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Patterns produced by dried droplets of protein binary mixtures suspended in water

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ABSTRACT

Patterns formed by the evaporation of a drop containing biological molecules have provided meaningful information about certain pathologies. In this context, several works propose the study of protein solutions as a model to understand the formation of deposits of biological fluids. Generally, dry droplets of proteins in a saline solution create complex aggregates. Here, we present an experimental study on the formation of patterns produced by the evaporation of droplet suspensions containing a protein binary mixture. We explore the structural aspect of such deposits by using optical and atomic force microscopy. We found that salt is unnecessary for the formation of complex structures such as crystal clusters, dendritic and undulated branches, and interlocked chains. Such structural features allow us to differentiate among protein binary mixtures. Finally, we discuss the potential use of this finding to reveal the presence of a protein suspensions, the folded and unfolded state of a protein, as well as their structural changes.

1. Introduction

The formation of patterns produced by the evaporation of drops containing biofluids is a topic of great relevance due to its potential use for diagnostic and screening of pathologies. For example, blood serum droplets of patients with leukemia, anemia, viral hepatitis type B, tuberculosis, burn disease and breast cancer produce complex and well-differentiated patterns [1–3]. A good approach to the formation of such deposits has been the study of biomolecules [4–7]. In particular, protein solutions have been used as a simple biological models [8–10].

The formation of protein deposits strongly depends on concentration, relative humidity, the size of the drop and contact angle [8–12]. The pattern formation starts with the deposition of colloidal particles at the periphery due to the capillary flow resulting from the higher evaporation rate at the contact line. This process is the so called “coffee ring effect” that can be seen as the pinned of the droplet on the glass slide. The final deposits are also affected by protein–protein and protein–substrate interactions which promote the aggregation of proteins on the substrate [9,13–20].

Salt promote the dehydration of proteins making stronger intermolecular interactions between proteins. This produces complex aggregates in the interior of the deposit, consisting of crystals, rosettes, scallops, dendrite shapes and surrounded by a crack ring in the final semi-liquid gel [21–25].

Despite the accumulated knowledge on these droplets studies, very little is known about how the interaction between proteins with different structure affects the drying process. Proteins can exhibit a broad range of interactions at different levels associated with their structure [26,27]. Indeed, a small change in them (as those produced by protein heating) can promote different interactions leading to a significant change in a biological system [28]. Protein–protein interactions (PPIs) usually concern a physical contact with molecular docking between two or more protein molecules, which occur in a cell or living organism as a result of biochemical events [29–31]. The associations between subunits of proteins are dominated by Van der Waals forces, hydrogen bonds and hydrophobic effects [32–34].

In this paper, we report the study of pattern formation during the evaporation process of protein solutions. We focused on the interaction between two different proteins. We used mixtures of BSA and lysozyme, both globular proteins. The combination of these proteins generates deposits with exceptional characteris-

Abbreviations: PPIs, protein–protein interactions; BSA, bovine serum albumin; LYZ, lysozyme; BSA*, BSA denatured; Lysozyme*, lysozyme denatured; AFM, atomic force microscopy; DLA, diffusion limited aggregation.

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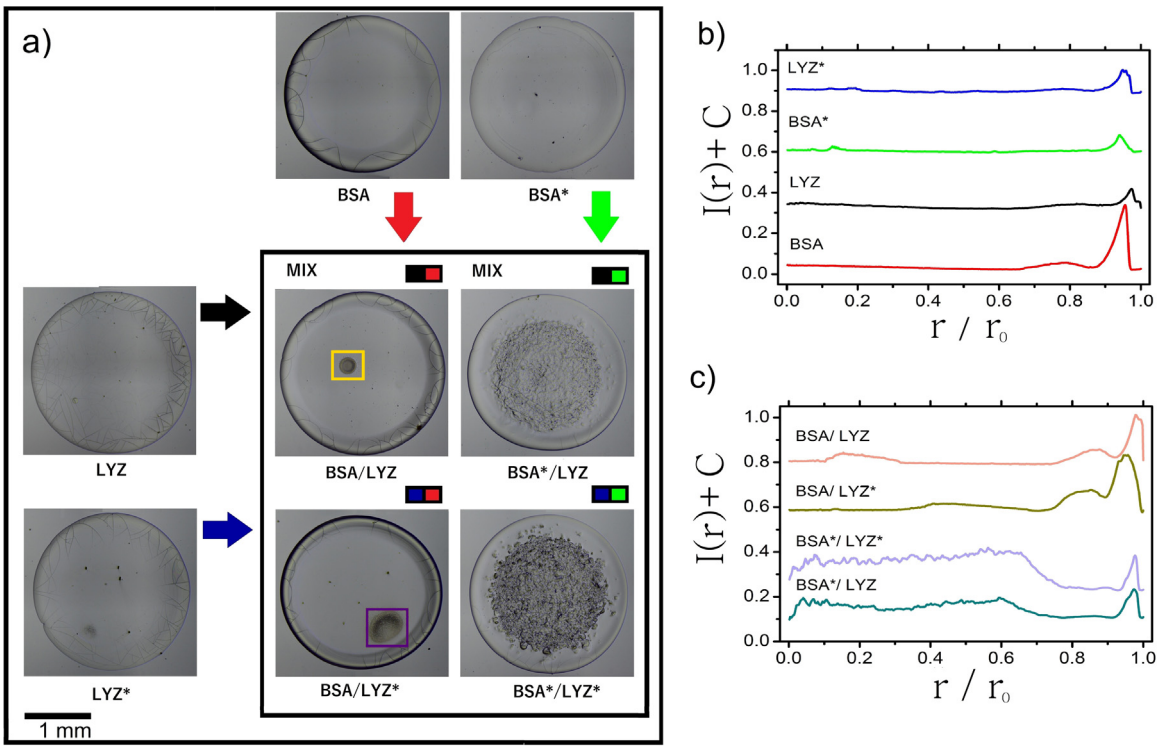

