Journal of Biotechnology 201 (2015) 69–74

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Enzymatically controlled material design with casein—From defined films to localized deposition of particles

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ARTICLE INFO

Article history:
Received 14 May 2014
Received in revised form 1 October 2014
Accepted 13 October 2014
Available online 20 October 2014

Keywords:
Casein
Coatings
Enzymes
Enzyme immobilization
Protein

ABSTRACT

A new concept for deposition and material design of coatings from biological compounds is presented. An enzymatic reaction triggers the specific coagulation of particles on a support surface. The first examined model system is casein and is based on the natural rennet reaction as applied in the process of cheese-making. The aspartic protease chymosin is immobilized on a support surface and cleaves the hydrophilic parts of the casein micelles, inducing deposition. The concept allows for a high level of control over film characteristics and enables the formation of site-specific film structures. The variability rages from formation of casein films with several micrometers film thickness to the targeted deposition of casein micelles.

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1. Introduction

1.1. General remarks

Biopolymers as coating materials have a great potential in many applications including biocompatibility, multi-functional surfaces, anti-fouling, and biodegradability. Frequently used substances are, e.g. cellulose, dextrans, chitin, casein, and other proteins (Juvonen et al., 2011; Khosravi et al., 2010; Klemm et al., 2011; Liebert et al., 2011; Müller-Buschbaum et al., 2006; Qi et al., 2012). Challenges that must be addressed are a high level of control over the film formation and the assembly of complex film structures, which is difficult to gain with common coating methods, like solution casting or spin coating. Moreover, it is desirable to get a better understanding of the film formation processes and the principles of adhesion. A method that combines these features could lead to the development of a new generation of biological coatings.

In this work, a novel approach to assemble protein coatings by means of an enzymatic reaction is presented, which has the potential to establish a new kind of material design. The process utilizes the altered solubility of certain colloidal particles after an enzymatic reaction. As a first example, we present the formation of casein coatings via enzymatic cleavage with the aspartic protease chymosin.

Casein micelles consist of four major proteins, which are α_{s1}-casein, α_{s2}-casein, β-casein, and κ-casein (Fox and Brodkorb, 2008). There are several models for the exact structure of those micelles that are still under discussion in the literature (Audic et al., 2003; Jollegs, 1966). In a very simple model, which is sufficient here, it can be assumed that the core of the micelle consists mainly of hydrophobic α_{s1}-, α_{s2}- and β-caseins, whereas κ-casein is accumulated on the outer plane due to its amphiphilic structure (Lucey, 2002; Horne, 2006). κ-Casein plays an important role, as it can be cleaved with the aspartic protease chymosin resulting in the loss of its hydrophilic part (caseinomacropeptide), whereas the hydrophobic para-κ-casein remains within the micelle (Lucey, 2002). This cleavage results in an increased hydrophobicity of the entire micelle and finally leads to agglomeration and precipitation. In fact, this utilized process is the natural rennet reaction, used in cheese-making and also occurring in the stomach of young mammals. The chymosin-sensitive peptide sequence of κ-casein is even used in protein engineering, e.g. as a cleavable linker in fusion proteins (Walsh and Swaisgood, 1996).

1.2. Concept

Casein as a material is known since the ancient Egypt and has, ever since then, been used in various applications, including coatings (Audic et al., 2003). In all previous coating applications though, casein is simply used as a binder agent. The presented approach

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http://dx.doi.org/10.1016/j.jbiotec.2014.10.019
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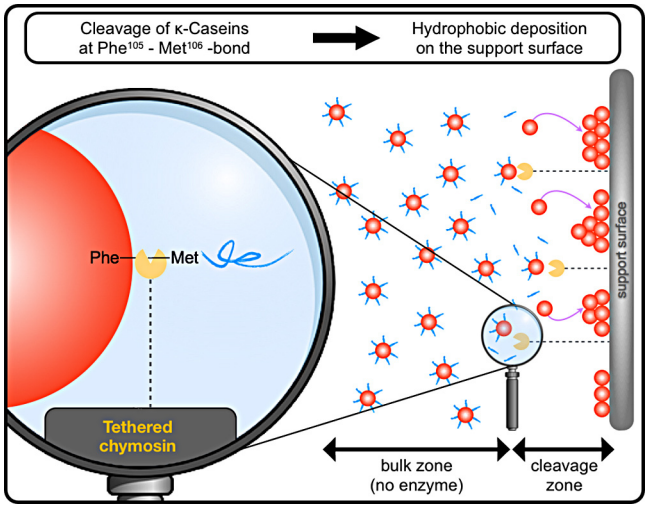


Scheme 1. Idealized mechanism of the enzymatically triggered film formation process. Enzyme is tethered onto the support surface; its diffusion ability defines the cleavage zone. Only casein micelles that enter this cleavage zone get in contact with the enzyme. In consequence, they loose their hydrophilic parts and form a coating on the support surface because of their increased hydrophobicity. The size of the cleavage zone depends on the enzyme tethering method. (The dashed lines indicate variability of the tethering method and the respective size of the cleavage zone and not a polymeric spacer.).

