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In Silico Characterization and Comparative Analysis of Allergenicity of Allergic Proteins from Different Food Sources

[1,2]Sourav Chakraborty, [1,2]Shakhawat Hossain Foysal, [1,2]Md. Nazmul Hasan and [1,2]Nahian Khan

[1]*Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet-3114, Bangladesh*
[2]*CANSi Research Institute, SUST, Sylhet, Bangladesh*

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Corresponding Author:
Sourav Chakraborty
Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet-3114, Bangladesh
Email: souravgeb91@gmail.com

Abstract: Allergy is a steadily increasing health problem for all age groups. In general, it's recommended that 10-35% of our daily calories come from protein. A complete protein source is one that provides all of the essential amino acids called high quality proteins. Although animal-based foods; for example, meat, poultry, fish, milk, eggs, are considered complete protein sources these are the common sources of causing allergenicity. In case of meat *Bos taurus*, prawn *Penaeus monodon* and egg *Gallus gallus* are found to be the most responsible for triggering allergenicity. The current study has disclosed the best alternative sources for meat, prawn and egg through in silico characterization and comparative analysis of allergic proteins (Myoglobin Ovomucoid, Lysozyme, Ovalbumin, Ovotransferrin, Tropomyosin) with other common sources of meat (*Capra hircus*, *Ovis aries*, *Gallus gallus*, *Sus scrofa*), prawn (*Fenneropenaeus merguiensis*, *Macrobrachium rosenbergii*, *Metapenaeus ensis*, *Pandalus borealis*) and egg (*Anas platyrhynchos*). Analyzing the results we found that *Gallus gallus*, *Macrobrachium rosenbergii*, *Anas platyrhynchos* would be the safe source for meat, prawn and egg respectively.

Keywords: Allergenicity, High Quality Proteins, Allergy, Allergic Proteins

Introduction

The term food allergy is used to describe an adverse immune response to foods (Johansson *et al*., 2004). Allergy is a steadily increasing health problem for all age groups in the United States. Food allergies, mostly against milk, eggs, peanuts, soy, or wheat, affect up to 8% of infants and young children (Sampson, 1999a; 2005). A 2008 Centers for Disease Control and Prevention report indicated an 18% increase in childhood food allergy from 1997 to 2007, with an estimated 3.9% of children currently affected. Branum and Lukacs, (2008). One hypothesis is that this late onset may be the result of individuals being sensitized by long-term exposure to environmental factors that contain proteins similar to those in the known triggers of allergenic response (Sampson, 1999b; Vanek-Krebitz *et al*., 1995; Scheurer *et al*., 1999; Rabjohn *et al*., 1999). Recent studies have identified common molecular features of proteins from different sources, which could account for clinically important cross-reactivity (Breiteneder and Ebner, 2000; Jenkins *et al*., 2005) and sensitivity (Ferreira *et al*., 2004; Mari, 2001). Some common animal proteins from meat, egg, shrimp, cow's milk (Das *et al*., 2005) have been identified and characterized as major allergens. Myoglobin protein of mammalian meats causes allergy may be based on subtle changes of amino acids. Other cause may be a heat-resistant nature of the protein of 17 kDa that could be implicated in those patients that do not tolerate well-cooked meat (Fuentes *et al*., 2004). The only major allergen (Pen a 1) identified in shrimp is the muscle protein, tropomyosin (Daul *et al*., 1994). Surprisingly, there is no report of *M. rosenbergii* allergy in any medical literature. From the anaphylaxis study at the Siriraj hospital, Thailand, there were subpopulations of shrimp allergic patients who developed anaphylaxis to freshwater shrimp but could tolerate seawater shrimp or vice versa

