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Buildup of biobased adhesive layers by enzymatically controlled deposition on the example of casein

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ABSTRACT

We present a method for the enzyme mediated buildup of protein adhesive layers. The process is based on our autodeposition-like formation of biological coatings. The presented example utilizes the enzymatic destabilization of casein micelles with the aspartic protease chymosin—the same process that is used in cheesemaking. If two adjacent support surfaces with adsorbed enzyme are immersed into a casein dispersion, protein layers will form on both surfaces and eventually form an adhesive layer between them. As a proof of concept, we show that the process works as intended, and that in fact the enzymatic process is the driving force. Several parameters are examined and the resulting layers are investigated via scanning electron microscopy.

1. Introduction

1.1. General remarks

The demand for environmentally friendly adhesives is increasing, since replacement of fossil fuel-based adhesives by alternative adhesives based on renewable materials is desired. Especially biobased polymers have a high potential in this area due to their intrinsic properties, such as biodegradability, biocompatibility, non-toxicity and sustainability [1,2]. Biopolymers are very specific concerning their evolutionary field of application and might exhibit competitive properties such as fast reversible adhesion, strong resistance to weathering and advanced underwater adhesion if compared to synthetic adhesive polymers [3].

As biobased adhesive polymers, proteins play an important role. They are produced by both animals and plants, and are abundantly occurring in nature. Proteins have a versatile reactive macromolecular structure which makes them an interesting source for innovative biomaterials. Popular resources for proteins used as adhesives are soy protein [4,5], wheat gluten [6,7], gelatin [8], and casein [9]. Especially casein has a very long tradition for gluing applications, as it has been used since the time of the ancient Egyptians [10]. Casein is the major protein occurring in mammalian milk and consists of four main proteins, namely α_{S1}-casein (36%), α_{S2}-casein (10%), β-casein (36%), and κ-casein (13%). Casein forms micelles in solution, with κ-casein located mostly on the outer plane. This κ-casein layer stabilizes the micelle due to its amphiphilic character [11].

.Polymer films made of caseins provide good mechanical and gas barrier properties, comparable to those of other used protein films, such as soy protein isolate and wheat gluten films [12]. Among other proteins, caseins have a very unique distribution of hydrophilic and hydrophobic regions resulting in specific surface-active properties. Accompanied by their flexible and mobile character, caseins are able to fulfill complex adhesion and association behavior [13].

With the dawn of synthetic polymers, casein adhesives have lost importance but are still used for some applications, e.g. as labeling adhesives in the bottling industry or in interior woodworking [14]. Besides the use as gluing agents, casein films and coatings find application as biocompatible and protective layers in packaging [15,16], and represent a promising biomaterial for medical applications such as drug delivery carrier systems and wound dressing membranes [17–19].

1.2. Concept

Recently, we developed a biological autodeposition process that enables the controlled deposition of biological particles on surfaces [20]. The process utilizes enzymatic reactions to change solubility of certain biopolymers. Our first examined system was the cleavage of casein micelles by the aspartic protease chymosin, which is the same process that is present in cheesemaking: the enzyme cleaves the hydrophilic parts of κ-casein, which results in destabilization of the casein micelles. In solution the cleaved casein now evades the aqueous environment by aggregation and precipitation. If this destabilization

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occurs in direct proximity to a support surface, immediate deposition follows due to hydrophobic interactions. Besides other results, it could be shown that the process yields continuous layers of casein if the enzyme is tethered by physical adsorption [20].

In this work, we present a proof of concept study for the applicability of this process in adhesion technology. If two supports with immobilized enzyme are brought into near proximity, the casein layer will grow from both sides until they meet. Eventually, a combined layer is generated, which holds the two supports together.

Fig. 1 shows the experimental setup. Two supports with a fixed distance are immersed into a casein dispersion. The lower part of the supports is functionalized with chymosin, the upper part is not functionalized. After the reaction a casein layer is expected to form only in the lower area, where enzyme is present. The upper part is expected to remain blank after washing. The vertical orientation of the supports ensures that the deposition is not due to sedimentation. If the casein layers extend far enough, adhesion of the two supports should derive. The proposed buildup process is shown in Fig. 2.

