



Amfep Fact Sheet on Protein Engineered Enzymes

Protein engineering is a technique to change the amino acid sequence of proteins in order to improve their specific properties. This paper addresses the background of this technique, why it is used in the case of industrial enzymes, as well as regulatory and safety aspects.

Natural evolution, biodiversity and traditional methods of improving industrial enzyme proteins

All proteins, including enzymes, are based on the same 20 different amino acid building blocks arranged in different sequences. Enzyme proteins typically comprise sequences of several hundred amino acids folded in a unique three-dimensional structure. Only the sequence of these 20 building blocks determines the three-dimensional structure, which in turn determines all properties such as catalytic activity, specificity and stability.

Nature has been performing 'protein engineering' for billions of years since the very start of evolution. Natural spontaneous mutations in the DNA coding for a given protein result in changes of the protein structure and hence its properties. This natural variation is part of the adaptive evolutionary process continuously taking place in all living organisms, allowing them to survive in continuously changing environments. Natural variants of enzyme proteins are adapted to perform efficiently in different environments and conditions. This explains why in nature enzymes belonging to the same enzyme family but isolated from different organisms and environments often show a variation in amino acid sequence of more than 50%.

The properties of enzymes used for industrial purposes sometimes also require some adaptations in order to function more effectively in applications for which they were not designed by nature. Traditionally, such enzyme optimisation is performed by screening naturally occurring microorganisms, followed by classical mutation and selection. The disadvantage of this method is, however, that it may take a very long time until the enzyme with the desired properties is found. This is why protein engineering was developed.

Protein engineering and how it is applied to enzymes

Protein engineering of enzymes is a faster, more controlled, more targeted and more accurate method to optimise the properties of enzymes for a specific industrial application than the traditional method described above. It makes it possible to sidestep the high number of natural isolate screenings that would otherwise be necessary to find the enzyme with the desired properties, and increases the likelihood that a suitable enzyme will be found.

The protein engineering technique involves genetic modification by means of recombinant DNA technology of the enzyme producing microorganism, in particular the enzyme encoding gene, resulting in substitution of one or more amino acids in the amino acid sequence of the enzyme protein.

Strategies for making such amino acid substitutions and developing protein engineered enzymes are based on the knowledge of the structure/function relationships of enzymes, computer modelling and techniques for creating and testing enzyme variants.

Benefits of protein engineered enzymes

Protein engineering enables faster development of optimized enzymes, offering benefits for industry, agriculture, consumers and the environment, such as:

- Reduced consumption of raw materials and energy
- Use of alternative and renewable raw materials
- Reduced CO₂ and other greenhouse gas emissions
- Improved performance of industrial processes
- Improved quality of foods and animal feeds
- Cost-effective production and sustainable development

Protein engineering allows more efficient use of raw materials and energy by enabling more efficient processes, and changing environmentally unfriendly processes into more sustainable ones by development of tailor-made enzymes for applications where no traditional enzymes are available. For example, enzymes can be designed to be active at high or low temperature, at very alkaline or acidic conditions, to have higher conversion rates, or to be compatible with other enzymes and chemicals, like detergents.

Protein engineered enzymes were introduced 20 years ago, mainly in laundry and automatic dish wash detergents to improve enzyme performance at washing conditions in compatibility with other ingredients like bleach. They are now widely used, offering additional cleaning and environmental benefits.

More recently, protein engineered enzymes have also been marketed for specific food applications. Many speciality enzymes are used in food processing and protein engineered enzymes hold great potential for improving food processing and food quality.

Safety assessment and regulation of protein engineered enzymes

Protein engineered enzymes and the genetically modified microorganisms (GMMs) used for their production undergo the same procedures for safety assessment as conventional enzymes. The GMMs as well as the commercial enzyme preparations are well characterised, certified as safe and widely assessed and approved by national and international authorities.

Since the protein structures of protein engineered enzymes fall within the spectrum of natural

amino acid sequence variation, there are no reasons to suspect or evidence to support unexpected or unusual safety characteristics, as long as the starting enzyme being subjected to modification is well-characterized.

Like conventional enzymes they are potential respiratory allergens. Relative respiratory allergenicity varies to some extent among naturally occurring enzymes. However, since the introduction of protein engineered enzymes twenty years ago, no significant difference has been observed between the human allergenic potential of protein engineered and conventional enzymes.

Respiratory enzyme allergy is an occupational risk only and effectively prevented by complying with product formulation, hazard labelling and handling standards. Many years of experience and numerous studies show that enzymes present no risk of causing allergies to the consumer.

In terms of environmental safety, protein engineered enzymes are produced according to the EU Directive on the contained use of GMMs. They are as readily biodegradable as conventional enzymes and without risk to the environment.

Consequently, it has not been found necessary to set up special provisions for protein engineered enzymes.

Amfep position and further information

Protein engineering is regarded by Amfep as a safe and useful tool in the development of improved enzyme products and processes that bring real benefits to manufacturers, consumers and society. Amfep promotes an open dialogue on the use of this technology. Further information on enzymes and protein engineering is available from Amfep and its member companies.

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