(Jirapongsananuruk *et al*., 2007). The specific role of egg ovalbumin has been found in patients allergic to cow milk, casein, along with other two milk proteins immunoreacted with IgE antibody (Szabo and Eigenmann, 2000). Although infants can, in theory, be allergic to any food, one of the major food allergies is hen's egg (Du Toit *et al*., 2009). Most people who are allergic to hen's eggs have antibodies which react to one of four proteins in the egg white: Ovomucoid, ovalbumin, ovotransferrin and lysozyme (Platts-Mills and Ring, 2005). Fish allergy is one of the most common food allergies mediated by IgE antibody. Consumption of fish products could lead to symptoms like skin rash, dermatitis, urticarial, angioedema, gastrointestinal problems, diarrhoea, respiratory distress and even fatal systemic anaphylactic reactions (Pascual *et al*., 1992; O'Neil *et al*., 1993). Allergenic proteins that have been isolated from primary food sources, such as egg (Mine and Rupa, 2004) (ovomucoid (Mizumachi and Kurisaki, 2003; Mine and Zhang, 2002; Mine *et al*., 2003) and lysozyme (Fremont *et al*., 1997), shrimp and related species (tropomyosins (Ayuso *et al*., 2002; Reese *et al*., 2002; Samson *et al*., 2004)). The present study is an attempt to analyze the componential and structural similarity among different allergic proteins present in different foods to compare allergenicity among different allergic protein and identify common motifs. The newly discovered sequence motif along with the analyses of the structures of these allergens will not only help in the understanding of structure-function relationship of these allergens but also in the identification of new allergic protein.

Materials and Methods

The amino sequences of 18 different allergic proteins were retrieved from the NCBI (http//www.ncbi. nlm.nih. gov/genomes/FLU/). The Protparam tool at ExPASy (http://www. expasy.org/tools/) was used to analyze physic-chemical properties of two proteins i.e., amino acid compositions in all the species under consideration. The SOPMA tool at ExPASy server was exploited for comparative secondary structure analysis. The protein sequences of meat allergens are aligned using the Clustal W2 program (http://www.ebi.ac.uk/tools/clustalw2). Phylogenetic trees were constructed by the neighbor-joining method. The computer software of the Molecular Evolution Genetic Analysis (MEGA), version 5.2 was utilized in this study for phylogenetic analysis of selected sequences. The MEME (http://meme.nbcr.net/meme/) software was used to elect the motifs from different protein sequences.

Results

The 18 protein sequences of allergens were retrieved from NCBI. The accession number of retrieved sequences along with species names is listed in Table 1. Multiple Sequence Alignment using ClustalW2 are shown in three separate figures (Fig. 1 to 3). The phylogenetic tree using neighbor joining mode revealed three major clusters of protein sequences (Fig. 4). Biochemical features for 18 allergic protein were obtained by using ProtParam are listed in Table 2. Secondary structure analysis were done by using SOPMA software and shown in Table 3. MEME analysis resulted in frequently observed 3 motifs (Table 4).

Discussion

The sequences were characterized for homology search, multiple sequences alignment, biochemical features, phylogenetic tree construction and motifs search using various bioinformatics tools. Multiple Sequence Alignment using ClustalW2 provided that the myoglobin protein of *Bos taurus* shows similarity score of 97.4 with *Capra hircus and Ovis aries*. The myoglobin protein of *Gallus gallus* and *Sus scrofa* shown similarity score of 72.73 and 88.31 respectively with *Bos taurus* (Fig. 1). The tropomyosin protein of *Penaeus monodon* showed similarity score of 100 with *Fenneropenaeus merguiensis* and with *Macrobrachium rosenbergii*, *Metapenaeus ensis*, *Pandalus borealis* it was ranges from 96.83-98.24 (Fig. 2). The egg allergic proteins-ovomucoid, lysozyme, ovalbumin, ovotransferrin of *Anas platyrhynchos* and *Gallus gallus* shown similarity score of 73.68, 80.27, 76.68, 79.45 respectively (Fig. 3).

The phylogenetic tree based on protein sequences revealed three major clusters. Cluster 1, a cluster containing 5 sequences under study, includes meat allergens (Fig. 4).

