

Studying B-Cell Epitopes of Olive Allergens Using Computational Methodology

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Abstract: Frequently a few proteins of the normal and fundamental sustenance segments or potentially their tree (dust, for example, wheat, shelled nut, olive, soybean, and soon have been found to instigate sensitivity in a few people. Acknowledgment of hypersensitivity causing epitopes in such proteins and understanding their cross reactivity is the initial phase toward keeping sensitivities from happening. Their in-vitro and in-vivo contemplates are tedious and expensive. Since the in-vivo thinks about rely on the living life form and subsequently comes about are species subordinate. Bioinformatics came as an awesome help to therapeutic science, where utilizing computational devices forecasts can be made with respect to the reasons for sickness and medications required. These computational procedures influence the procedure to time proficient and financially savvy. They clear way for in-vitro and in-vivo analyzes. One such field where it is helping restorative sciences is expectation of sensitivity causing B-cell epitopes and finding their cross-reactivity. Cross-reactivity predicts other conceivable sustenance which may cause hypersensitivity. In the present investigation, IgE interceded hypersensitivity causing B-cell epitopes of olive (*Olea europaea*) have been recognized, to be specific 'Ole e 1', 'Ole e 2', 'Ole e 3', 'Ole e 4', 'Ole e 9', 'Ole e 10' and 'Ole e 11.0101.' with a specific end goal to distinguish direct and conformational epitopes, an aggregate seven web-servers were utilized to be specific BCPred, BcePred, ABCPred, CBTOPE, BepiPred 1.0b, DiscoTope 2.0 and ElliPro. Programming foreseeing the conformational epitopes requires protein structure. Structures for 'Ole e 9' and 'Ole e 10' were not accessible in information base and consequently homology based demonstrating was likewise done with the guide of the Modbase webserver, using modeler 9 and these displayed structures were utilized for forecast of conformational epitopes. An aggregate of 47 straight epitopes and 28 conformational epitopes have been anticipated in the present investigation, which were additionally contemplated for properties, for example, hydrophilicity, extremity and surface uncovered zone, utilizing PepCalc and NetSurfP 1.1. Anticipated epitopes were examined for cross reactivity, from the 'Basic Database of Allergenic Proteins' (SDAP) database, which demonstrated the anticipated epitopes to contain a lot of comparability with different nourishment parts, for example, strawberry, privet, potato and Castor bean allergens and additionally with allergens found in cow-like lungworm and two assortments of parasites.

Keywords: Food Allergy, Olive allergens, epitope mapping, IgE binding, cross-reactivity.

I. INTRODUCTION

In-vitro and in-vivo analysis in life sciences are quite time consuming and costly affair. Both time and cost involved can be reduced to a great extent if computational tools are used to predicted the causes of illness. Hence in recent past, bioinformatics tools are gaining great popularity. Numerous algorithms are being developed and new data bases and computational web servers are being designed, to help medical science. Using these computational tools, causes of disease can be predicted easily and in-vitro and in-vivo can be planned in better way. The present paper discusses the allergy causes of olive and its cross-reactivity. Before moving further a small discussion of allergy is essential. The immune system of the human body is devised in order to fortify the body against foreign bodies, pathogens and disease-causing agents. However, the immune system often tends to identify various otherwise harmless substances, as potentially disease-causing, and thus induces the production of histamine in the body. Histamine production in turn, results in the occurrence of symptoms such as headache, skin rash, sneezing, swelling, runny nose, nausea, diarrhoea, etc. The occurrence of these symptoms in the body is referred to as allergic reactions [1], and the substances which are responsible for the immune system to induce histamine production which in turn leads to this, are labelled as allergens. To name a few we have, grass pollen, animal dander, dust mites, insect stings, etc. Substances in food components such as egg, milk, soybean, etc have been characterised as allergens as well [2]. Olive is one such common food item which is reported to induce a number of allergies (pollen, fruit and pollen-food allergy) in the human body [3]. Hence, in the present study we have focussed on the allergies caused due to Olive (*Olea europaea*). Olive, being a widespread food component as well as rich in a number of essential nutrients is an important part of most diets and hence being produced in large scale. The most common allergy caused by olive is due to the olive plant. The pollen released by the olive plant during spring season, can result into a number of respiratory problems ranging from mild to serious [4]. This makes people susceptible to olive induced allergies wherever olive grows.

