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A. M. Venger[1], O. O. Venger[2], A. S. Zaitsev[1], O. A. Hruzevskyi[1]

MODULATION OF STRUCTURE AND FOLDING HOMOLOGS OF *Amb a 6* ALLERGEN OF *AMBROSIA ARTEMISIIFOLIA*

[1] The Odessa National Medical University, Odesa, Ukraine,

[2] The Plant Breeding and Genetics Institute — National Center of Seed and Cultivar Investigation, Odesa, Ukraine

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А. Н. Венгер[1], О. А. Венгер[2], А. С. Зайцев[1], А. А. Грузевский[1]

МОДУЛИРОВАНИЕ СТРУКТУРЫ И ФОЛДИНГОВЫХ ГОМОЛОГОВ *Amb a 6* АЛЛЕРГЕНА *AMBROSIA ARTEMISIIFOLIA*

[1] Одесский национальный медицинский университет, Одесса, Украина,

[2] Селекционно-генетический институт — Национальный центр семеноведения и сортоизучения, Одесса, Украина

Введение. *Amb a 6* аллерген *Ambrosia artemisiifolia* является одной из главных причин аллергических заболеваний в Северной Америке и Европе. Структура данного аллергена неизвестна.

Целью нашего исследования была модуляция трехмерной структуры и поиск фолдинговых гомологов аллергена *Amb a 6 A. artemisiifolia* биоинформатическими методами.

Материал и методы. Моделирование структуры и поиск гомологов проводили при помощи программ SWISS-MODEL и ProMod3.

Результаты и обсуждение. Создана трехмерная структура аллергена *Amb a 6*. В качестве фолдинговых гомологов аллергена *Amb a 6* был обнаружен белок неспецифического переноса липидов кукурузы.

Ключевые слова: *Ambrosia artemisiifolia*, аллерген *Amb a 6,* трехмерная структура, модуляция фолдинговых гомологов, биоинформатика.

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Introduction. *Amb a 6* allergen of *Ambrosia artemisiifolia* is a ragweed allergen (a principle cause of late summer hayfever in North America and Europe). The weed has recently become spreading as a neophyte in Europe, while climate change may also affect on the growth of the plant and additionally may also influence pollen allergenicity. In Ukraine, the number of diseases caused by this allergen has recently increased. Still the three-dimensional structure of *Amb a 6* allergen has been yet undescribed.

The aims of our study were the modulation of the three-dimensional structure and the search of folding-homologs of *Amb a 6* allergen of *A. artemisiifolia* by bioinformatics methods.

Material and Methods. Template search with Blast and HHBlits has been performed by the SWISS-MODEL template library. Models are built based on the target-template alignment using ProMod3. Coordinates which are conserved between the target and the template are copied from the template to the model. Insertions and deletions are remodelled using a fragment library. Side chains are then rebuilt. Finally, the geometry of the resulting model is regularized by a force field. In case loop modelling with ProMod3 fails, an alternative model is built with PROMOD-II.

Results and Discussion. Three-dimensional structure of *Amb a 6* allergen has been successfully built. As folding-homologs of *Amb a 6* allergen the maize nonspecific-lipid transfer protein was found. This protein has ability to bind and transfer lipids. Information about the three-dimensional structure and partial analogy with nonspecific-lipid transfer protein may help to thoroughly understand the properties and the spatial configuration of *Amb a 6* allergen.

Key words: *Ambrosia artemisiifolia, Amb a 6* allergen, three-dimensional structure, folding-homologs modulation, bioinformatics.

It has already become a well-known fact that a major cause of hay fever and associated asthma may appear the pollen of common ragweed *(Ambrosia artemisiifolia).* Recently ragweed has started to spread in many parts of central Europe, where it has become a serious health problem for a lot of people. Several initiatives were formed to prevent its further spread in Austria, France, Southern Germany, etc. In Ukraine, the number of diseases caused by this allergen has recently increased. The *Asteraceae* or *Compositae* family is known to be one of the largest families of flowering plants. Among them only a few plants are strong allergic sources, namely: *Ambrosia* (ragweed), *Artemisia* (mugwort), *Helianthus* (sunflower), and *Parthenium* (feverfew). Furthermore it was demonstrated that sera of mugwort allergic patients show considerable cross-reactivity with ragweed pollen extracts [6]. Ragweed pollen extract, which indicates close homology of the essential allergens in ragweed and mugwort pollen, has inhibited effectively IgE-binding to mugwort allergens in immunoblots. Thus, there were found six groups of allergens in ragweed pollen. If patients reacted with the pectate lyases of the *Amb a 1/2* group, they were classified as ragweed allergic. Moreover the homologous pectate lyase *Art v 6* in mugwort has been reported to play only a minor role in allergic disease [7]. The small proteins *Amb a 6* (lipid transfer protein), *Amb a 8* (profilin), *Amb a 9* and *Amb a 10* (both calcium-binding proteins) are belonging to the group of well-known cross-reactive pan-allergens, while the proteins *Amb a 7* and the fragment *Amb a 3* are plastocyanins described only as minor ragweed allergens [5].

