CHAPTER 12

Recent Applications of Enzymes in Personal Care Products

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INTRODUCTION

Enzymes, unique protein molecules that catalyze most of the reactions in living organisms, are rightly termed as catalytic machinery of the living system. Current applications of enzymes are focused on many different markets including pulp and paper, leather, detergents, textiles, pharmaceuticals, chemicals, food and beverages, biofuels, animal feeds, and personal care, among others (Adrio and Demain, 2014). At the same time, the end use market for industrial enzymes is extremely widespread with numerous industrial commercial applications (Adrio and Demain, 2005). Over 500 industrial products are being made using enzymes (Johannes and Zhao, 2006; Kumar and Singh, 2013). It was in 1833 that scientists discovered that a thermolabile substance was able to convert starch into sugar and called it "diastase," which is now known as "amylase." Later, in 1926 the protein nature of enzymes were finally confirmed when Summer (1926) successfully crystallized urease enzymes from Jack beans. Then an era of utilizing cell-free enzymes started with renin, an aspartic protease in cheese making. In the last 20 years, the global beauty market has grown by 4.5% a year on average (CAGR), with annual growth rates ranging from around 3–5.5%, also known as cosmetics and toiletries or personal care products (PCPs) (Barbalova, 2011). The world's cosmetic industry is worth tens of billions of US dollars, and the industry is constantly seeking new products with ingredients that have specific actions for which enzymes have been the most preferred choice for enhancement of PCPs. The first commercial enzyme was prepared by Rohm in Germany in 1914. The trypsin enzyme was isolated from animals; it degraded proteins and was used in detergents. The larger-scale commercialization of enzymes started with microbial protease derived from *Bacillus*, which was used in washing powders. In 1959, Novozyme of Denmark made it a huge business when they started manufacturing detergents with these microbial enzymes. In addition to the cheese industry, enzymes had been used in various fields, such as food industries and manufacturing of fruit juice, since 1930, however, a major breakthrough started around the 1960s when enzymes were industrially manufactured and were used in the starch industry. The traditional acid

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hydrolysis of starch was completely replaced by alpha amylases and glucoamylases that could entirely convert starch into glucose. The starch industry became the second largest industry to use enzymes after the detergent industry. Over the years, biotechnology has shown that it is now possible to utilize and harness the use of these enzymes in diverse fields. Enzymes have recently been started to be used by cosmetic scientists in developing PCPs for wide acceptability as they have been found to have good consumer appeal and improved performance. However, they have always been poorly evaluated for their functionality in cosmetic science. Proteolytic enzymes like bromelain, papain, etc. have been used in PCPs for skin peeling and smoothing for many years, however, the general problem associated with such use is the irritation caused by some enzymes on the skin surfaces due to their proteolytic activities. The area where the topical applications of enzymes are widely explored and have shown significant benefits is in skin protection, with enzymes having excellent stability. The enzymes used for skin protection have profound abilities to capture free radicals caused by environmental pollution, microorganisms, sunlight, radiations etc. The recent trend of application of enzymes in PCPs shows ample variability in terms of enzymes used from different types of classes for their specific roles and function. Studies of enzyme formulations suitable for topical use have also shown that such dosage forms are relatively easy to handle. However, the choice of base, surface active agent, etc., is important to provide for a stable formulation, and proper vehicle selection is also critical for the proper activity. Another futuristic approach to cosmetics and skin care product development is to increase the efficacy of existing ingredients that might improve skin functioning. Many new topical ingredients—from mushrooms to salmon caviar to sea urchin spines to green algae to knotweed—have been placed in complex antiaging formulations (Draelos, 2012). Nanoparticles are revolutionizing many areas of chemistry, physics, and possibly cosmetic formulation. The long-term effects of nanoparticles in the oceans of the world are not currently known. Yet, nanoparticles could be the next frontier in cosmetic dermatology (Sonneville-Aubrun et al., 2004). Nanoparticles have great potential to create topical cosmeceutical formulations that behave in ways that enable better penetration of active skin ingredients. Someday in the not-too-distant future we may be using nanoparticle therapy, nanoemulsions, polymeric nanoparticle spheres, and nanoliposomes to improve the appearance of the skin (Tadros et al., 2004). Nanotechnology may allow ingredients to exhibit new skin effects, improving cosmetics and skin care product efficacy. We will discuss a few of the specific and widely used enzymes, with the main focus on the nature of their activity (Table 12.1).

Superoxide Dismutase

Superoxide dismutase (SOD) belongs to the oxidoreductase class of enzymes and catalyzes oxidation and reduction reactions. SOD is one of the most effective and popular tropical enzymes so far being used in skin care products. After its discovery as a blue/

Table 12.1 Use of Enzymatic Activities in Cosmetic Products (Ugo Citernesi and Kathe Andersen; www.iralab.it/download/pubblicazioni/New_trends_in_drug.pdf)

Enzyme	Source	Cosmetic Use
Protease	Fungi	Peeling/antiaging/ antiwrinkle
Lipases	Bacteria	Anticellulitis
Hyaluronidase	Bacteria	Moisturising agent
Tyrosinase	Yeast (recombinant) *Tenebrio molitor* Fungi	Tanning agent
Superoxide dismutase	Yeast (Recombinant)	Antifree radicals
Peroxidase	Horseradish, bacteria and yeast (recombinant)	Antifree radicals
Alkaline Phosphatase	Yeast and fungi (recombinant)	Antiwrinkle Energetic