Olive (pollen and fruit) comprises of a number of proteins which the human immune system recognises as foreign and are acted against in the case of allergic individuals. In this paper, the olive allergens which have been studied include,

'Ole e 1', 'Ole e 2', 'Ole e 3', 'Ole e 4', 'Ole e 9', 'Ole e 10' and 'Ole e 11.0101' [5, 6].

Allergies can be characterised into three parts, a) Immunoglobulin E or IgE mediated and b) Non-IgE mediated and c) Mixed [7, 8, 9]. Allergies due to olive are IgE mediated allergies. A person susceptible to IgE mediated allergy will be allergic to a variety of food items as long as they contain the same protein to which the individual is allergic to [10]. This phenomenon is known as cross-reactivity. This can be taken advantage of as, if the epitopic regions causing such allergies can be identified and neutralised, the person shall become immune to a number of food components which otherwise could have lead to a potential allergic reaction in his/her body.

The contemporary age of research and development has seen emergence of improved methodology for immunotherapy and vaccine development in order to counter food allergies. The utilisation of hypoallergenic variants, for immunotherapy and as potential vaccine candidates, has aided the process in becoming both simple in its means as well as highly time efficient, all the while having an advantageous feature of being capable of inducing the necessary immune response in order to reduce IgE binding. The foremost attribute in order to make predictions regarding the components involved in the IgE cross-linking, is the identification of the epitopes of the concerned allergy. This can be achieved through a number of experimental techniques however they are very costly as well as time taking. Hence, the most efficient technique in both these regards proves to be the in-silico approach [11].

An epitope consists of the set of the antigenic amino-acid residues which are in direct contact with residues belonging to an antibody (the paratope). Thus, epitope identification is the primary step in the procedure of medication of allergy. The epitopes present are of two varieties; the linear epitopes, present as a continuous chain of amino acids that result in eliciting the immune response in an allergic patient's body and the discontinuous epitopes, which by functionality are similar, however instead of being continuous chain of amino acids, are instead spatially clustered and are exposed on the surface of the 3-D structure of the protein [11]. In the pursuit of determining the allergenic potential of a species, the identification of both linear as well as conformational epitopes is of utmost importance.

Utilizing the modern biological computational soft-wares and web servers which are available, mapping of linear and conformational IgE binding epitopes of olive allergens have been carried out. Various physio-chemical properties like hydrophilicity, surface exposed residues, polarity, etc have also been analysed in order to draw comparisons and make predictions regarding the allergy causing potential of the various allergens [12].

II. MATERIALS AND METHODS

A. Sequence retrieval: The National Centre for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov) data base is used for retrieving amino acid sequences for different olive allergens namely 'Ole e 1', 'Ole e 2', 'Ole e 3', 'Ole e 4', 'Ole e 9', 'Ole e 10' and 'Ole e 11.0101'.

B. Mapping linear IgE binding epitopes: Four different web servers were utilised in order to have an best possible mapping of linear IgE binding epitopes. The web servers employed were, ABCPred [13], BCPred [14, 15, 16], BepiPred 1.0b [17] and BcePred [18]. Various notions including those of artificial neural network, hydrophilicity scale with a hidden markov model, support vector machine, surface probability, amino acid propensity scales of hydrophilicity, antigenic index and flexibility are involved by the web servers which take the allergens' primary sequence as the input (in FASTA format) in order to predict their behaviour.

C. Peptide similarity search for identified linear epitopes: Continuous/Linear epitopes predicted for the various olive allergens were assessed for analogy with other known allergens in the SDAP (Structural Database of Allergenic Proteins) database [5]. In order to consider only those showing a significant amount of similarity, allergens which had a PD value of less than 2 were included in the current study.

D. Homology Modelling: Homology based 3D modelling was carried out for two olive allergens namely, 'Ole e 9' and 'Ole e 10' with the help of ModBase web server, which makes use of Modeller 9. The obtained models were successfully submitted at PMDB (Protein Model DataBase) database and were also verified by RAMPAGE Ramachandran Plot Analysis Web server.