Biochemical features for this cluster are listed in Table 2. The total number of amino acid residues was 154 with variable molecular weights. pI values of this cluster ranged from 6.63 to 7.96. Variations among various allergens in this group in terms of other physiochemical parameters like positively charged and negatively charged residues, hydropathicity (GRAVY) are given in Table 2. Aliphatic index analysis reveals uniformity in this group of allergens within the range of 79.87 to 86.88. Aliphatic index of protein measures the relative volume occupied by aliphatic side chains of the amino acids: Alanine, valine, leucine and isoleucine. Globular proteins with high aliphatic index have high thermo stability and an increase in aliphatic index increases protein thermo stability (Ikai, 1980; Rawlings *et al*., 2006).

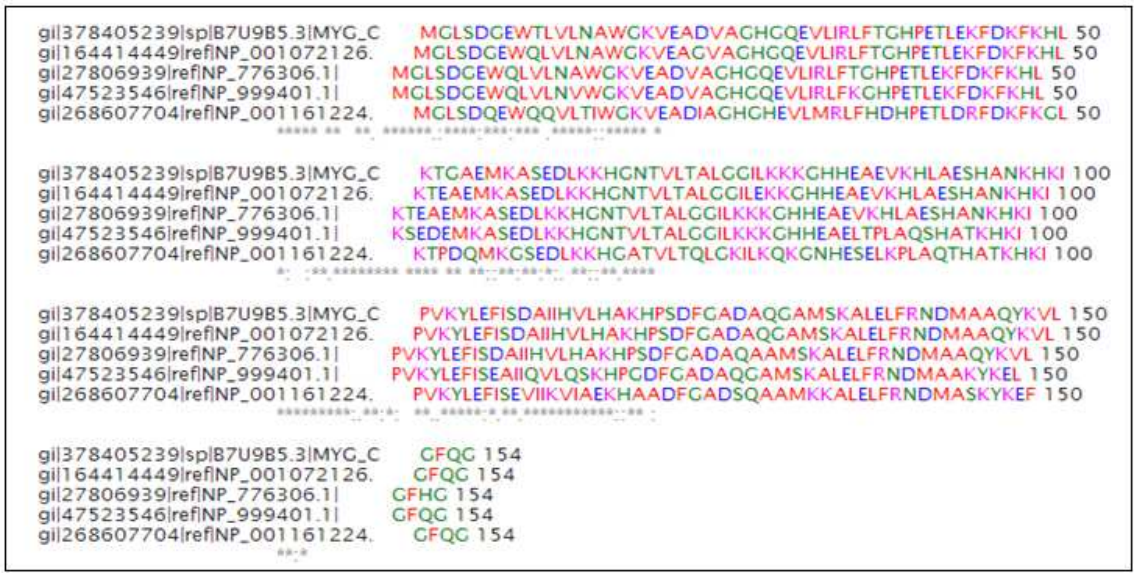

Fig. 1. Alignment result of meat allergic proteins

Fig. 2. Alignment result of shrimp allergic proteins