green protein in 1938 by Mann and Leilin and its subsequent characterization as an enzyme and named as superoxide dismutase by McCord and Fridovitch in 1969, this enzyme has been frequently used in various fields for its effective role in catalyzing superoxide free radicals. Reactive oxygen species (ROS) are produced by cells during normal metabolic activities such as mitochondrial oxidative phosphorylation; however, levels of ROS vary with UV exposure and levels of antioxidant enzymes. Without inactivation, ROS damages macromolecules including lipid, proteins, and DNA (Zastrow et al., 2009). Numerous studies have tested the effects of solar radiation and oxidative stress on the skin (Lan et al., 2013), and oxidative stress has been linked to age-related loss of skin elasticity (Nylor et al., 2011), defective cellular signaling, and photoaging (Lee et al., 2012). Antioxidant enzymes mediate the removal of ROS, with different enzymes functioning in specific compartments thereby preventing ROS from reacting with DNA and other cell signal proteins, impairing their function (Fig. 12.1). Functionally, SODs are characterized as potential oxidizing and reducing agents, and many studies have demonstrated their applications in cosmetics and PCPs for younger looking skin. L'Oréal, a cosmeceutical company, was the first to obtain a European Union (EU) patent for this enzyme for its general use in cosmetics in the year 1973 (EU patent no. 2 287 889) and ever since the SOD from marine sources has been in use in developing PCPs. The main factor involved in utilizing enzymes as an active component of any PCP is its side effects and acceptability; many of the SODs derived from different sources were not effective or not considered suitable in the early days because they were reported to cause skin irritation. In 1987 Brooks Industries developed CuZn-SOD derived from yeast, which was formulated as a powder (Biocell SOD-Yeast CuZn-SOD) containing approximately 600 IU SOD activity. This form of yeast protein-bonded SOD is known to have excellent stability at 45°C in aqueous solution in comparison to pure forms of SOD.

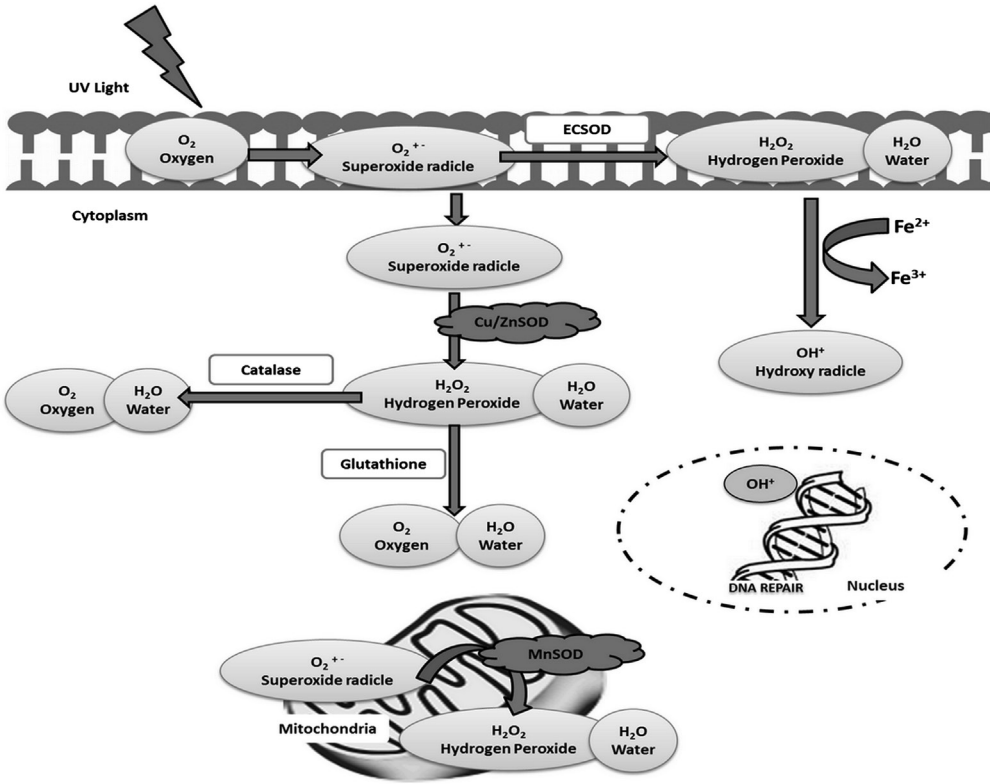

Figure 12.1 A schematic representation of different antioxidant enzyme functioning, in specific cell compartments for removal of reactive oxygen species. *ECSOD*, extracellular superoxide dismutase; *Cu/Zn SOD*, copper/zinc superoxide dismutase; *MnSOD*, manganese superoxide dismutase. *Reproduced with input from Amaro-Ortiz, A., Betty Yan, B., D'Orazio, J.A., 2014. Ultraviolet radiation, aging and the skin: prevention of damage by topical cAMP manipulation. Molecules 19 (5), 6202–6219.*

This yeast SOD was also found to be nonirritating and nonsensitizing both in powder form and as an active 1% yeast CuZn-SOD liposome. Studies have also shown that these yeast-derived SODs are excellent antioxidants much better than commonly used antioxidants, like tocopherol and polyphenols. A few of the examples of products containing SOD as an active ingredient are presented in Table 12.2.

Peroxidase

There are two different types of hydroxyl free radical–scavenging enzymes, known as peroxidase and catalase belonging to the oxidoreductase class of enzymes. Plants are known to have heme-containing peroxidases, which are nonspecific peroxidases and are capable of acting on a variety of substrates including hydrogen peroxide. Similar nonspecific enzymes in animals are lactoperoxidase (thiocyanate ion oxidation), myeloperoxidase (phagocytosis), and thyroid peroxidase (iodine ion oxidation). However, the

Table 12.2 Commercial Products Containing Superoxide Dismutase as an Active Ingredient and Their Use (http://cosmetics.specialchem.com)