E. Mapping conformational IgE binding epitopes: The PDB structures (ID) of the olive allergens were utilised in order to carry out the prediction of conformational IgE binding epitopes. The web servers utilised for this task were, DiscoTope 2.0 [19], Ellipro [20] and CBTOPE [21].

F. Hydrophilicity and Polarity: Study regarding the hydrophilicity and polarity of the identified linear epitopes were carried out using the PepCalc web server which is available at Innovagen (<http://>

+//www.innovagen.se/custompeptidesynthesis/peptidepropertycalculator/peptidepropertycalculator.asp).

G. Surface exposed residues: NetSurf ver1.1 ([http:// www.cbs.dtu.dk/services/NetSurfP/](http://www.cbs.dtu.dk/services/NetSurfP/)) was incorporated in order to identify the Surface exposed residues amongst the predicted epitopic regions.

III. RESULTS AND DISCUSSION

In the present era of technological and scientific advancements, computational biology has grown to have a significant impact over research and development. Various tools and softwares have emerged in the past decades which have led to highly efficient in-silico research to be made possible in the field of biology. In contrast to the traditional in-vivo and in-vitro research and experimentation methodologies, in-silico research has not only proven to be cost efficient, but is also highly time efficient while producing significantly accurate results.

Therefore, in the present study, in-silico methodologies have been incorporated in order to study about the food allergens present in olive. Although, the computational tools are used in abundance for identification and determination of epitopes and the results of the in-vivo/in-vitro and the in-silico studies are unanimous [22, 23] but still in the opinions of the authors the results presented in the computational study should be validated experimentally.

In order to map the B-cell epitopes (both linear and conformational) of the various olive allergens, a number of web servers have been utilised, (ABCPred, BCPred, BcePred, BepiPred 2.0, CBTOPE, DiscoTope 2.0 and Ellipro). Linear epitopes of the allergens are those which are present as a continuous chain of amino-acids, hence are named as continuous epitopes. Web servers ABCPred, BCPred, BcePred and BepiPred 2.0 were utilised in order to map the potential linear epitopes of various olive allergens. The amino-acid sequences are given as the input by the user, and the web servers utilise numerous algorithms in order to carry out the epitope predictions. As the different web servers incorporate different algorithms, the intersection of the epitope prediction has been taken in the study in order to ensure high level of accuracy. TABLE I represents the various allergens taken into consideration for the study and the epitopes of each allergen as taken from the consensus of the four incorporated web servers.

In order to identify the conformational or discontinuous epitopes of the allergens, the PDB structure of its protein is essential. The PDB structures of allergens ‘Ole e 1’, ‘Ole e 2’, ‘Ole e 3’, ‘Ole e 4’, and ‘Ole e 11.0101’ were taken from NCBI. Due to the unavailability of the PDB structures of ‘Ole e 9’ and ‘Ole e 10’, homology based 3D modelling was carried out for these two allergens and also validated by RAMPAGE giving 88 % residues in favourable region and 8 % in allowed region for ‘Ole e 9’ and 86 % residues in favourable region and 10 % in allowed region for ‘Ole e 10’. Fig. 1a depicts the generated 3D model of ‘Ole e 9’ and Fig. b depicts the generated 3D model of ‘Ole e 10.’ After the PDB structures of all the allergens were obtained, they were used as input for the DiscoTope 2.0 and Ellipro web servers which aided in predicting their conformational epitopes. CBTOPE utilised the amino-acid sequence of the allergens in order to carry this out. Further, to ensure a high degree of accuracy of the predicted epitopes, the intersection of the results given by the three webservers was incorporated in the study. TABLE II represents the allergens and their predicted conformational epitopes.

Experimentally it has been observed that the residues present as both the linear and conformational epitopes determine the true potential of an allergenic species. Therefore, consensus regions were identified on comparing linear and conformational epitopes as shown in Table III. Only 4 out of seven predicted allergenic sequences of olive showed linear and conformational consensus.

