction of immediate type skin reactions against recombinant Indianmeal moth arginine kinase and recombinant birch pollen allergen Bet v 1. A and C, moth and birch pollen-allergic patient AH11; B and D, Birch pollen-allergic and indoor allergic patient H60 without sensitization to moth proteins.



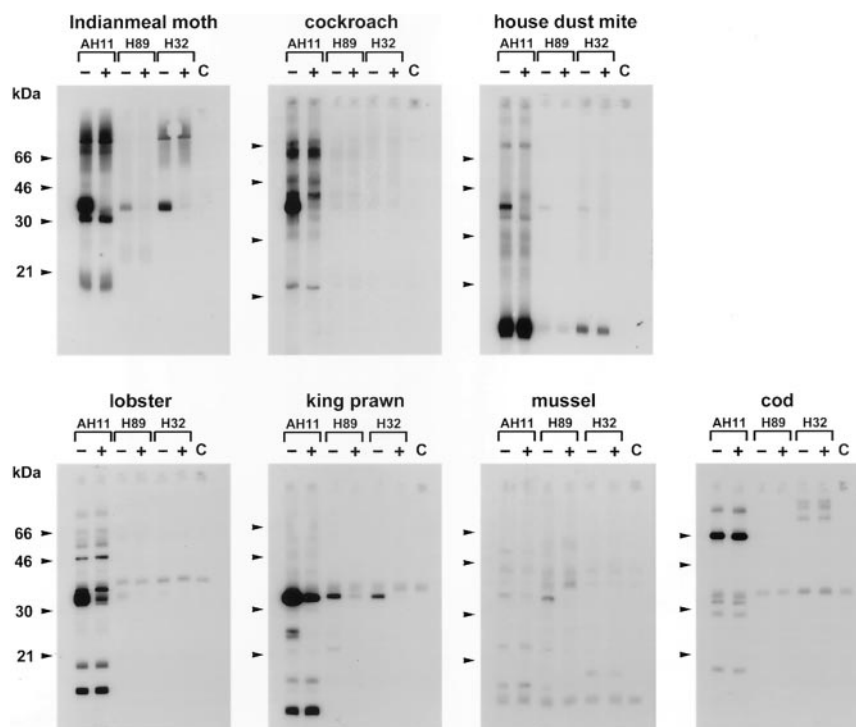


FIGURE 7. IgE immunoblot inhibition experiment. Nitrocellulose-blotted extracts from moth, cockroach, house dust mite, lobster, king prawn, mussel, and cod were probed with three arginine kinase-positive sera (AH11, H89, and H32), pre-incubated either with recombinant arginine kinase (+) or with buffer only (-). The positions of molecular mass markers are indicated at the left side. Lanes C represent the buffer controls without added serum.

Further screening experiments using other patients' sera led to the identification of several other moth allergens (B. Hayek, unpublished data).

Arginine kinases (EC 2.7.3.3) catalyze the reversible transfer of a high-energy phosphate from ATP to L-arginine yielding ADP and *N*-phospho L-arginine. In various invertebrate species, excess energy can thus be stored as arginine phosphate (31, 35), whereas vertebrate species use creatine phosphate to store energy and therefore possess creatine kinases. To our knowledge, arginine kinases have not been described as allergens; however, Lin et al. (36) obtained partial peptide sequences from a shrimp (*Parapenaeus fissurus*) allergen, which shared similarity with the arginine kinase sequence published soon afterward by Dumas and Camonis (31).

We produced and purified the recombinant arginine kinase and demonstrated its enzymatic activity. IgE immunoblot experiments showed that 13% of all indoor allergic patients and 25% of the moth-allergic patients had specific IgE to the recombinant allergen.

Previous studies by Baldo and Panzani (10) and by Komase et al. (11) had demonstrated strong IgE-binding to a 40-kDa component in immunoblots of various insects or silkworm moth, but this component was not identified. In the present study, the allergenic activity of the recombinant moth arginine kinase was demonstrated by its ability to induce the specific and dose-dependent release of histamine from basophils down to a concentration of 10 pg ml⁻¹, and to give specific skin reactions. We therefore suggest a tentative designation of Plo i 1 for this first identified allergen from the Indianmeal moth.

