Technology Offer

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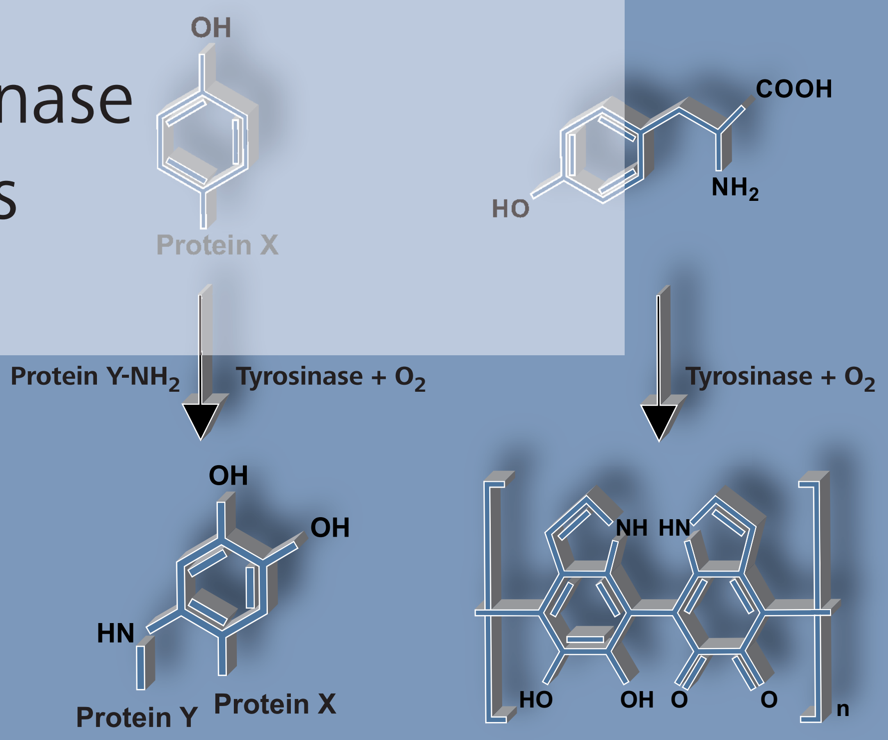
EMPA Materials Science & Technology

Bacterial Tyrosinase for Biomaterials



Invention

The invention provides an isolated highly purified tyrosinase enzyme from the bacterium *Verrucomicrobium spinosum* and mutants of the enzyme having greatly enhanced activities. A method for the recombinant production of high yields of the novel bacterial tyrosinase has been developed and the enzyme applied in the formation of biomaterials such as cross-linked proteins and melanin.

Background

Tyrosinases are copper containing enzymes that play a central role in the formation of the naturally occurring pigment, melanin. Melanins have the ability to absorb UV radiation, metals, sound and also have anti-oxidant and semi-conductor properties. These complex biopolymers have found applications in sunscreens, cosmetics, water purification, as UV filters in sunglasses and may prove useful in the development of photovoltaics. Alternatively, melanins can also be synthesized from the corresponding phenols and hydrogen peroxide. However, the disadvantage of this method is the high unspecific reactivity of hydrogen peroxide.
Currently a crude preparation of mushroom tyrosinase is utilised in the majority of the above described applications, although comparatively inexpensive the high level of contamination by other proteins and bimolecules in such preparations can present a disadvantage in some situations.

Advantages

The recombinant tyrosinase can be easily expressed and purified (>95%) from its host organism, *Escherichia coli*, in relatively high yields (10-20mg of pure protein per litre of shake flask culture). Given its recombinant form the enzyme can also be easily mutated if so desired allowing it to be tailored for specific applications. The tyrosinase can be produced in an apo (inactive) form in bacteria which greatly simplifies purification by avoiding the formation of melanin (which is usually a hindrance in the production of such enzymes). Activity can be restored by simply incubating the enzyme in the presence of chloride or sulphate salts of copper.

Applications

Due to their ability to utilise a wide variety of mono- and di-phenolic compounds tyrosinases have been used for making phenol biosensors, cross-linking proteins (including food proteins like casein) and removal of phenols from waste waters. As well as these applications, the recombinant *V. spinosum* tyrosinase protein can be used to produce tailor-made melanin and other polyphenolic materials using various phenols and catechols as starting materials. Such biomaterials may have a variety of applications as organic semi-conductors or in photovoltaics. The tyrosinase enzyme can also be used to produce cross-linked proteins, allowing enzyme biocatalysts such as lipase to be easily recycled. Tyrosinase can also be used to cross-link food proteins causing changes in consistency and texture. Stamping of the tyrosinase enzyme onto plastic surfaces allows the formation of thin films of melanin directly in-situ, which may find applications in tissue culture, as such films have been shown to be conducive to nerve cell growth. It has also been demonstrated that melanin has a bacteriostatic effect; thus, such thin films may be useful in the prevention of bacterial contamination.

Ownership

Empa, Swiss Federal Laboratories for Materials Science and Technology, Überlandstrasse 129, CH-8600 Dübendorf; *patent pending*

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Keywords

Anti-bacterial Coating, Conductive Biopolymer, Organic Semi-Conductor, Enzyme Immobilization, Melanin, Metal Chelation, Sound Absorption, Tyrosinase, *Verrucomicrobium spinosum*.

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