Promising Perspectives for Transglutaminase In “Bioplastics” Production

Raffaele Porta*, Prospero Di Pierro, Angela Sorrentino and Loredana Mariniello

Department of Food Science, University of Naples “Federico II”, Portici, Napoli, Italy

When Heinrich B. Waelsch [53,13], Laszlo Lorand [33] and John E. Folk [20] – the latter who passed away on Dec 27th 2010 and to whom we wish to dedicate the present Editorial - began to investigate at the middle of the past century on the calcium dependent transamidating activities contained in the guinea pig liver and blood, no one could imagine that transglutaminase (TG, EC 2.3.2.13) would become at the beginning of the new millennium one of the most interesting biocatalyst for industrial applications in several different fields. An accurate historical overview of the first 50 years of TG research was recently reported by Beninati et al. [3] in occasion of the “IX Conference on Transglutaminase and Protein Crosslinking” held in Marrakesh in the early days of September 2007.

The complete name of the enzyme, R-glutaminyl-peptide:amine-γ-glutamyltransferase, indicates that the catalysis consists of the acyl transfer of γ-glutamyl residues, present in protein or peptide substrates (acyl donor), to an acyl acceptor substrate, resulting in a variety of different products depending on the involved molecules [21]. The transamidation reaction occurs when the acyl acceptor is either the ε-amine group of an endoprotein lysine residue or a low molecular mass primary amine, generating ε-(γ-glutamyl)lysine crosslinked linear or branched homo- and heteropolymers, in the first case, or protein-amine deriva- tives in the latter. The wide range of interest on TG biological role is mostly related to the existence of multiple molecular forms of the enzyme which are present in different organisms, such as bacteria, plants, invertebrates and vertebrates [39,41,42, 49].

Although TG was mostly studied for the ability to post-translationally remodel many different proteins in vivo, its reaction has attracted the attention of many scientists in the last twenty years as an effective way to manipulate the structure of proteins of different origin outside the living cells. Therefore, applied investigations with the different enzyme isoforms have been carried out in different fields, from biomedicine and cosmetics to food, leather, and textile industry [41]. As far as the human health applications, concentrated Factor XIII, a zymogenic form of the enzyme, is used as therapeutic agent to correct blood coagulation in a rare genetic condition due to its deficiency and to reduce bleeding risks [36]. Factor XIII was the first TG isoform used to modify protein and peptide substrates in vitro [45]. In addition, the so-called “tissue” TG (type 2) is an effective biomedical technological tool exploited for the diagnosis of an autoimmune pathology like the celiac disease [15,19]. Also this isoform was extensively tested to modify both structure and biological properties of several polypeptides in vitro [41]. However, both Factor XIII and type 2 TG are not really attractive for industrial uses, since their production is expensive and they cannot be easily manipulated outside of their natural environment. Moreover, further molecular forms of the enzyme extracted from plant or animal tissues were never suggested for biotechnological applications. Keratino cyt TG, for example, needs a complex post-translational modification to be fully active [9] and was proposed only as a possible target for gene therapy since it is involved in lamellar ichthyosis [26].

The scenario suddenly changed about twenty years ago, when a microbial TG (mTG) was isolated from Streptverticillium sp. and its characterization indicated that such enzyme is quite different from the other isoforms, having smaller molecular mass (about 40 kDa despite 80–100 kDa of mammalian TG) and very low-sequence identity [1]. Although the hydrophobic environment of the catalytic site, including a single cysteine residue, was found to be similar to that of the other isoforms, no sequence identity with the calcium-binding domain was detected. In fact, mTG possesses a calcium-independent activity and, furthermore, exhibits a wide substrate specificity being also active over a wide range of temperature and pH values [61]. Therefore, because of the low-cost mass production by traditional fermentation technology and since it has been “Generally Recognized As Safe” and its addition is allowed in food, mTG has been largely utilized in the last decade as a biotechnological tool, mostly in the food field [31,62] and numerous investigations have been carried out by modifying with it proteins of different origin [41]. Ajinomoto Co. Inc. actually produces several preparations of mTG that are commercialized with different names. They differ in stabilizer composition in relation to the type of food for the production of which they have been designed. In agreement with the EU legislation (Directive 89/107/EC), Ajinomoto declared that mTG could be considered as a processing aid and, thus, its presence does not need to be indicated in the finished products. As a consequence, mTG is currently used to improve texture, mechanical properties, and emulsifying characteristics of food proteins for their best utilization as ingredients of complex foods [31].