Arginine kinases with highly similar amino acid sequences have been identified in various invertebrate species. The results from our IgE immunoblot inhibition experiments identify arginine kinase-related allergens of 40 kDa in mite (*D. pteronyssinus*), cockroach (*B. germanica*), king prawn (*P. monodon*), lobster (*H. gammarus*), and mussel (*M. edulis*). No homologous cross-reactive component was observed in the cod, a vertebrate species. These data suggest that arginine kinases represent a novel class of invertebrate cross-reactive pan-allergens which may be implicated in

the induction and maintenance of respiratory and food allergy in polysensitized patients (10, 36).

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References

- Kay, A. B. 1997. *Allergy and Allergic Disease*. Blackwell Science, Oxford, U.K.
- Thomas, W. R., and W. Smith. 1999. Towards defining the full spectrum of important house dust mite allergens. *Clin. Exp. Allergy* 29:1583.
- Rosenstreich, D. L., P. Eggleston, M. Kattan, D. Baker, R. G. Slavin, P. Gergen, H. Mitchell, K. McNiff Mortimer, H. Lynn, D. Ownby, and F. Malveaux. 1997. The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. *N. Engl. J. Med.* 336:1356.
- Arruda, L. K., L. D. Vailes, D. C. Benjamin, and M. D. Chapman. 1995. Molecular cloning of German cockroach (*Blattella germanica*) allergens. *Int. Arch. Allergy Immunol.* 107:295.
- Van Wijnen, J. H., A. P. Verhoeff, D. K. Mulder Folkerts, H. J. Brachel, and C. Schou. 1997. Cockroach allergen in house dust. *Allergy* 52:460.
- King, T. P., and M. D. Spangfort. 2000. Structure and biology of stinging insect venom allergens. *Int. Arch. Allergy Immunol.* 123:99.
- Vaughan, W. T. 1928. Some causes for failure in the specific treatment of allergy. *J. Lab. Clin. Med.* 13:955.
- Urbach, E., and P. M. Gottlieb. 1941. Asthma from insect emanations. *J. Allergy* 12:485.
- Stevenson, D. D., and K. P. Mathews. 1967. Occupational asthma following inhalation of moth particles. *J. Allergy* 39:274.
- Baldo, B. A., and R. C. Panzani. 1988. Detection of IgE antibodies to a wide range of insect species in subjects with suspected inhalant allergies to insects. *Int. Arch. Allergy Appl. Immunol.* 85:278.
- Komase, Y., M. Sakata, T. Azuma, A. Tanaka, and T. Nakagawa. 1997. IgE antibodies against midge and moth found in Japanese asthmatic subjects and comparison of allergenicity between these insects. *Allergy* 52:75.
- Kino, T., and S. Oshima. 1978. Allergy to insects in Japan. I. The reaginic sensitivity to moth and butterfly in patients with bronchial asthma. *J. Allergy Clin. Immunol.* 61:10.
- Kino, T., and S. Oshima. 1979. Allergy to insects in Japan. II. The reaginic sensitivity to silkworm moth in patients with bronchial asthma. *J. Allergy Clin. Immunol.* 64:131.
- Suzuki, M., H. Itoh, K. Sugiyama, I. Takagi, J. Nishimura, K. Kato, S. Mamiya, S. Baba, Y. Ohya, H. Itoh, et al. 1995. Causative allergens of allergic rhinitis in Japan with special reference to silkworm moth allergen. *Allergy* 50:23.
- Reichmuth, C., M. Schöller, and C. Ulrichs. 1997. *Vorratsschädlinge im Getreide*. Th Mann, Gelsenkirchen, Germany, p. 119.