TABLE I: LIST OF LINEAR IGE BINDING EPITOPES PREDICTED BY ONLINE WEB SERVERS

Allergen	No. of epitopes	Start position	Predicted epitope residues	No of residues
Ole e 1	1	133	QVYNKLGMY	9
	2	122	FFKKEALPKC	10
	3	72	DHKNE	5
	4	24	AGFITELSEFIP	12
	5	94	PTEGWAKPSLKFKLNT	16
	6	84	SSGRKDCNEIPTGWA	16
Ole e 2	1	14	DIEGHEDH	8
	2	42	FPQFKPE	7
	3	58	NEPGHLA	7
	4	110	EETVTP	6
Ole e 3	1	19	ANGDGKISSS	10

	2	39	SVTPEEIQ	8
	3	54	TDGDGFISFE	10
	4	73	RGLVK	5
Ole e 4	1	5	GVEIVSIDTYLFLSLYD	16
Ole e 11.0101	1	353	WLLPPRV	7
	2	320	GPGANM	6
	3	321	PGANMEKRAKFKVRLS	16
	4	327	KRAKFKVRLSDAEAKQ	16
	5	298	EGWILVKPEH	10
	6	292	VDAIHPEGWILVK	13
	7	167	RPDGKRVGA	9
	8	135	TAAEYGT	7
	9	74	SGDFK	5
	10	57	LVTAEGQT	8
	11	50	KATMDPALVTAEGQT	15
	12	30	SNSAQLNSWFDGI	13
	13	21	LSDDRAPIPSNSAQLN	16
	14	19	IVLSDDRAPI	10
Ole e 9	1	446	TNPSYGAC	8
	2	428	AGRNSWNC	9
	3	397	DCGPIQPGGACFE	13
	4	377	PGVSDQ	7
	5	357	TTPATPTPKAAGS	15
	6	347	VLLKNTQNPTTPATP	16
	7	341	GSTTYDVGLLK	11
	8	322	EDKKTGASSE	10
	9	302	VGTPMPG	8
	10	226	PNAGQVDSGNH	12
	11	210	YKNQTPDT	9
	12	168	SQSYPPSSGVFNP	13
	13	128	GDQK	4
	14	92	ASNPV	6
	15	38	SDNLPSLQ	8
Ole e 10	1	110	TSSDPSNGSCS	11
	2	95	KGRNDFDCDF	10
	3	64	CGPIQANGACFNPNT	15
	4	5	AGVPDQPVPTPTP	13

The continuous/linear epitopes of olive showing analogy with the physio-chemical characteristics available in SDAP database were recognised. This helped in order to predict the possible cross-reactivity showed by olive allergens to those of other food or non-food components. However, only those showing a property distance (PD) value of less than 2, were incorporated in the study as to only consider those epitopes which show a significant amount of analogy. The predicted epitopes were found to contain a significant amount of similarity with various food components, such as strawberry, privet, potato and castor bean allergens as well as with allergens found in bovine lungworm and two varieties of mites. The results for this analysis have been depicted in the TABLE IV.

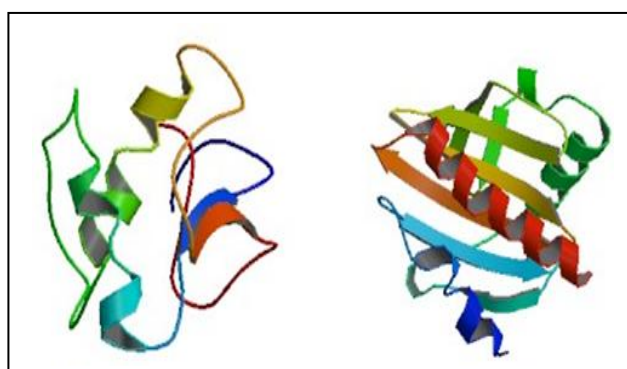


Fig. 1a). Depicting the homology modeled ‘Ole e 9’, b). Depicting the homology modeled ‘Ole e 10’