Fig. 3. Alignment result of egg allergic proteins

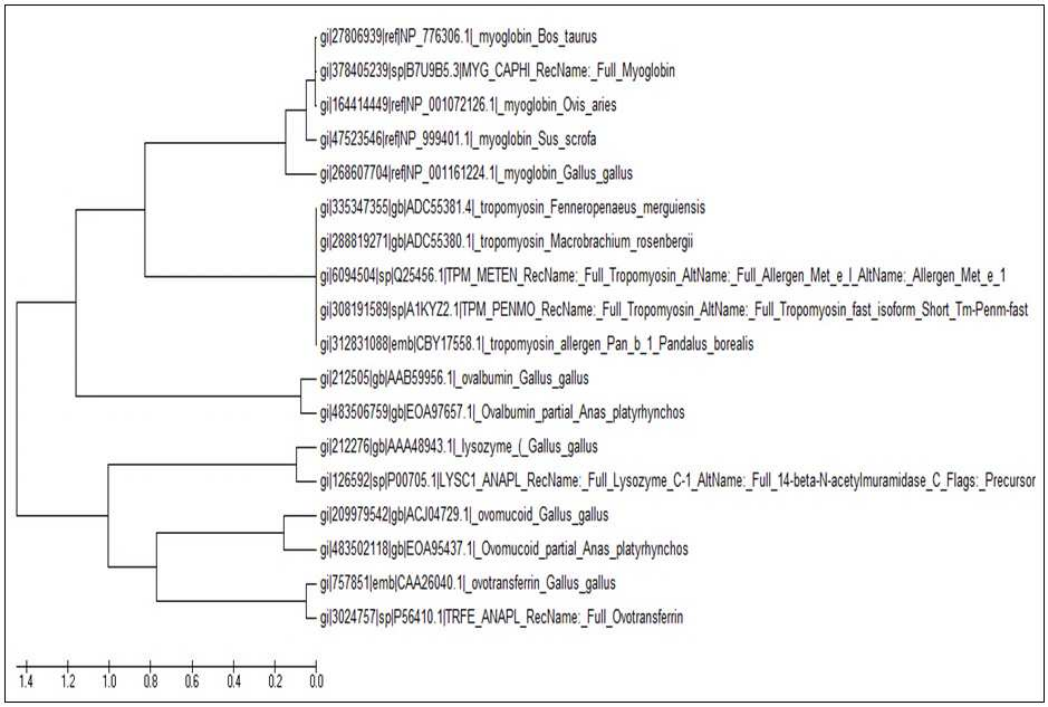

Fig. 4. Phylogenetic tree constructed by NJ method based on allergic protein sequences

Table 1. List of retrieved protein sequences from NCBI their accession number

Source organism	Protein	Accession No
Bos taurus	Myoglobin	NP_776306.1
Capra hircus	Myoglobin	B7U9B5.3
Ovis aries	Myoglobin	NP_001072126.1
Gallus gallus	Myoglobin	NP_001161224.1
Sus scrofa	Myoglobin	NP_999401.1
Gallus gallus	Ovomucoid	ACJ04729.1
Gallus gallus	Lysozyme	AAA48943.1
Gallus gallus	Ovotransferrin	CAA26040.1
Gallus gallus	Ovalbumin	AAB59956.1
Anas platyrhynchos	Lysozyme	P00705.1
Anas platyrhynchos	Ovotransferrin	P56410.1
Anas platyrhynchos	Ovalbumin	EOA97657.1
Anas platyrhynchos	Ovomucoid	EOA95437.1
Fenneropenaeus merguiensis	Tropomyosin	ADC55381.4
Macrobrachium rosenbergii	Tropomyosin	ADC55380.1
Metapenaeus ensis	Tropomyosin	Q25456.1
Penaeus monodon	Tropomyosin	A1KYZ2.1
Pandalus borealis	Tropomyosin	CBY17558.1