Fig. 1. Protein mixtures deposits. (a) Deposits formed during the evaporation of a droplet containing one and two types of proteins (at a relative concentration ϕ_r = 50:50, see the inner black square) on a substrate at T = 37 °C and ϕ = 1.6 wt%. The color of the arrows indicates the type of protein (nature or denatured) in the mixture. The small circular structure and the cavity are shown in the small yellow and purple boxes, respectively. (b) Radial density profile of the deposits formed by a dried droplet containing one type of protein. (c) Radial density profile of the protein mixture deposits. The curves were separated by a constant to appreciate their differences. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

tics. Interestingly, the interaction between native and denaturated proteins increases the complexity of the patterns. We discovered that the addition of salts in the solution is not necessary for the formation of crystals and dendritic structures, as has been generally reported. Indeed, a high relative concentration of lysozyme interacting with BSA promotes the formation of crystal clusters, undulated branches, and interlocked chains. Finally, we studied the pattern formation of proteins and found that the diversity of the complex structures appears as a result of intermolecular interactions between subunits of different types of proteins (total or partially unfolded).

2. Materials and methods

2.1. Proteins preparation

High purity lysozyme (Sigma–Aldrich, L6876) and bovine serum albumin (BSA) powders (Sigma–Aldrich, A2153) were used to prepare lysozyme and BSA stock solutions. These powders as received were dissolved in deionized water (Mili-Q, 18.2 MΩ cm) at a concentration of 2.00 wt% and 25 °C. The stock solutions were diluted according to the desired concentrations. The mixed protein solutions were prepared by mixing single protein solutions. They were thermally denatured at 90 °C for 100 min. We refer to them with these notations: BSA* and Lysozyme*. The solutions were stored at 2 °C, thereafter thermalized to room temperature prior to deposition.

2.2. Droplet evaporation

The droplets of solutions were placed onto clean glass slides using a micropipette; the volume of the drops was 2 μl. The droplets were evaporated under controlled ambient conditions: T = 37 °C and relative humidity of 30%. The evaporation process was recorded at 30 fps with a digital camera (Nikon Digital, SLR Camera D3200). The deposits were observed after evaporation in ambient conditions using a microscope (Velab, VE-M4, 4× and 10×).

2.3. Image analysis

We use the radial density profile $I(r)$ to carry out the structural analysis of the patterns. This quantity describes a profile of integrated intensities produced by concentric circles as a function of radial distance [7]. For 2D objects this quantity is given by the following expression:

$$I(r) = \frac{1}{2\pi}\int_0^{2\pi} i(r,\theta)\, d\theta, \tag{1}$$

where $i(r, \theta)$ is the local light intensity contained in a circle of radius r. Each value of $I(r)$ represents the sum of the pixel intensities around a circle with radius r.

In order to quantify the complexity of the small circular structures inside of the protein deposit, we measure the fractal dimension of mass d using the equation $m = r^d$, where d is the exponent of the power law and m is the mean mass in a box of side r. Here, the number of pixels enclosed within the area of an image is correlated to the mass of an object in a delimited region. The analysis of the clusters is carried out using the corresponding photographs in binary scale. The number of pixels in an element area corresponds to the mass contained in the same element, which is estimated in 580 equally separated concentric squares. This procedure is repeated in different sites of the structure. Finally, the average of the number of pixels for each size box is calculated. The slope of $log(m)$ as a function of $log(r)$ is the corresponding mass