A number of other physio-chemical properties such as, hydrophilicity, polarity and surface exposed area were also analysed using PepCalc and NetSurfP 1.1 web servers. The epitopes showing a higher amount of hydrophilicity are more likely to act as possible allergens; likewise those showing a higher amount of polarity and surface exposed area are also more likely to behave as potential allergens. From the allergens incorporated in our study we found out that, ‘Ole e 2’ is the most hydrophilic with 32.14% of its residues falling in the hydrophilic region, followed by ‘Ole e 1’ and ‘Ole e 3’ with 27.94% and 27.27% respectively. They are followed by, ‘Ole e 11.0101’ with 25.82%, ‘Ole e 4’ with 18.75% and finally ‘Ole e 10’ and ‘Ole e 9’ with 14.28% and 14.09% each respectively. The polarity of ‘Ole e 9’ was found to be the greatest with 34.22% of its residues present in polar region, which was followed by ‘Ole e 10’ with 32.65%, ‘Ole e 3’ with 27.27%, ‘Ole e 4’ with 19.75%, ‘Ole e 1’ with 19.11%, ‘Ole e 11.0101’ with 18.54% and finally by ‘Ole e 2’ with 10.71%. Similarly the surface exposed area was also assessed for all the seven allergens incorporated in study, the result showed ‘Ole e 2’ to have a 96.42% followed by ‘Ole e 9’ with 94.63%, ‘Ole e 3’ with 90.90%, ‘Ole e 1’ by 86.76%, ‘Ole e 10’ by 83.67%, ‘Ole e 11.0101’ by 83.44% and finally ‘Ole e 4’ by 56.25%. The result has been depicted in both graphical and tabular format in the Fig. 2 and TABLE V.

IV. CONCLUSION

Olive is one of the most important food components. Due to its widespread availability and high nutritional value, it has become an essential part in every healthy diet. The medicinal property of olives and olive oils also contribute to its heavy utilization in maximum households and hence are produced at large scale. Unfortunately, (pollen, food and pollen-food/ Oral Allergic Syndrome) allergic problems associated with it have come out to become a major problem in the recent times. In order to address these allergy related problems, the first step is the identification of epitopes of allergens present in olive so that respective antibodies can be developed. The in-vivo and in-vitro methodologies for this purpose prove to be both time and cost inefficient and thus, it is important to carry out its in-silico study in the first stage so that a more planned subsequent studies can be carried out in the future. In the present paper, allergy causing epitopes of seven olive’s allergens have been identified using a total of seven different web servers. Out of the predicted epitopes, a total of 47 epitopes are linear or continuous in nature where as 28 of them are conformational in nature. The various physio-chemical properties of the epitopes have also been studied in order to find the possibility of being a potential allergy causing substance. For this, their properties such as ‘hydrophilicity’, ‘polarity’ and ‘surface exposed area’ have also been identified. This gives us a good estimate into the possible allergy causing epitopes present in olive allergens and also a rough estimate on how to carry out their in-vitro in-vivo and analysis.

TABLE II: LIST OF CONFORMATIONAL IGE BINDING EPITOPES PREDICTED BY ONLINE WEB-SERVERS

Allergen	No of epitopes	Start position	Predicted epitope residues	No of residues
Ole e 1	1	12	HIQGQVYCDTCR	12
	2	49	GDVTFTEV	8
	3	69	VERDHKNE	8
	4	91	NEIPTEGW	8
	5	133	QVYNKLGMYPPNM	13
Ole e 2	1	130	VEQGM	5
Ole e 3	1	1	MADDPQEVAEH	11
Ole e 11.0101	1	27	PIPSNSA	7
	2	37	SWFDGII	7
	3	72	DGSGDFKS	8
	4	94	RVILS	5
	5	206	GKHFYK	6
	6	255	SEDTGYY	7
Ole e 9	1	26	SQSFLGVNYGQ	11
	2	91	LASNP	5
	3	132	LISQL	5
	4	230	QVDSGNGHKYTNMF DAQV	18
	5	313	DTYLF	5
	6	278	NEVGPSLDN	9
	7	339	PDGSTT	6
	8	380	SDDQLTGNINY	11
	9	420	VMNLYYQSAGRNS WNCDFSQTATLTNT	41

			NPSYGACNFPSGSN	
Ole e 10	1	37	WCVPKAE	7
	2	45	TDAQLQSNIDYVCSQ SG	17
	3	63	DCGPIQANGA	10
	4	77	NTVRAHA	7
	5	90	WYQSK	5
	6	106	TGAISSDPSNGSC	14