Products	Purpose	Manufacturer
Dismutin BT	Skin care:antiaging, antiinflammatory	DSN Nutritional Products, LLC
Dismutin BT 5000	Skin care- antiaging and antioxidant	DSN Nutritional Products, LLC
Liposystem complex	Skin care- Antioxidant Moisturizer	IRA Istituto Ricerche Applicate
Zymo Radical	Skin care- Smoothing Moistening Antiwrinkling	IRA Istituto Ricerche Applicate
Zymo Radical MD	Skin care- Antiwrinkling Moistening Smoothing	IRA Istituto Ricerche Applicate
Detox Duo	Skin care Protection Antioxidant	MD Skincare
Arch-Biocell SOD	Body lotion –Skin care antiinflammation Protective Antioxidant Soothing agent	LONZA
Brookosome SOD	Body lotion –Skin care antioxidant	LONZA
Chronosphere SOD	Body Lotion-skin care Antioxidant	LONZA
ProCircul8	Skin care Protective Antioxidant Antiinflammatory	LONZA

most studied one is the horseradish peroxidase obtained from the roots of horseradish. These free radical–scavenging enzymes are been extensively used in PCPs. For instance, fennel seed extracts containing peroxidase are being used in cosmetics because of their high-lipid peroxidation activities and low odor. The pale yellow/green liquid extract is also shown to have nonirritating and nonsensitizing activity and has shown much better protection activity than tocopherol. Lignin peroxidase, a novel skin-lightening active agent derived from a fungus is being studied with some interest for developing as an ingredient in products to treat pigmentation disorders. Some pigmentation disorders resulting from excessive sun exposure leading to solar lentigo are notoriously

difficult to treat. Melanin is a very durable compound, and researchers have been largely unsuccessful in finding ways to break down melanin to reduce unwanted skin pigment. The existing topical treatments for skin lightening focus on the prevention of melanin formation by blocking tyrosinase and inhibiting its biosynthesis; by preventing the stimulation of melanocytes by UVA; or by blocking the transfer of melanosomes to keratinocytes via the PAR-2 receptor. The enzyme lignin peroxidase (LIP) was first identified by Gold et al. in 1984 and has been researched for many years as a potential agent to break down lignin to whiten wood pulp in paper production (Fig. 12.2). It was later found to break down eumelanin, which has a chemical structure similar to lignin. The development of lignin peroxidase as a skin-lightening agent resulted from these discoveries (US Patent and Trademark Office Patent Application 20060051305). This novel skin-lightening active ingredient is produced extracellularly during submerged fermentation of the fungus *Phanerochaete chrysosporium* (Woo et al., 2004) and then purified from the fermented liquid medium (Lonza of Switzerland). The LIP enzyme (trademarked as Melanozyme) identifies eumelanin in the epidermis and specifically breaks down the pigment without affecting melanin biosynthesis or blocking tyrosinase. Although there are other types of lignin peroxidase enzymes, at

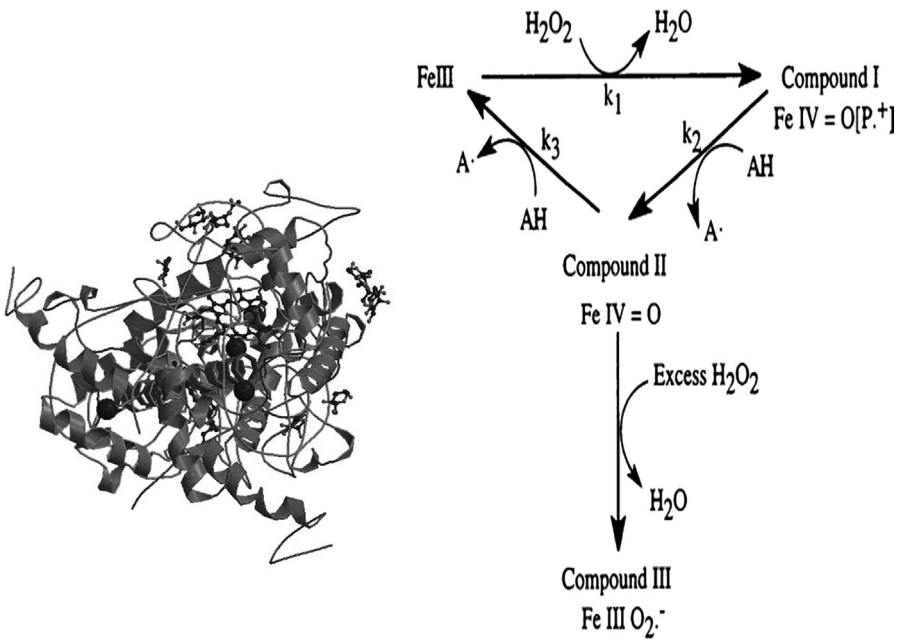

Figure 12.2 The crystal structure of lignin peroxidase at 1.70 Å resolution obtained from Protein Data Base and catalytic cycle of lignin peroxidase. *Adapted with permission from Gold, M.H., Wariishi, H., Valli, K., 1989. Extracellular peroxidases involved in lignin degradation by the white rot basidiomycete* Phanerochaete chrysosporium. *In: Whitaker, J., Sonnet, P. (Eds.), Biocatalysis in Agricultural Biotechnology. Toronto, Ontario, Canada: American Chemical Society, 127–140.*

this point, Melanozyme is the only one that has been developed and proved to be effective for skin lightening. Melanozyme is a glycoprotein active at pH 2–4.5. Melanozyme is currently proprietary and is available only in a new skin-lightening product known on the market as Elure. The safety of lignin peroxidase as a skin-lightening active ingredient has been demonstrated in preclinical studies with doses that are 17,000 times the recommended dose without prompting any side effects. LIP is nonmutagenic and nonirritating to eyes. The potential for skin irritation is very low, and in studies of 50 subjects each, there were no reports of skin irritation during acute sensitivity or cumulative sensitivity, or when used in sensitized skin. A few examples of products containing peroxidase as active ingredient are presented in Table 12.3.