16. Wittich, F. W. 1940. The nature of various mill dust allergens. *Journal-Lancet* 60:418.
17. Hanifin, J. M., and G. Rajka. 1980. Diagnostic features of atopic dermatitis. *Acta Derm. Venereol. Suppl.* 92:44.
18. Short, J. M., J. M. Fernandez, J. A. Sorge, and W. D. Huse. 1988. λ ZAP: a bacteriophage λ expression vector with in vivo excision properties. *Nucleic Acids Res.* 16:7583.
19. Pearson, W. R., and D. J. Lipman. 1988. Improved tools for biological sequence comparison. *Proc. Natl. Acad. Sci. USA* 85:2444.
20. Genetics Computer Group. 1994. *Program Manual for the Wisconsin Package, Version 8*. Madison, WI.
21. Blom, N., S. Gammeltoft, and S. Brunak. 1999. Sequence- and structure-based prediction of eukaryotic phosphorylation sites. *J. Mol. Biol.* 294:1351.
22. Hofmann, K., P. Bucher, L. Falquet, and A. Bairoch. 1999. The PROSITE database, its status in 1999. *Nucleic Acids Res.* 27:215.
23. Hansen, J. E., O. Lund, N. Tolstrup, A. A. Gooley, K. L. Williams, and S. Brunak. 1998. NetOglyc: prediction of mucin type O-glycosylation sites based on sequence context and surface accessibility. *Glycoconj. J.* 15:115.
24. Kunkel, T. A., J. D. Roberts, and R. A. Zakour. 1987. Rapid and efficient site-specific mutagenesis without phenotypic selection. *Methods Enzymol.* 154:367.
25. Hochuli, E. H. Dobeli, and A. Schacher. 1987. New metal chelate adsorbent selective for proteins and peptides containing neighboring histidine residues. *J. Chromatogr.* 411:177.
26. Anosike, E. O., B. H. Moreland, and D. C. Watts. 1975. Evolutionary variation between a monomer and a dimer arginine kinase. *Biochem. J.* 145:535.
27. Gill, S. C., and P. H. van Hippel. 1989. Calculation of protein extinction coefficients from amino acid sequence data. *Anal. Biochem.* 182:319.
28. Valent, P., J. Besemer, M. Muhm, O. Majdic, K. Lechner, D. Maurer, and P. Bettelheim. 1989. Interleukin-3 activates human blood basophils via high affinity binding sites. *Proc. Natl. Acad. Sci. USA* 86:5542.
29. Wang, Y. E., P. Esbensen, and D. Bentley. 1998. Arginine kinase expression and localization in growth cone migration. *J. Neurosci.* 18:987.
30. Kucharski, R., and R. Maleszka. 1998. Arginine kinase is highly expressed in the compound eye of the honey bee, *Apis mellifera*. *Gene* 211:343.
31. Dumas, C., and J. Camonis. 1993. Cloning and sequence analysis of the cDNA for arginine kinase of lobster muscle. *J. Biol. Chem.* 268:21599.
32. Pereira, C. A., G. D. Alonso, M. C. Paveto, A. Iribarren, M. L. Cabanas, H. N. Torres, and M. M. Flawia. 2000. *Trypanosoma cruzi* arginine kinase characterization and cloning: a novel energetic pathway in protozoan parasites. *J. Biol. Chem.* 275:1495.
33. Reddy, S. R., A. Houmeida, Y. Benyamin, and C. Roustan. 1992. Interaction of scallop muscle arginine kinase with filamentous actin. *Eur. J. Biochem.* 206:251.
34. Zhou, G., T. Somasundaram, E. Blanc, G. Parthasarathy, W. R. Ellington, and M. S. Chapman. 1998. Transition state structure of arginine kinase: implication for catalysis of bimolecular reactions. *Proc. Natl. Acad. Sci. USA* 95:8449.
35. Wyss, M., D. Maughan, and T. Wallimann. 1995. Re-evaluation of the structure and physiological function of guanidino kinases in fruitfly (*Drosophila*), sea urchin (*Psammechinus miliaris*) and man. *Biochem. J.* 309:255.
36. Lin, R.-Y., H.-D. Shen, and S.-H. Han. 1993. Identification and characterization of a 30 kd major allergen from *Parapenaeus fissurus*. *J. Allergy Clin. Immunol.* 92:837.