The aims of our study were the modulation of the three-dimensional structure and the search of folding-homologs of *Amb a 6* allergen of *A. artemisiifolia*.

Material and Methods

Template Search

Template search with Blast and HHBlits has been performed against the SWISS-MODEL template library. The target sequence was searched with against the primary amino acid sequence contained in the SMTL. A total of 27 templates were found [1].

An initial HHblits profile has been built using the procedure outlined in, followed by 1 iteration of HHblits against NR20 [9]. The obtained profile has then be searched against all profiles of the SMTL. A total of 32 templates were found.

Template Selection

For each identified template, the template's quality has been predicted from features of the target-template alignment. The templates with the highest quality have then been selected for model building.

Model Building

Models are built based on the target-template alignment using ProMod3. Coordinates which are conserved between the target and the template are copied from the template to the model. Insertions and deletions are remodelled using a fragment library. Side chains are then rebuilt. Finally, the geometry of the resulting model is regularized by using a force field. In case loop modelling with ProMod3 fails, an alternative model is built with PROMOD-II [4; 10].

Model Quality Estimation

The global and per-residue model quality has been assessed using the QMEAN scoring function [2]. For improved performance, weights of the individual QMEAN terms have been trained specifically for SWISS-MODEL.

Ligand Modelling

Ligands present in the template structure are transferred by homology to the model when the following criteria are met: (a) the ligands are annotated as biologically relevant in the template library, (b) the ligand is in contact with the model, (c) the ligand is not clashing with the protein, (d) the residues in contact with the ligand are conserved between the target and the template. If any of these four criteria is not satisfied, a certain ligand will not be included in the model. The model summary includes information on why and which ligand has not been included.

Oligomeric State Conservation

Homo-oligomeric structure of the target protein is predicted based on the analysis of pairwise interfaces of the identified template structures. For each relevant interface between polypeptide chains (interfaces with more than 10 residue-residue interactions), the QscoreOligomer is predicted from features such as similarity to target and frequency of observing this interface in the identified templates [8]. The prediction is performed with a random forest regressor using these features as input

parameters to predict the probability of conservation for each interface. The QscoreOligomer of the whole complex is then calculated as the weight-averaged QscoreOligomer of the interfaces. The oligomeric state of the target is predicted to be the same as in the template when QscoreOligomer is predicted to be higher or equal to 0.5.

Results and Discussion

Three-dimensional structure of *Amb a 6* allergen has been built (Fig. 1).

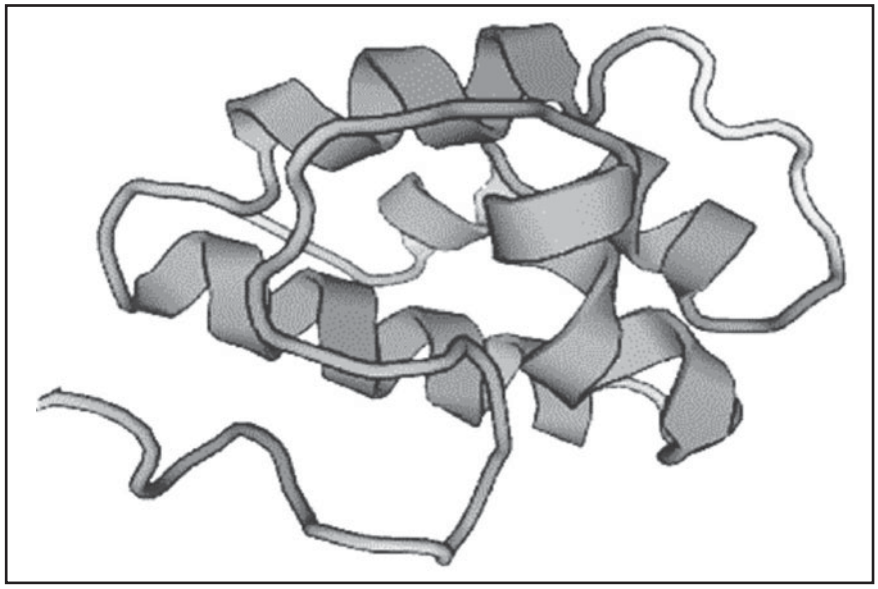

Fig. 1. Three-dimensional structure of *Amb a 6* allergen

As folding-homologs of *Amb a 6* allergen the maize nonspecific-lipid transfer protein (MNLTP) was found. MNLTP is known for its ability to bind and transfer lipids. Proteins belonging to the MNLTP subfamily are larger (~90 residues) and bind various phospholipids, fatty acids, and glycolipids, while the smaller (~70 residues) but more structurally flexible members of the MNLTP subfamily can bind to bulkier sterol molecules as well [3].

Information about the three-dimensional structure and the partial analogy with nonspecific-lipid transfer protein may help to thoroughly understand the properties and the spatial configuration of *Amb a 6* allergen.

Ключові слова: *Ambrosia artemisiifolia*, алерген *Amb a 6,* тривимірна структура, модуляція фолдінгових гомологів, біоінформатика.

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