Tyrosinase

Tyrosinase, an oxidase that is the rate-limiting enzyme for controlling the production of melanins is mainly involved in two distinct reactions of melanin synthesis: (1) the hydroxylation of a monophenol, and (2) the conversion of an *o*-diphenol to the corresponding *o*-quinone. *o*-quinone undergoes several reactions to eventually form melanin (Hideya et al., 2007; Kumar et al., 2011) (Fig. 12.3). Melanin synthesis in melanocytic cells is ultimately regulated by tyrosinase, a membrane-bound copper-containing glycoprotein, which is the critical rate-limiting enzyme. Tyrosinase is produced only by melanocytic cells, and following its synthesis and subsequent processing in the endoplasmic reticulum and Golgi, it is trafficked to specialized organelles, termed melanosomes, wherein the pigment is synthesized and deposited. In the skin and hair, the melanosomes are transferred from melanocytes to neighboring keratinocytes and are distributed in those tissues to produce visible color (Hideya et al., 2007). During the past years, the cosmetic industry increasingly worked with substances involved in natural melanin formation. The advantages here are obvious. Unlike the

Table 12.3 A Few of the Commercial Products Containing Peroxidase as an Active Ingredient and Their Uses (http://cosmetics.specialchem.com)

Products	Purpose	Manufacturer
Liposystem complex	Antioxidants Moisturizing agents Nourishing agents	IRA Istituto Ricerche Applicate
Zymo radical MD	Antiwrinkle agents Moisturizing agents Smoothening agent	IRA Istituto Ricerche Applicate
Zymo radical	Antiwrinkle agents Moisturizing agents Smoothening agent	IRA Istituto Ricerche Applicate
ABS fennel extract	Antiaging Antistress/Relaxing agents	Active Concepts

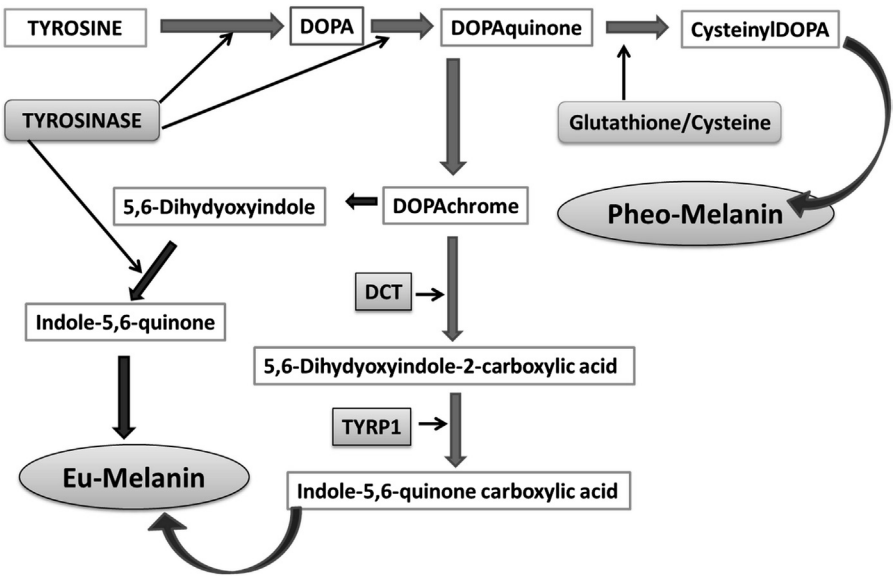

Figure 12.3 Schematic overview of melanin synthetic pathway and the involvement of melanogenic enzymes. Initial melanin synthesis is catalyzed by tyrosinase and is then divided into eumelanogenesis or pheomelanogenesis. The other melanogenic enzymes, that is, L-3,4-dihydroxyphenylalanine (DOPA) chrome tautomerase (DCT) and tyrosinase-related protein 1 (TYRP1), are involved in eumelanogenesis.

melanoidin process, a natural tan is induced and protection against UV radiation also is provided. It is a well-known fact that the enzyme tyrosinase transforms the amino acid tyrosine into dihydroxyphenylalanine (DOPA) and into its quinoid form, the DOPA quinone, which is the base for the formation of both the melanin types, eumelanin (dark brown) and pheomelanin (reddish yellow). The combination of both the types is responsible for the skin tone, which varies from skin to skin. The tyrosinase is controlled by UV radiation and induced by the α-melanocytes stimulating hormone (α-MSH). Further tyrosinase stimulators are the β-endorphins. Endorphin-related substances can be found in specific vegetable extracts as, eg, the chaste berry or chaste tree (vitex agnus castus), and together with synthetic acetyl tyrosine, a tyrosine pro-drug, they are able to induce the UV independent formation of melanin. Additional UV radiation will speed up and stimulate the melanin formation process after the product has been applied. New developments concentrate on additional tyrosinase activators and adequate transport systems to integrate the substances into the skin (Lautenschltens, 2007). Zymo-tan complex, a tanning activator, consists of tyrosine amino acids (precursors of melanine) and tyrosinase. Tyrosinase is an enzyme that catalyzes the reaction forming the melanin in the presence of solar radiation. The enzyme, present in several plants, has been also isolated from leucocytes, yeast, and milk. Some of the tyrosinase-based products are presented in Table 12.4.

Table 12.4 A Few of the Commercial Products Containing Tyrosinase as an Active Ingredient and Their Use (http://cosmetics.specialchem.com)

Products	Purpose	Manufacturer
Hydrosoluble zymo tan complex	Sunscreen agents, self-tanning agents	IRA Istituto Ricerche Applicate
Zymo tan complex PF	Sunscreen agents, self-tanning agents	IRA Istituto Ricerche Applicate
Zymo tan complex	Sunscreen agents, self-tanning agents	IRA Istituto Ricerche Applicate
Brookosome	Sunscreen agents, self-tanning agents	Lonza