A more recent and promising exploitation of mTG is related to the production of the so-called “bioplastics” both biodegradable and edible. Most of the biodegradable plastics are produced utilizing renewable biomass sources, whereas fossil-fuel plastics (poly-ethylene, -vinyl acetate) and since it has been “Generally Recognized As Safe” and its addition is allowed in food, mTG has been largely utilized in the last decade as a biotechnological tool, mostly in the food field [31,62] and numerous investigations have been carried out by modifying with it proteins of different origin [41]. Ajinomoto Co. Inc. actually produces several preparations of mTG that are commercialized with different names. They differ in stabilizer composition in relation to the type of food for the production of which they have been designed. In agreement with the EU legislation (Directive 89/107/EC), Ajinomoto declared that mTG could be considered as a processing aid and, thus, its presence does not need to be indicated in the finished products. As a consequence, mTG is currently used to improve texture, mechanical properties, and emulsifying characteristics of food proteins for their best utilization as ingredients of complex foods [31].

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Figure 1: TG-crosslinked bioplastics.
nyl, -propylene, -stylene, -amide derivatives) are petrochemical-based materials. Bioplastics development is happening in the context of the industrial chemistry “greening”, as a consequence of the need to derive more carbon for chemical processes from renewable molecules instead of oil reserves [24]. Interest in using biodegradable materials strongly increased also because of the consumer’s awareness of environmental damage caused by plastics of petrochemical origin, more than 250 million tons of which are still produced each year throughout the world [52]. Many leading companies and newcomers have announced investments in expanding the production capacities of bioplastics within the upcoming years and, accordingly, the global production capacity of bioplastics could reach approximately 1.5 million tons in 2012. Biodegradable polymers have been used so far for various applications in medicine [8], since several biomaterials have been developed as surgical implants in vascular and orthopaedic surgery and as implantable matrices for the controlled long-term release of drugs inside the body. Some of them have also been commercialized in food industry and agriculture. BASF in Germany has developed bioplastics trademarked as Ecovio® [58] and its derivative Evcoflex® (containing up to 75% renewable raw materials) (www.plasticker.de), that can be broken down chemically by the action of a multitude of common microorganisms in the soil. Different biodegradable films, well known as Mater-Bi®, have been produced and commercialized by Novamont in Italy [24]. In particular, these bioplastics have been proposed for shopping or organic waste bags (where they can be composted together with the food or green waste), as vehicles to carry specific substances to monitor and/or influence the quality of wrapped foods thus realizing an active packaging, or as mulch films for agricultural applications [45,56]. It is noteworthy that over 3.6 million tons of traditional plastics are used in agriculture worldwide each year [50], mulching films accounting for around 40% of this use. Other mechanically more resistant bioplastics are used for the production of diaper back sheets, cotton swabs, toothpaste tubes, fast-food tableware, containers for meat, eggs and vegetables, as well as bottles for soft drinks and dairy products and blister foils for fruit. In 2003 Sanyo introduced a CD sample based on biodegradable polyactic acid, and one year later the Japanese electronics group Pioneer developed a cornstarch-based optical media, a 1.2 millimetres thick “bio-disk” with a capacity of maximum 25 gigabytes (www.european-bioplastics. org). More recently, Fujitsu commercialized even a computer mouse completely made with two wood-based materials, lignin and cellulose, that can be disposed of in industrial compost settings and the remains can be recycled as well (www.greenbize.com). However, bioplastics still cover less than 10% of the European current plastic market (about 50, 000 tons), the development costs being still high and having not yet the benefit of “economies of scale” [54]. But, although the new biomaterials are still more expensive than their petro-based relatives, the gap is decreasing and bioplastics competitiveness is greatly improving. The increased biomass exploitation as industrial raw material seems to be extremely expedient mostly for the European countries, possessing limited crude oil and natural gas resources.

The main components of bioplastics also include biopolymers of mineral origins, like polypeptides and polyvinylalcohols, while those of natural origin contain polysaccharides, proteins, lipids and polypeptides synthesized by several microorganisms [7]. For natural film preparation one way is to recycle agricultural industry by-products, that are very rich of both carbohydrates and proteins, with the concurrent goal to manage lower waste amounts and reduce environmental pollution [44]. Starch, cellulose, chitosan and pectin - besides exudate, seed or microbial fermentation gums (arab, guar, xanthan and gelan gums), and seaweed extracts (carrageenans and alginates) - are the most used polysaccharides so far utilized [29,6]. Thermoplastic starch is the most widely used component, constituting almost the fifty percent of the current bioplastics market. Conversely, polypeptide-based biodegradable materials include collagen, gelatin, casein, zein, wheat gluten, and egg white, whey and soy proteins [30,5]. It has been demonstrated that films prepared with polysaccharides are quite resistant but exhibit poor water vapor barrier features, owing to their hydrophilic nature [29], whereas protein-based films show superior oxygen barrier characteristics [28]. However, since petrochemical-based materials are known to exhibit good mechanical performance, heat sealability, effective barrier to gases, and are available at low cost, increasing efforts to both identify additional biopolymers and develop new methods for preparing market compatible bio-based materials are needed.