Table 2. Biochemical characteristics of allergic proteins

Protein name	organisms	Source amino acids	Number of MW	Theoretical pI	Total number of negatively charged residues (Asp + Glu)	Total number of positively charged residues (Arg + Lys)	Instability index	Aliphatic index	Gravy
Myoglobin	*Bos taurus*	154	17077.5	6.90	21	20	15.13	86.88	-0.364
Myoglobin	*Capra hircus*	154	16955.4	7.23	20	20	11.93	86.23	-0.342
Myoglobin	*Ovis aries*	154	16997.4	6.63	21	19	16.36	86.23	-0.357
Myoglobin	*Sus scrofa*	154	17084.6	6.76	22	21	20.50	84.94	-0.444
Myoglobin	*Gallus gallus*	154	17422.0	7.96	22	23	10.76	79.87	-0.542
Ovomucoid	*Gallus gallus*	210	22608.4	4.67	30	18	28.99	64.00	-0.170
Lysozyme	*Gallus gallus*	147	16238.6	9.36	9	18	19.86	81.70	-0.150
Ovalbumin	*Gallus gallus*	386	42881.2	5.19	47	35	37.11	89.95	-0.001
Ovotransferrin precursor	*Gallus gallus*	705	77893.6	6.50	91	88	35.68	74.28	-0.354
Lyozyme DL1	*Anas platyrhynchos*	147	16362.7	9.41	11	21	26.44	78.37	-0.276
Ovotransferrin	*Anas platyrhynchos*	686	75632.8	6.19	92	86	37.44	74.78	-0.386
Ovalbumin	*Anas platyrhynchos*	420	47749.5	4.92	55	38	48.01	77.52	-0.158
Ovomucoid	*Anas platyrhynchos*	171	18629.0	4.69	24	15	56.96	52.92	-0.436
Tropomyosin	*Fenneropenaeusmerguiensis*	274	31704.0	4.66	71	46	39.46	79.85	-1.109
Tropomyosin	*Macrobrachium rosenbergii*	284	32846.4	4.73	73	49	38.61	77.08	-1.129
Tropomyosin	*Metapenaeus ensis*	274	31704.9	4.66	71	46	37.58	78.43	-1.138
Tropomyosin	*Penaeus monodon*	284	32849.4	4.72	72	49	39.00	79.12	-1.094
Tropomyosin	*Pandalus borealis*	284	32753.3	4.70	73	49	37.74	78.45	-1.088

Table 3. Secondary structure of allergic proteins

Serial no.	Accession No	Alpha helix (Hh) (%)	Extended strand (Ee) (%)	Beta turn (Tt) (%)	Random coil (Cc) (%)
1	NP_776306.1	69.48	1.30	9.74	19.48
2	B7U9B5.3	69.48	1.30	9.74	19.48
3	NP_001072126.1	67.53	1.30	9.74	21.43
4	NP_001161224.1	70.13	2.60	7.14	20.13
5	NP_999401.1	66.23	0.65	11.04	22.08
6	ACJ04729.1	20.00	12.86	2.86	64.29
7	AAA48943.1	43.54	14.97	12.24	29.25
8	AAB59956.1	45.85	14.51	5.96	33.68
9	CAA26040.1	31.35	19.15	7.52	41.99
10	P00705.1	32.65	15.65	8.16	43.54
11	P56410.1	29.74	19.39	7.00	43.88
12	EOA97657.1	42.86	16.67	6.19	34.29
13	EOA95437.1	11.70	9.36	2.92	76.02
14	ADC55381.4	100.00	0.00	0.00	0.00
15	ADC55380.1	99.30	0.00	0.00	0.70
16	Q25456.1	99.27	0.00	0.00	0.73
17	A1KYZ2.1	99.30	0.00	0.00	0.70
18	CBY17558.1	100.00	0.00	0.00	0.00

Table 4. Distribution of commonly observed motifs in different allergic protein sequences

Motifs number	Motif present in number of sequence	Sequence
1	5	"HKIPVKYLEFIS[DE][AV]II[HKQ]V[LI] [HAQ] [AES]KH[PA][SAG] DFGAD[AS]Q[GA]AM[SK] KALELFRNDMA[AS][QK]Y"
2	5	"AEARAEFAERSVQKLQKEVDRLEDELVNEKEKYKSITDELDQTFSE LSGY"
3	6	[GQ][KG][LV][CE][RS][IQR]C[EFK][GLQ][ADT]A[AK]][DMT][KQ] [ACR][LR][EGR][LN][DI][NP][YS][RSW][GV][YE]S[GLQ] [AGT] [N F][GW]