Proteases

Proteases (also known as peptidases or proteinases), their substrates and inhibitors are of great relevance to biology, medicine, and biotechnology. Proteases are referred to as a group of enzymes that hydrolyze the protein bonds of amino acids (proteolysis). Proteases have evolved multiple times, and different classes of protease can perform the same reaction by completely different catalytic mechanisms (Gupta and Khare, 2007; Kalpana Devi et al., 2008). Proteases constitute the largest group of enzymes in bioindustry with an array of applications. They play an important role in industrial biotechnology, especially in detergents, foods, pharmaceuticals, and in PCPs. Proteolytic enzyme is essential for several physiological processes like digestion of food proteins, protein turnover, cell division, blood clotting cascade, signal transduction, processing of polypeptide hormones, etc. (Li et al., 2013). The vast variety of proteases, with specificity of their action and application, have attracted worldwide attention to exploit their physiological as well as biotechnological applications (Poldermans, 1990). They are considered eco-friendly because the appropriate producers of these enzymes for commercial exploitation are nontoxic and nonpathogenic and are designated as safe (Gupta et al., 2002). Proteases are used extensively in the pharmaceutical industry for preparation of medicines, such as ointments for debridement of wounds. They are also used in denture cleaners and as contact lens enzyme cleaners (Ogunbiyi et al., 1986). Proteases that are used in the food and detergent industries are prepared in bulk quantities and are used as crude preparations; whereas those used in medicine are produced in small amounts but require extensive purification before application (Bholay and Patil, 2012). The thermostability and their activity at high pH and the alleviation of pollution characteristics have made proteolytic enzymes an ideal candidate for laundry applications. Alkaline proteases are supplemented in different brands of detergents for use in home and commercial establishments. Enzymes have been added to laundry detergents for the last 50 years to facilitate the release of proteinaceous material in stains, such as those of milk and blood. The proteinaceous dirt coagulates on the fabric in the absence of proteinases as a result of washing conditions. The enzymes remove not only the stain, such as blood, but also

other materials including proteins from body secretions and food, such as milk, egg, fish, and meat. An ideal detergent enzyme should be stable and active in the detergent solution and should have adequate temperature stability to be effective in a wide range of washing temperatures (Aurachalam and Saritha, 2009). A few examples of products containing different types of proteases as active ingredient are presented in Table 12.5.

Lipases

Lipases belong to hydrolases and exert their activity on the carboxyl ester bonds of triacylglycerols and other substrates. Their natural substrates are insoluble lipid compounds prone to aggregation in aqueous solution. Lipases are ubiquitous enzymes present in all types of living organisms. In eukaryotes they may be confined within an organelle (ie, the lysosome), or they can be found in the spaces outside cells and play roles in the metabolism, absorption, and transport of lipids. In lower eukaryotes and bacteria, lipases can be either intracellular or be secreted in order to degrade lipid substrates present in the environment, and in some pathogenic organisms (*Candida albicans*, *Staphylococcus* and *Pseudomonas* species, *Helicobacter pylori*) they can even act as virulence factors. Most bacterial lipases are sourced from *Pseudomonas, Burkholderia, Alcaligenes, Acinetobacter, Bacillus*, and *Chromobacterium* species; widely used fungal lipases are produced by *Candida*, *Humicola*, *Penicillium*, *Yarrowia*, *Mucor*, *Rhizopus*, and *Aspergillus* sp. Among the lipases from higher eukaryotes, porcine pancreatic lipase has been in use for several years as a technical enzyme (Lotti and Alberghina, 2007). Active lipases can mainly be found in cosmetics for surficial cleansing (anticellulite treatment) or overall body slimming, where they are responsible for the mild loosening and removal of dirt and/or small flakes of dead corneous skin (ie, peeling) and/or assist in breaking down fat deposits, often in combination with further enzymes, such as proteases. Further applications have been described for nose cleansing, makeup beauty masks, and hair care. Based on the broad variety of compounds derived from fats and carboxylic acids in cosmetic products, lipases and their hydrolytic, esterifying, and acylating activities show enormous potential for implementation in the production of cosmetic ingredients. In fact, a multitude of possible lipase-catalyzed syntheses have been described to date, and a variety of products have actually been commercialized. For classification, specialty esters, aroma compounds, and functional actives can essentially be distinguished (Marion et al., 2013). A few examples of products containing lipase as active ingredient are presented in Table 12.6.

Hyaluronidase

Hyaluronidase, enzymes that catalyze the hydrolysis (chemical decomposition involving the elements of water) of certain complex carbohydrates, such as hyaluronic acid (HA) and chondroitin sulfates, have been found in insects, leeches, snake venom, mammalian tissues (testis being the richest mammalian source), and in bacteria. HA has gained much importance in cosmetics for its popularity in cosmetic facial augmentation. HA is a naturally occurring glycosaminoglycan disaccharide present in skin, joint synovia,

Table 12.5 A Few of the Commercial Products Containing Protease as an Active Ingredient and Their Uses (http://cosmetics.specialchem.com)

Products	Purpose	Manufacturer
Protease	Antiaging agents Antiwrinkle agents Nourishing agents	Green Tech
Prozymex HBT LS 9142	Antiaging agents Lightening and whitening agents Exfoliants/peeling agents Smoothing agents	Laboratories Serobiologiques
Zymo acids	Conditioning agents	IRA Istituto Ricerche Applicate
Depil enzyme	Depilatory agents Antihair regrowth agents	IRA Istituto Ricerche Applicate
Zymo hair MD	Moisturizing agents	IRA Istituto Ricerche Applicate
Zymo lift MD	Antiwrinkle agents Moisturizing agents	IRA Istituto Ricerche Applicate
Okoumyrrhine	Antiaging agents Antiwrinkle agents Smoothing agents Antiinflammatory	Naturactiva
Dub karite	Antioxidants Antiaging agents Antiwrinkle agents Smoothing agents Antiinflammatory Moisturizing agents Healing agents Shining agents Regenerating agent Healing agent	Stearinerie dubois
PromaCare TA	Lightening and whitening agents	Uniproma chemical
Prozymex HBT LS 9142	Antiaging agents Whitening agents Exfoliants/peeling agents Smoothing agents	Laboratoires Serobiologiques
Bromelain	Lightening and whitening agents Smoothing agents	Spec-chem Industry
BioNatural enzyme SK 320 P	Moisturizing agents Exfoliants/peeling agents Smoothing agents Antiwrinkle agents Conditioning agents	Bio-organic concepts
Eperuline PW LS 9627	Firming agents Toning agents Antiaging agents Antiinflammatory	BASF

Table 12.6 A Few of the Commercial Products Containing Lipase as an Active Ingredient and Their Uses (http://cosmetics.specialchem.com)