Although some of the described bioplastics disadvantages were partially obviated by the preparation of biopolymer composite (containing protein/polysaccharide/lipid) [5,56] and nanocomposite (incorporating mineral fillers such as clay, silica or talc) [52] materials, their permeability and mechanical properties might be greatly improved by physical, chemical or enzymatic crosslinking of target bioplastics components [6,22]. Thus, several studies were carried out in the last decade in this direction [14,48]. γ-irradiation was found to be an effective method to crosslink proteins and to improve both barrier and mechanical properties of protein-based edible films [32,35,47]. Furthermore, many chemical crosslinkers - such as formaldehyde, glutaraldehyde, glyoxal and genipin - were tested to improve film properties [23,43,51,25,57] but, due to their toxicity, the addition of these molecules is not advisable. Conversely, a great interest was devoted to investigate the possibility to introduce crosslinks into the film network “enzymatically” and, thus, both peroxidase and tyrosinase have been utilized but with not enough encouraging results [11,12,55]. For these reasons TG is stimulating an increased deal of interest as potential tool to modify structure and characteristics of protein containing bioplastics.

Mahmoud and Savello [37], Mahmoud and Savello [38] followed by Yildirim and Hetiarachchy [60] were the first to suggest mTG as crosslinker to produce homo- and heteropolymers into soy- and whey protein-based edible films. Then, [34] demonstrated that mTG was effective in introducing covalent bonds into films obtained from slightly deamidated gluten, producing high mol. wt. polymers responsible for the decreased solubility of the obtained films and for their increased integrity and capacity to stretch. Babin and Dickinson [2] observed that mTG treatment determined positive or negative effects on the strength of gelatin types A and B, depending on the order in which the crosslinks were formed. [46] used the enzyme to produce whey protein or casein films by incorporating zein hydrolysate and observed that the obtained films exhibited higher elongation values and lower tensile strength. Yi et al. [59] reported that the addition of mTG into fish gelatin solutions increased the tensile strength and the oxygen barrier property of the obtained films, decreasing the percent of their elongation. Chambi and Grosso [10] demonstrated that the introduction of mTG-catalyzed crosslinks in the casein and gelatin films induced the formation of high mol. wt. polymers and caused an increased elongation of the films and decreased their water vapor permeability.

Further researches have been concentrated on the use of mTG as crosslinking agent in reticulating proteins of hydrocolloid multi-component edible films to obtain bioplastics with desired mechanical and gas barrier properties. In particular, films of either pectin or chitosan were produced in the presence of different plant and milk proteins able to act as mTG substrates [49]. It was demonstrated that the enzyme-catalyzed formation of protein crosslinks into the film network gener-
ally determines a decreased solubility of the bioplastics and increases its capacity to stretch. Concerning the pectin-based materials, the effect of mTG was shown both to significantly increase the barrier properties to both oxygen and carbon dioxide and to confer to these bioplastics a moderate permeability to water vapour [41,42]. These modifications of bioplastics characteristics suggest their possible use in a variety of situations, one of which could be the edible wrapping of high-moisture food to prevent their quality changes. Moreover, the markedly improving of both mechanical and water vapor permeability properties exhibited by bioplastics produced with a pectin-putrescine conjugate enzymatically crosslinked to soy proteins Di Pierro et al. [18] confer further potential industrial applications to the multicomponent TG-crosslinked bioplastics not only in food industrial sector but also for pharmaceutical and agricultural applications. A further possible application in the agricultural field of a pectin-based bioplastics is represented by that produced with fennel waste and containing in its matrix TG-crosslinked phaseolin, a seed storage protein of the common bean. The latter material was suggested as a promising candidate for the production of an environmentally-friendly sheet mulching [40]. A significant improvement in the mechanical resistance of TG-crosslinked bioplastics was observed by using chitosan in the presence of different proteins [16,17]. In fact, the protein crosslinking inside the chitosan network seems to reduce the intermolecular chain mobility of the polysaccharide matrix, thus increasing the tensile strength and reducing the extensibility of the produced bioplastics. In addition, the reported marked decrease in their permeability to oxygen and carbon dioxide, as well as the lower water vapor permeability, are probably associated with the resulting more compact structure of such biomaterial and with the changes in its hydrophilic properties.

In conclusion, sustained multidisciplinary researches are necessary both to exploit the hitherto produced crosslinked bioplastics for specific industrial applications and to develop new ecofriendly materials to widen their potential use. Thus, the possibility to obtain with the aid of TG a "tailored" molecular network conferring desired features to specific bioplastics remains an attractive and promising perspective in this field of investigations.

References