Cluster 2 includes 5 protein sequences and represents shrimp allergen sequences. The total number of amino acid residues was in the range of 274 to 284 and the pI values range from 4.66 to 4.73. It has less variation in its pI as compared to cluster 1 sequences. Aliphatic index of this cluster sequences was uniform in the range of 77.08 to 79.85. Cluster 3 includes 8 protein sequences and represents egg allergen sequences. Various biophysical parameters for this group of sequences reveal amino acid residues ranging from 147 to 705, while pI value of the majority of sequences was in range of 4.67 to 6.50except for lysozyme [*Gallus gallus*] (9.36). Aliphatic index of this group of sequences reveals in the range of 64.0 to 81.70 and ovalbumin was the highest thermo stable allergen (90.18) among all three clusters. Secondary structure analysis exhibits that the instability index is used to measure in vivo half-life of a protein (Guruprasad *et al*., 1990). The proteins which have been reported as *in vivo* half-life of less than 5 h showed instability index greater than 40, whereas those having more than 16 h half-life (Rogers *et al*., 1986) have an instability index of less than 40. Instability index of allergic protein sequences under the study was found less than 40 (Table 2).

Secondary structure analysis demonstrated that the myoglobin protein of beef was more similar with goat and sheep meats. In case of shrimp Alpha helix ranges from 99.27-100.00% and Random coil is 0.70-0.73%, except CBY17558.1, ADC55381.4. Extended strand and Beta turn are absent in all the sequences of the cluster2. In egg allergen Alpha helix ranges from 11.70-45.85%, Extended strand 9.36-19.39%, Beta turn 2.86-12.24%, Random coil 76.02-29.25% (Table 3).

MEME analysis provided that five amino acid residues of Cluster1 representing motif 1.Cluster 2, representing motif 2 in its sequences, it contains a 50 amino acid residues long unique motif. Motif 3 was present in 6 protein sequences representing cluster 3.

Conclusion

Comparing variation among biochemical features of myoglobin from different organisms we get that goat, sheep and pig meat are most similar with beef than chicken meat in causing allergenicity. Heat stability of an allergic protein is one of the most important reasons of becoming more allergic and it depends on the value of aliphatic index. The aliphatic index of myoglobin protein of *Gallus gallus* is lower than other four organisms. So, chicken meat can be a convenient alternative to beef allergic peoples. In case of shrimp the biochemical features of tropomyosin of *Penaeus monodon* shows more similarity with *Fenneropenaeus merguiensis, Metapenaeus ensis* and *Pandalus borealis* than *Macrobrachium rosenbergii*. However, the aliphatic index of tropomyosin of *Macrobrachium rosenbergii* is lower than the other. As for that *Macrobrachium rosenbergii* can be a suitable substitute to *Penaeus monodon* allergic peoples. The allergic proteins of hen's egg white are more thermo stable because of having comparatively high aliphatic index than duck. So, duck egg can be an appropriate alternative to hen's egg allergic infants. Beside these phylogenetic clustering and variation among biochemical features of different allergens might contribute in further classification of highly diverse allergens and their selection for various application purposes. Conserved sequences in motifs may be utilized for designing specific degenerate primers for identification and isolation of type and class of allergens as numerous allergens are being isolated to assure food security. Variation in biochemical features may be a key source of information for the screening of novel allergens and comparison with other classes of allergens. Functional attributes are needed to verify experimentally for conserved motifs found. This in silico analysis might be used for future genetic engineering of assuring food security.

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Author's Contributions

Sourav Chakraborty: Manuscript writing and result verification.

Shakhawat Hossain Foysal: Secondary structure analysis by using SOPMA software.

Nazmul Hasan: Biochemical features data preparation for 18 proteins using ProtParamsoftware.

Nahian Khan: Phylogenetic tree construction and common motif identificationusing MEGA5.2 and MEMEsoftware respectively.

Ethics

This article is original and contains unpublished materials. I'm Sourav Chakraborty-the corresponding author confirms that all of the other authors have read and approved the manuscript.

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