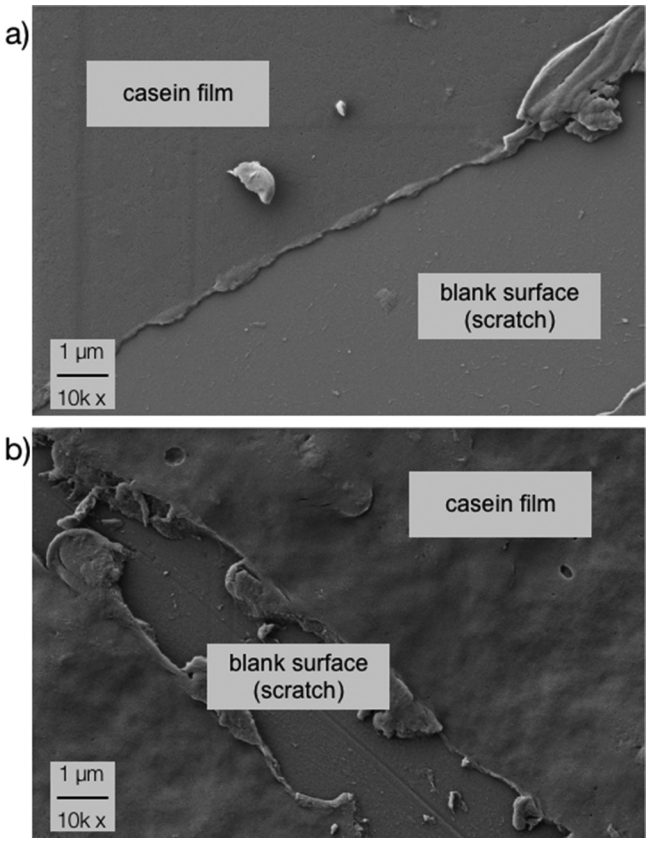


Fig. 1. SEM pictures of casein films formed by enzymatic reaction with adsorbed chymosin. (a) Complete film formation, thin film; (b) complete film formation, thick film.

brings a completely new aspect to the use of casein in this area, as it utilizes the complex structure of the micelles and their ability to change its solubility. More precisely, it mimics the natural process of the enzymatic casein cleavage, in order to form biological coatings with high level of control.

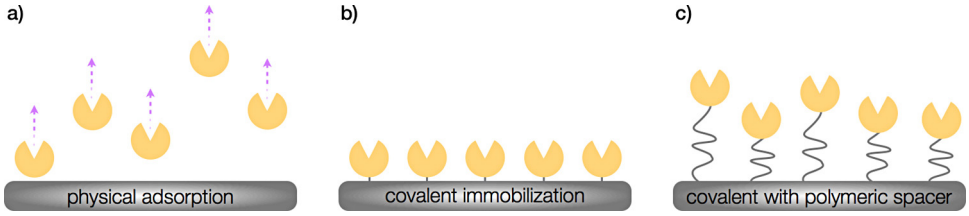
The concept of this approach is illustrated in Scheme 1. The crucial point is the cleavage of the casein micelles in direct proximity to the support surface. This prevents uncontrolled agglomeration and precipitation. Instead of that, the cleaved micelles deposit on the support surface due to hydrophobic interactions. This restriction of the "cleavage zone" is achieved by immobilization of the enzyme onto the support surface (Barbosa et al., 2013; David et al., 2006; Guisan, 2006; Shindo and Arima, 1979; Wang et al., 2010). The cleavage zone defines the area where casein can be cleaved. Its size can be controlled by variation of the enzyme tethering method.

1.3. Used enzyme tethering methods

As described above, the key parameter in the presented approach is the maximum distance of the enzyme molecules from the support surface. To control the degree of diffusion of the enzyme, and consequently the film formation process, the enzyme molecules have to be tethered to the support surface. Scheme 1 shows an idealized illustration, with single-enzyme molecules responsible for specific casein cleavage.

In this work, two different approaches, physical adsorption (reversible tethering) and covalent immobilization (irreversible tethering), are applied (Scheme 2a and b). At the beginning of the reaction ($t = 0$), all enzyme molecules are located directly on the support surface ($d = 0$), independent of the used tethering method. For $t > 0$, the distance of the reversibly immobilized enzymes increases ($d > 0$) due to diffusion. The irreversibly immobilized enzymes remain on the support surface ($d = 0$) as they cannot diffuse. The cleavage zone is therefore restricted to the immediate surface. Those different diffusion patterns have a high impact on the resulting film characteristics. A logical evolution of the process would be the use of polymeric spacers (Scheme 2c), which gives a cleavage zone that is variable in size but consistent over time. This approach though, has not yet been examined, but will be subject to future research.

As indicated in Scheme 1, an ideal process would use single-enzyme molecules. However, chymosin tends to form aggregates in solution, which are utilized here to provide a proof of principle. Nevertheless, single-enzyme molecules are desirable for maximum efficiency and are a goal in future research.



Scheme 2. Different tethering methods for enzyme molecules on the support surface. Arrows indicate possible diffusion of the enzyme into the solution. (a) Reversible immobilization by physical adsorption; (b) irreversible immobilization by covalent binding; (c) irreversible immobilization with variable distance to the support surface by use of polymeric spacers.

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