Products	Purpose	Manufacturer
CycloLipase	Slimming agents	Sederma Croda International Group
Zymo hair MD	Moisturizing agents	IRA Istituto Ricerche Applicate
Sopholiance	Antimicrobial and deodorants	Soliance
Zymo cell MD	Moisturizing agents Slimming agents	IRA Istituto Ricerche Applicate
Zymo clear MD	Moisturizing agents Antiacne agents	IRA Istituto Ricerche Applicate
Uncaryl	Antiaging agents Antiinflammatory Antiacne agents Slimming agents Antioxidants Sunscreen agents/UV filters	Cobiosa
Pheoslim	Slimming agents	Codif
Pheoslim G	Slimming agents	Codif
Lipocel-ErasePB	Slimming agents	PROTEOS Biotech
Lipocleansing-Erase HydraPB	Antiacne agents Peeling agents Smoothing agents	PROTEOS Biotech
Lipocleansing-SensitivePB	Antiacne agent Antiallergenic agent	PROTEOS Biotech
Spec-Chem-Climbazole	Antidandruff agents Antimicrobials	SpecChem Industry
Lipocel-Erase HYDRA PB	Slimming agents	PROTEOS Biotech
Facial cleaner	Antimicrobial Anticellulite Treatment/ Surfacial cleansing	JUJU Cosmetics
Revue SebumSoap	Antimicrobial Anticellulite Treatment/ Surfacial cleansing	Kanebo Cosmetics
Silhouette Sculptant Exfoliating Mousse 402	Anticellulite treatment	Maria Galland
Double Minceur Cible'e	Anticellulite treatment	Guinot
Bath additive with fat dissolving enzymes	Slimming agents	Ishizawa Laboratories

cartilage, and vitreous (Kablik et al., 2009). For its use as dermal filler, HA is chemically cross-linked to achieve the manufacturer's desired composition, which determines the filler's structure, longevity, and other properties. The properties of HA are adjusted in the manufacturing of different commercially available HA fillers, leading to their differing

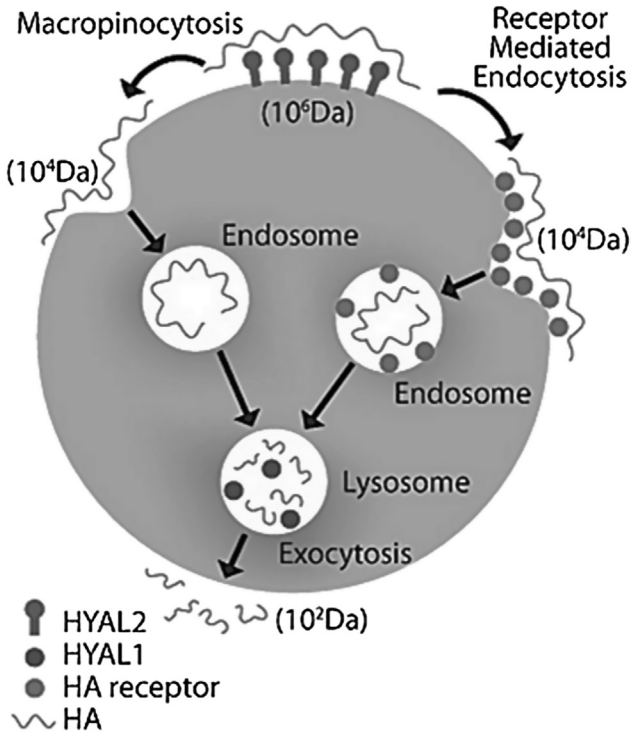

Figure 12.4 Schematic overview of hyaluronic acid (HA) endocytosis and processing. HMW- HA (~106 Da) is first degraded by hyaluronidase 2 (HYAL2) into smaller 104 -Da-sized fragments before it is taken up by a cell. The cell can either utilize surface HA receptors for receptor-mediated endocytosis or macropinocytosis. Once internalized the HA is degraded by hyaluronidase 1 (HYAL1) into small 102 Da fragments and then exocytosed. *Reproduced with input from Racine, R., Mummert, M.E., 2012. Hyaluronan endocytosis: mechanisms of uptake and biological functions. In: Brian, C. (Ed.), Biochemistry, Genetics and Molecular Biology – Molecular Regulation of Endocytosis. ISBN:978-953-51-0662-3.*

structural properties. These varying properties may inform clinicians as to which HA filler would be most appropriate for a specific clinical use. For example, a more highly cross-linked HA filler would likely be resilient in its ability to hold its form, making it suitable for the correction of deep wrinkles. In addition, a more monophasic filler might cleanly retain its form and clinically have a smoother appearance. Hyaluronidase is a naturally occurring enzyme capable of local degradation of HA (Fig. 12.4), thereby providing a means for correction or alteration of injected fillers. It is US Food and Drug Administration (FDA) approved as a temporary dispersion agent for injectable fluids, typically local anesthetics during retrobulbar blocks. It has been used clinically for over 60 years (Silverstein et al., 2012). In the event of complications with HA fillers, hyaluronidase has been used in attempt to reverse HA fillers (Lambros, 2004). Hyaluronidase hydrolyzes HA by splitting the bond between C1 of an N-acetylglucosamine moiety and C4 of a glucuronic acid moiety. It is FDA approved as an agent to increase tissue

Table 12.7 A Few Commercial Products Containing Hyaluronidase as an Active Ingredient and Their Use (http://cosmetics.specialchem.com)

Products	Purpose	Manufacturer
Hyaluronidase	Moisturizing agent	IRA Istituto Ricerche Applicate
Alpaflor Edelweiss B	Antiaging Antimicrobial Antioxidants	DSM
EcoCare Telmesteine	Antiinflammatory Antiaging	Ecochem
Phytessence Wakame	Anti-Inflammatory Antiaging Moisturizing agent Antioxidants	Croda
Hyaldrain PB	Slimming agents	PROTEOS Biotech
Hyalucorrect PB	Conditioning agents	PROTEOS Biotech

permeability to facilitate subcutaneous hydration, drug dispersion, and reabsorption of radiopaque dyes, so its use to reverse HA fillers is off-label. Different formulations of hyaluronidase are available, including a human recombinant agent and an ovine agent (Rao et al., 2014). A few examples of products containing lipase as active ingredient are presented in Table 12.7.