TABLE III: CONSENSUS OF LINEAR AND CONFORMATIONAL IGE BINDING EPITOPES OF OLIVE ALLERGENS

Allergen	No. of epitopes	Start position	Predicted epitope residues	No of residues
Ole e 1	1	133	QVYNKLGMY	9
	91	65	NEIPTEGW	8
Ole e 9	1	230	QVDSGNHG	8
	2	380	SDDQ	4
	3	428	AGRNSWNCD	9
	4	446	TNPSYGAC	8
Ole e 10	1	64	CGPIQANGA	9
	2	110	TSSDPSNGSC	10
Ole e 11.0101	1	27	PIPSNSA	7

TABLE IV: LINEAR EPITOPES PREDICTED FOR 'OLE E 1' AND 'OLE E 9' SHARES SIMILARITY WITH THE PEPTIDES OF KNOWN ALLERGENS IN SDAP DATABASE HAVING PD VALUES LESS THAN 2.0.

Allergen	PD Index	Start Residue	Matching Region	End Residue
Ole e 1 - QVYNKLGMY				
Fra e 1.0101	0.00	133	QVYNKLGMY	141
Lig v 1	1.41	133	QVFNKLGMY	141
Ole e 1 -NEIPTEGW				
Fra e 1.0101	0.74	91	DEIPTEGW	98
Lig v 1	0.74	91	DEIPTEGW	98
Ole e 9- SDDQ				
Dic v a	0.00	826	SDDQ	829
Sola t 4	0.00	182	SDDQ	185
Pen c 19	1.48	300	SDNQ	303
Ric c 1	1.48	191	SDNQ	194
Cla h 5.0101	1.48	431	SDNQ	434
Blo t 2.0104	1.81	38	SDDH	41
Eur m 14	1.96	1017	NDDQ	1020

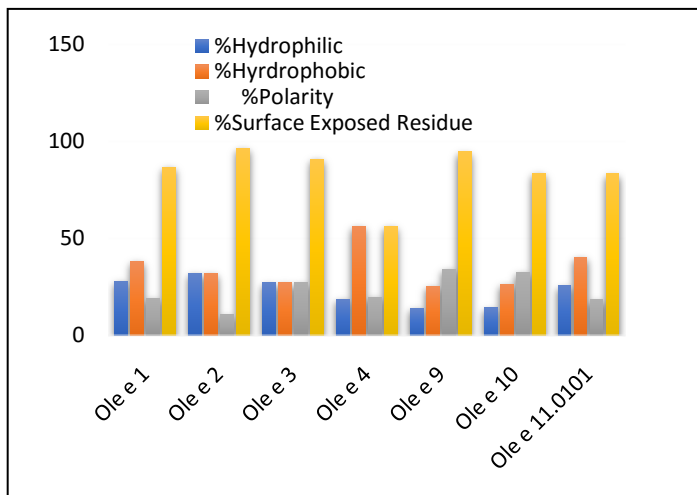


Fig. 2 Bar diagram depicting Hydrophilicity, Hydrophobicity, polarity, surface exposed residue for predicted allergens

TABLE V. VALUE OF HYDROPHILICITY, HYDROPHOBICITY, POLARITY, SURFACE EXPOSED RESIDUE FOR PREDICTED ALLERGENS

Allergen	%Hydrophilic	%Hydrophobic	%Polarity	%Surface Exposed Residue
Ole e 1	27.94%	38.23%	19.11%	86.76%
Ole e 2	32.14%	32.14%	10.71%	96.42%
Ole e 3	27.27%	27.27%	27.27%	90.90%
Ole e 4	18.75%	56.25%	19.75%	56.25%
Ole e 9	14.09%	25.5%	34.22%	94.63%
Ole e 10	14.28%	26.53%	32.65%	83.67%
Ole e 11.0101	25.82%	40.39%	18.54%	83.44%

V. FUTURE PROSPECT

In the present study, the prediction of allergy causing epitopes of olive have been analysed with the help of available biological computational tools in a cost and time efficient manner. The prediction of epitopes of any allergen is the primary step towards devising a therapeutic option against the allergen. In order to neutralise the occurrence of possible allergy, we need to find methodologies in order to neutralise the epitopes leading to that allergic reactions. These methods can involve tablets, vaccines or be as simple as consuming other food components along with the allergy causing ones which have the potential to neutralise the said allergy causing epitopes. Therefore, considering the impact of the olive allergy on human health and its widespread cross reactivity present study is of immense importance and paves the way towards the invention of allergy prevention methodologies.

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