The study of chitinolytic enzymes, aiming at constructing an artificial chitinosome

The main aim of the project is the construction of an “artificial chitinosome”, a complex of connected enzymes for degrading chitin more efficiently than a mixture of individual enzymes. In order to reach this goal we shall first study selected chitinolytic enzymes in terms of their structure, enzymatic properties and stability under varying conditions, especially changing temperature. We selected the enzymes for our research from extremophilic organisms, especially hyperthermophiles and psychrophiles (cold-adapted), and, for comparison, from mesophiles (i.e. organisms living in moderate temperatures). We shall also prepare and investigate specific mutants of those proteins. This research is aimed not only at characterising those natural enzymes, but mainly to select from them suitable elements for assembling, using the ‘lego’ method, into larger enzymatic structures of desired properties. We selected for this research thermophilic enzymes because they are unusually stable, and psychrophilic enzymes because they are especially efficient enzymatically. From the selected structural elements we shall construct enzymes which will have extended functionality in comparison to their initial form. For instance, by attaching a chitin-binding domain to a chitinolytic enzyme that does not have it we hope to increase its substrate affinity, or by attaching a ‘spacer domain’ (Ig-like) we should increase the enzyme’s possibility to explore the surface of chitin, which should also increase it’s activity. We also plan to increase enzymes’ stability by exchanging thermolabile domains with more thermostable homologues taken from thermophilic proteins. In the next step we plan to construct compound enzymes that catalyse more than one reaction from the process of chitin degradation. We expect that owing to the proximity of the catalytic domains the product of one domain will find its way more easily to the second one, where it will undergo further degradation. Therefore, such a multienzyme should be more effective than a mixture of individual enzymes.

We want to prove that thanks to modern scientific methods and the choice of a suitable research model one can manipulate in a rational manner even as complex and delicate structures as protein, and construct functional structures not existing in nature. It is important for the success of the project that we work on a well defined biological process and that chitinolytic enzymes often have a multi-domain structure, which makes them easier to manipulate. This is a conclusion from our preliminary research which showed that specially constructed deletion mutants, in which one or more domains have been deleted, retain structural stability to the extent that they can be crystallised and analysed in detail by crystallographic methods.

Another reason why we chose chitinolytic enzymes for our studies is the fact that they catalyse one of the most important biological process taking place in the biosphere, because chitin is the second most abundant biopolymer. The global production of chitin is estimated at 10^{10}-10^{11} tons. Chitin is the building material of cell wall in fungi, exoskeletons of insects and crustaceans, and some elements of the organisms of centipedes and molluscs. Chitin is a derivative of glucose and consists of long chains of connected sugar residues. As a polymer it is similar to cellulose and forms a dense network of hydrogen bonds between the chains, which makes a compact and stable structure, which in turn makes it unusually recalcitrant as a substrate. Chitinolytic enzymes are therefore interesting examples of enzymatic optimisation. In addition to the active site, where the enzymatic reaction takes place, they can have an ability to destabilise the ordered structure of chitin, so that the reaction can proceed more easily. Chitin is degraded by bacteria, archaea and, to a lesser extent by fungi, for this it is a valuable source of energy and the most basic macroelements. Chitin is only to small extent exploited by man.

No natural multienzymes have been discovered for chitin degradation, but such structures, known as cellulosomes, have been discovered in organisms degrading cellulose, which is similar to chitin and produced on an even greated scale. We hope that our work on constructing an ‘artificial chitinosome’ will enrich out knowledge about an important biological process and will contribute to the development of a new scientific speciality known as synthetic biology, whose aim is to design and create artificial biological systems modelled on nature.