APPLICATION OF NANOPARTICLES FOR ENZYME IMMOBILIZATION

Nanotechnology plays a crucial role in developing elegant and effective cosmeceuticals by using smaller particles that are readily absorbed into the skin and repair damage easily and more efficiently (Singh et al., 2013a). Incorporation of nanotechnology in cosmeceuticals is aimed toward making incense of perfumes last longer, sunscreens to protect the skin, antiaging creams to fight back the years, and moisturizers to maintain the hydration of skin. Some of the innovations brought by nanotechnology intervention in the cosmeceutical arena are nanoemulsions (which are transparent and have unique tactile and texture properties), nanocapsules (which are used in skin care products), nanopigments (that are transparent and increase the efficiency of sunscreen products), liposome formulations (which contain small vesicles consisting of conventional cosmetic materials that protect oxygen or light sensitive cosmetic ingredients), niosomes, nanocrystals, solid lipid nanoparticles, carbon nanotubes, fullerenes, and dendrimers (Fig. 12.5). The primary advantages of using nanoparticles in cosmeceuticals include improvement in the stability of cosmetic ingredients (eg, vitamins, unsaturated fatty acids, and antioxidants) by encapsulating within the nanoparticles; efficient protection of the skin from harmful ultraviolet (UV) rays; aesthetically pleasing products (eg, in mineral sunscreens, using smaller particles of active mineral allows them to be applied without leaving a noticeable

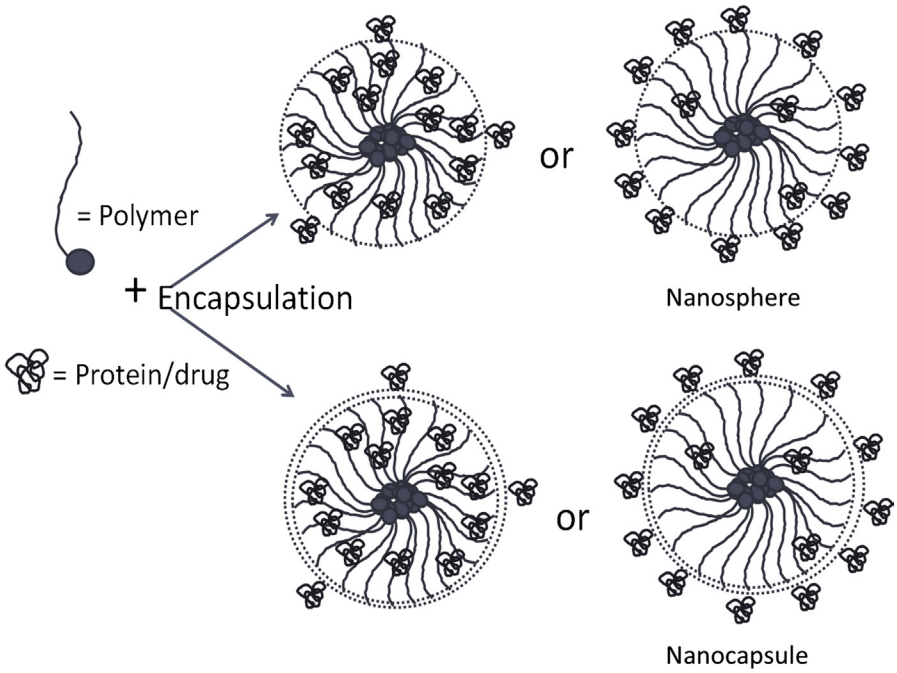

Figure 12.5 Schematic overview of different types of nanoencapsulations.

white cast); targeting of active ingredient to the desired site and controlled release of active ingredients for prolonged effect (Padamwar and Pokharkar, 2006; Mu and Sprando, 2010). LifePak Nano is a perfect example of a commercial product in which nanoencapsulation increases bioavailability of coenzyme Q10, protecting cells, tissues, and organs in the body against the ravages of aging (Lohani et al., 2014). There has been considerable interest in the development of enzyme immobilization techniques because immobilized enzymes have enhanced stability compared to soluble enzymes, and can easily be separated from the reaction. A few examples of nanoimmobilized enzymes are presented in Table 12.8. Approaches used for the design of immobilized enzymes have become increasingly more rational and are employed to generate improved catalysts for industrial applications. There are a variety of methods used to immobilize enzymes, the three of the most common being adsorption, entrapment, and cross-linking or covalently binding to a support. Currently, the major focus of enzyme immobilization has been in the development of robust enzymes that are not only active but also stable and selective in organic solvents. The ideal immobilization procedure for a given enzyme is one that permits a high-turnover rate of the enzyme while retaining high-catalytic activity over time. Proteins are immobilized either by physical adsorption to the surface of the nanoparticle or by covalent bonding to previously functionalized nanoparticles. Application of nanoparticles in formulations for PCPs has paved the way for utilizing these

Table 12.8 Examples of Nanoimmobilized Enzymes With Enhanced Activity (Singh et al., 2013b)

