s8a.m9.p1 **The structure of Retro-Diels-Alder catalytic antibody crystals and their evil twins.** M. Hugot, J.-L. Reymond and U. Baumann. *Department of Chemistry and Biochemistry of the University of Bern, Freiestrasse 3, CH-3012 BERN (Switzerland).*
Keywords. catalytic antibody, haptens, twinning.

Antibodies with enzyme-like catalytic properties can be obtained from immunisations against stable transition state analogues of chemical reactions [1]. Recently the Reymond group reported nitroxyl synthase catalytic antibodies by immunisation against certain haptens [2, 3]. The goal of this project is to determine the structure of nitroxyl-synthase catalytic antibodies by X-ray crystallography, and so to elucidate the catalytic mechanism of these biocatalysts.

Four monoclonal antibodies (9D9, 10F11, 27C5, 8E5) have been produced in large quantities and purified to homogeneity. Papain cleavage conditions for the catalytic antibodies 9D9 and 10F11 were optimised and the catalytically active fragments Fab of both antibodies were purified by Fast Protein Liquid Chromatography.

Currently, crystals of Fab 9D9 without hapten and 10F11 complexed with four different haptens (corresponding to different transition state analogues of the reaction) were obtained. 9D9 without hapten is a single crystal with space group $P2_12_12_1$ and cell constants a= 48.703, b= 80.355, c=125.103 and one molecule per asymmetric unit. The crystals diffract to 2.4Å resolution.

In the case of 10F11, well-diffracting crystals (resolution from 1.8 to 2.4Å) were obtained easily but most of them are pseudo-merohedrally twinned. The apparent twinned spacegroup is orthorhombic with cell constants a=40.633, b= 139.885, c= 85.202 , but the true spacegroup is $P2_1$ with two molecules in the asymmetric unit and the twinning operation (h, -k, -l) is nearly identical to the NCS-operation.Twinning fractions vary roughly from 0.3 to 0.4 and structure determination have been achieved by detwinning and molecular replacement.

Parallel to structure determination the genes for the antibodies have been cloned by phage display methods [4] to determine the amino acid sequence and to perform site-directed mutagenesis.

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s8a.m9.p2 **Structural Investigations of the Major Timothy Grass Pollen Allergen Phl p7**. P. Verdino[1], A. Twardosz[2], R. Valenta[2], and W. Keller[1], *[1]Institute of Chemistry / Structural Biology, Karl-Franzens-University Graz, Heinrichstraße 28, A-8010 Graz, Austria, [2]Institute of General and Experimental Pathology, University of Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria, email: petra.verdino@kfunigraz.ac.at*
Keywords: allergen, EF hand, IgE binding.

Almost 25 % of the population in industrialized countries are affected by type I allergy. This disease is based on the recognition of innocuous airborne antigens by specific immunoglobulin E and leads to allergic symptoms comprising rhinitis, conjunctivitis, dermatitis, asthma and even anaphylactic shock. The interaction of allergens with IgE-antibodies depends on the fold-recognition of the antigens' 3D structures by IgE. Hence the variation of the conformational features of allergens can be used as a tool for diagnosis and perhaps treatment of allergic diseases. This requires accurate understanding of the structural properties of the allergens and their recognition by the antibodies.

A very interesting allergen is the 8.6 kDa timothy grass pollen protein Phl p7. It belongs to a calcium-binding protein family consisting of allergens of various origins like pollens, fish-proteins and even human auto-allergens. Members of this protein family share common properties like low molecular weight, water solubility and are surprisingly stable when exposed to heat, denaturating agents or enzymes. A common structural feature is the so-called EF-hand calcium-binding motif.

Here we present structural investigations of the native Phl p7 protein and a mutant form thereof. Circular dichroism spectroscopy was performed with the calcium-bound and apo forms of the proteins. The recombinant native protein shows mainly α-helical secondary structure and a very high thermal stability. Depletion of calcium results in destabilization and a loss of fold. The secondary structure of the mutant is significantly reduced compared to the native protein and is only marginally affected by calcium depletion. This already indicated that the mutant has a decreased calcium binding ability, which was subsequently confirmed by mass spectrometry. These results point at the great contribution of bound calcium to the overall protein fold and stability and are closely correlated to findings resulting from IgE-binding studies. There the less folded proteins showed a reduced IgE binding capacity which corresponds to a decreased allergic potential.

For further investigation we employed protein crystallography and already managed to crystallize the Phl p7 protein. We were able to collect a native data set to 2.2 Angstrom and determined the space group and the cell constants. Data collection on heavy atom derivatives is in progress.