2. Materials and methods

2.1. Materials

Casein from bovine milk was purchased from SIGMA-ALDRICH and used without further purification. Chymosin was obtained as rennet powder from RENCO NEW ZEALAND through the European supplier BICHSEL-AG (Switzerland). The amount of chymosin was 5% (w/w) as indicated by the manufacturer. In order to increase

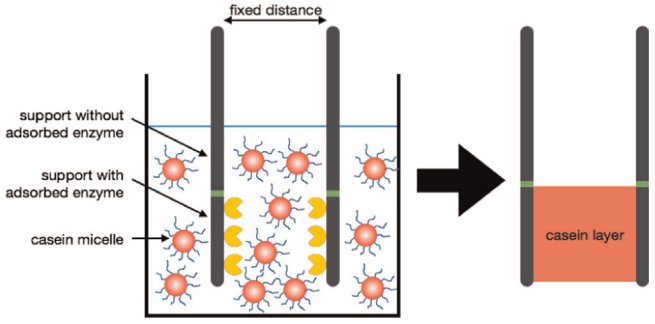

Fig. 1. Model picture of the experimental setup and the expected result. Casein will form an adhesive layer between two supports. This layer will only be built in that area which has been functionalized with enzyme.

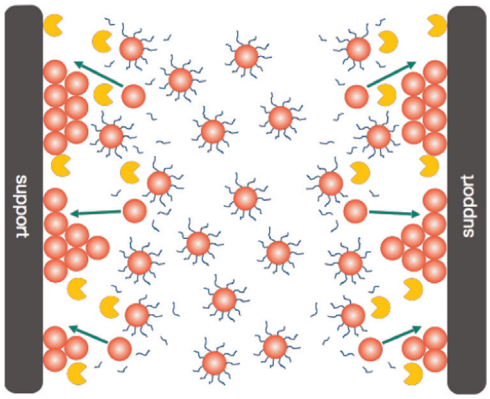

Fig. 2. Model of the casein deposition process. Enzyme (yellow) is adsorbed on the supports and diffuses into the solution over time. If it comes into contact with a casein micelle, κ-casein (blue) is cleaved. Consequently, the remaining micelle core (red) gets destabilized and deposits on one of the supports. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the chymosin content and therefore the effectiveness of the enzymatic reaction, the salt content was reduced by ultrafiltration. To this end, the rennet powder was dissolved in DI water and centrifuged three times at 5000 × *g* and 25 °C for 2 h using Amicon® Ultra-15 Centrifugal Filter Devices with a cutoff of 3000 g/mol. The enzyme content was increased to 67% (w/w), measured with thermal gravimetric analysis (TGA). Glass slides (75 × 25 × 1 mm^3) were purchased from VWR and used as support material in all experiments. All other chemicals were obtained from the usual suppliers and used without purification.

2.2. Casein deposition on single glass slides

Glass slides were cleaned with ethyl acetate (EtOAc) and DI water. Physical adsorption of chymosin was performed by dripping an aqueous enzyme solution (3 g/L; 600 μL) onto an area of 25 × 25 mm^2 of the support surface and subsequent drying of the solution. The glass slide with the adsorbed chymosin was immersed in an aqueous casein solution (10 g/L) at pH 3 and 40 °C for 80 min. After the deposition process, the glass slide was removed, washed with DI water, and dried. The control sample was made under the same conditions, with two modifications: first, no enzyme was present and second, the control was not washed because the film is not stable under the washing conditions.

2.3. Adhering of glass slides by cleaved casein

Glass slides were cleaned with EtOAc in an ultrasonic bath for 45 min and washed with DI water. An aqueous chymosin solution (25 g/L; 625 μL) was trickled onto an area of 25 × 25 mm^2 of each glass slide and dried. After that, two glass slides with adsorbed chymosin were placed in special holders and fixed. A variable amount of bar spacers with a thickness of 125 μm each was used to adjust the varying distance between the slides from 125 μm up to 500 μm and was removed after fixation of the slides. Subsequently, the two glass slides were immersed in a casein dispersion (varying concentrations from 1 g/L up to 20 g/L; 200 mL) at 40 °C for 80 min. The exact parameters for each experiment can be found in Table 2. After the cleavage reaction, the adhered glass slides were washed with DI water and dried. The drying was performed in open air with the slides still vertically fixed by the holders for at least 24 h.

2.4. Contact angle measurements

Contact angle measurements of sessile water drops were performed with a “Contact Angle Measuring System G10” from KRÜSS. The contact angle was measured one second after placing the drop onto the support surface. All reported contact angles are average values calculated from three single measurements.

2.5. Scanning electron microscopy

The obtained casein layers and adhesion samples were examined by means of scanning electron microscopy using a ZEISS “Neon 40“ scanning electron microscope. Pictures of the samples were obtained by applying the SE2-detector (high topography contrast) at an acceleration voltage of 2 kV. The samples were sputtered with 3 nm of a gold-palladium coating.

2.6. Measurement of adhesive strength

Adhesive strength of the casein layer was measured with an Epprecht twistometer by application of torsion force. Values were measured three times for samples with a plate distance of 125 μm.