Enzyme	Applications	Kinetic Parameters	Nanoparticles Used
α-Chymotrypsin	Proteolysis (cleave peptide amide bonds)	Immobilized enzyme: $K_m = 31.7\,\mu M$, kcat = $20.0\,s^{-1}$; Soluble enzyme: $K_m = 47.8\,\mu M$, kcat = $17.8\,s^{-1}$	General
Glucose oxidase	Estimation of glucose level up to $300\,mg\,mL^{-1}$	Immobilized enzyme: $K_m = 3.74\,mM$, Soluble enzyme = 5.85 mM	Gold NPs
Diastase	Starch hydrolysis	Immobilized enzyme: $K_m = 8414\,mM$, $V_{max} = 4.92\,\mu mol\,min^{-1}\,g{-}1$; Soluble enzyme: $K_m = 10{,}176\,mM$, $V_{max} = 2.71\,\mu mol\,min^{-1}\,mg^{-1}$	Fe impregnated silica NPs
Keratinase	Synthesis of keratin	Immobilized enzyme: Specific activity = $129.0\,U\,mg^{-1}$; Soluble enzyme: Specific activity = $37\,U\,mg^{-1}$	Iron oxide NPs
Horseradish peroxidase	catalyzes the conversion of chromogenic substrates (eg, TMB, DAB, ABTS) into colored products	Immobilized enzyme: $K_m = 0.8\,mM$, $V_{max} = 0.72\,\mu mol\,min^{-1}\,mg^{-1}$; Soluble enzyme: $K_m = 0.43\,mM$, $V_{max} = 0.35\,\mu mol\,min^{-1}\,mg^{-1}$	Nanoporous Cu NPs
Glucose oxidase	Estimation of glucose level	Immobilized enzyme: $K_m = 2.7\,mM$, $V_{max} = 28.6\,U\,\mu g^{-1}$; Soluble enzyme: $K_m = 9\,mM$, $V_{max} = 6.2\,\mu mol\,min^{-1}\,mg^{-1}$	Silver NPs
β-1,4-Glucosidase (*Agaricus arvensis*)	Lignocellulose hydrolysis	Immobilized enzyme: $K_m = 3.8\,mM$, $V_{max} = 3347\,\mu mol\,min^{-1}\,mg^{-1}$; Soluble enzyme: $K_m = 2.5\,mM$, $V_{max} = 3028\,\mu mol\,min^{-1}\,mg^{-1}$	Silica NPs
Diastase α-amylase	Hydrolyzing soluble starch	Immobilized enzyme: $K_m = 10.3\,mg\,mL^{-1}$; $V_{max} = 4.36\,\mu mol\,mL^{-1}\,min^{-1}$; Soluble enzyme: $K_m = 8.85\,mg\,mL^{-1}$; $V_{max} = 2.81\,\mu mol\,mL^{-1}\,min^{-1}$	Ag NP's doped gum acacia-gelatin-silica nanohybrid
Laccase	Bioremediation of environmental pollutants	Immobilized enzyme: K_m ($10^{-2}\,mM$) = 10.7, V_{max} ($10^{-2}\,mM\,min^{-1}$) = 14.0; Soluble enzyme: K_m ($10^{-2}\,mM$) = 5.69, V_{max} ($10^{-2}\,mM\,min^{-1}$) = 7.7	General
α-galactosidase (*Aspergillus terreus* gr.)	Animal feed	Immobilized enzyme: $K_m = 1.40\,mM$, $V_{max} = 20.16\,U\,mL^{-1}$; Soluble enzyme: $K_m = 4.2\,mM$, $V_{max} = 16.33\,U\,mL^{-1}$	Calcium alginate (Beads)

particles for developing these products with enhanced enzyme activity. For example, LifePak Nano, a nutritional antiaging program formulated to nourish and protect cells, tissues, and organs in the body with the specific purpose of guarding against the ravages of aging, has developed a product (Face Gel- Pharmanex/USA) that uses nanoparticles to enhance enzyme activity and in this product; nanoencapsulation increases bioavailability of coenzyme Q10 by 5–10 times (Lohani et al., 2014).

FUTURE PERSPECTIVES

The global personal care market, estimated at about $300 billion at the retail level, is a highly attractive segment of the consumer products space. The market has seen steady growth of 4.5% per annum in the last few years, from low-capital-intensive asset base, providing high return on capital to investors. Organics-based products is the fastest growing segment of the global personal care industry. Rising concerns for health safety, increasing go-green consciousness and growing consumer awareness toward hazards of synthetic chemicals have fueled the demand for organic PCPs, and increasing health awareness among consumers will continue to drive the growth of the organic personal care market during the forecast period. Among the organic and natural products, enzymes derived from various sources have found their specific utility in formulations of PCPs, which have been increasing rapidly. Application of enzymes in a given topical application depends on the nature of product as well as the limitations associated with the enzyme activity. Shorter shelf life of enzyme-based PCPs is a factor limiting consumer demand. Synthetic products are loaded with a large amount of preservatives in order to conserve their attributes. Enzyme-based PCP manufacturers have a hard time sourcing organic ingredients as an alternative to synthetic preservatives. The organic products containing enzymes as an active ingredient with natural preservatives have a short shelf life or need to be refrigerated. With the advent of nanotechnology, the effectiveness and durability of enzyme-based PCPs have been enhanced. Utilization of nanoparticles for enzyme stability and action is now being considered as an effective measure to address the problems that enzyme-based PCPs face. The enzyme groups that have proved to be quite useful are the oxidoreductases, proteases, and hydrolases, In addition, the search for new enzymes still continues. According to estimates by Kline & Co, a leading consultancy firm, the antiaging segment is the single largest product type in the personal care market and is the key growth engine. Skin care and hair care—the two largest segments of the market—are also the fastest growing, providing sizeable growth opportunities for suppliers. Cosmeceuticals, which are cosmetic products with drug-like benefits, has become the fastest-growing segment of the cosmetics and personal care industry. The global cosmeceuticals market offers huge potential among Asian countries, like Japan, China, and India, which are set to attract major players in the future. Though the market is at a nascent

stage in developing countries, such as India and China, there remains a large untapped potential, with the desire to look young and fair.

LIST OF ABBREVIATIONS

Cu/Zn SOD Copper/zinc superoxide dismutase
DOPA Dihydroxyphenylalanine
ECSOD Extracellular superoxide dismutase
HA Hyaluronic acid
HYAL1 Hyaluronidase 1
HYAL2 Hyaluronidase 2
LIP Lignin peroxidase
MnSOD Manganese superoxide dismutase
NP Nanoparticle
PCP Personal care products
ROS Reactive oxygen species
SOD Superoxide dismutase
TYRP1 Tyrosinase-related protein 1
UV Ultraviolet

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