Radiation Physics and Chemistry 144 (2018) 231–237

Contents lists available at ScienceDirect

Radiation Physics and Chemistry

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

Effect of gamma-irradiation of bovine serum albumin solution on the formation of zigzag film textures

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ARTICLE INFO

Keywords:
Gamma-irradiation
Bovine serum albumin
Conformation
Film
Texture
Fluorescence

ABSTRACT

Formation of patterns on the surface of dried films of saline biopolymer solutions is influenced by many factors, including particle size and structure. Proteins may be modified under the influence of ionizing radiation. By irradiating protein solutions with gamma rays, it is possible to affect the formation of zigzag (Z) structures on the film surface. In our study, the films were obtained by desiccation of bovine serum albumin (BSA) solutions, which were irradiated by a ^{60}Co gamma-source at doses ranging from 1 Gy to 12 kGy. The analysis of the resulting textures on the surface of the films was carried out by calculating the specific length of Z-structures. The results are compared against the absorption and fluorescence spectroscopy and dynamic light scattering (DLS) data. Gamma-irradiation of BSA solutions in the 1–200 Gy range practically does not influence the amount of Z-structures on the film surface. The decrease in fluorescence intensity and increase in absorbance intensity point to the destruction of BSA structure at 2 and 12 kGy, and DLS shows a more than 160% increase in particle size as a result of BSA aggregation at 2 kGy. This prevents the formation of Z-structures, which is reflected in the decrease of their specific length.

1. Introduction

The films resulting from desiccation of biopolymer solutions have found application in a number of fields, ranging from molecular biology research (Gorza et al., 2014; Dugas et al., 2005), drug screening (Takhistov and Chang, 2002) and disease diagnostics (Brutin et al., 2011; Killeen et al., 2006; Chen et al., 2016; Sikarwar et al., 2015) to product quality analysis (Kokornaczyk et al., 2008, 2011; Andersen et al., 1999; Busscher et al., 2010; Kim et al., 2012) and microelectronics engineering (Dai et al., 2005; Khatir et al., 2011).

The nature of the textures formed on the surface of films, which are obtained from solutions of biopolymers (proteins, DNA, starches), is discussed in a number of papers (Mayeres et al., 1995; Yakhno, 2011, 2015; Chen and Mohamed, 2010; Glibitskiy et al., 2012a, 2012b; López et al., 2008). In a survey by Zhong et al. 2015, dynamic evaporation of nanofluid droplets, self-assembly and patterns formed by nanoparticles are investigated. Depending on the experimental conditions of nanofluid droplet drying, different patterns can be obtained: cubic crystallites (Choudhury et al., 2013), diffusion-limited aggregation structures (Choudhury et al., 2013; Ben-Jacob and Garik, 1990), dense-branching/fingering patterns (Chen and Mohamed, 2010; Ben-Jacob and Garik, 1990), dendrites (Choudhury et al., 2013; Darwich et al., 2012), snowflake patterns (Ben-Jacob and Garik, 1990), honeycomb/hexagonal/zigzag patterns (Raz et al., 1991; Haidara et al., 2001).

It should be noted that patterns of the same type can be obtained both from solutions of biopolymers, and from suspensions or colloid

Abbreviation: BSA, bovine serum albumin; DNA, deoxyribonucleic acid; DLVO, Derjaguin, Landau, Verwey, Overbeek; SDS–PAGE, sodium dodecyl sulfate – polyacrylamide gel electrophoresis; DLS, dynamic light scattering

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http://dx.doi.org/10.1016/j.radphyschem.2017.08.019
Received 2 February 2017; Received in revised form 20 June 2017; Accepted 20 August 2017
Available online 26 August 2017
0969-806X/

solutions of water-insoluble nanoparticles. For example, dendrite ("alginate/Au NPs" solutions (Darwich et al., 2012), common wheat grain leakages (Kokornaczyk et al., 2011), lysozyme solutions (Gorr et al., 2013)) and hexagonal (aqueous drop containing polystyrene microspheres (Bhardwaj et al., 2009), aqueous solution of NH_4Cl (Raz et al., 1991)) structures forming on the film surface indicate the universality of the self-organizational processes in different systems (Kokornaczyk et al., 2011). Another example is the zigzag structures resulting from desiccation of NH_4Cl solutions (Raz et al., 1991), saline solutions of bovine serum albumin (BSA) and DNA (Glibitskiy, 2014; Glibitskiy et al., 2015) and nanoparticle suspensions (Haidara et al., 2001; Kokornaczyk et al., 2011; Bhardwaj et al., 2010; Sommer et al., 2004).

The type of pattern is primarily determined by the mechanisms governing the fluid flow and particle transport in the drying drop (Zhong et al., 2015): radial flow, DLVO (Derjaguin, Landau, Verwey, Overbeek) force and Marangoni recirculation loop (Sommer et al., 2004). The type and form of pattern is also influenced by a multitude of parameters, such as: composition of the droplet (proportion of components and impurities) (Choudhury et al., 2013, 2015; Raz et al., 1991), particle size (Darwich et al., 2010), nature of the solvent (Choudhury et al., 2013), viscosity (Choudhury et al., 2013), pH (Dutta et al., 2013), temperature (Choudhury et al., 2013, 2015; Darwich et al., 2010), humidity (Choudhury et al., 2013, 2015), drying mode (Darwich et al., 2012), nature of the substrate (wetting/nonwetting, roughness, porosity) (Choudhury et al., 2013, 2015; Mougin and Haidara, 2002), actual volume fraction of the drop in the late step of the drying (Mougin and Haidara, 2002). Controlling all these factors would allow one to manipulate the process of the drop drying and, consequently, the formation of the patterns (Zhong et al., 2015).

Protein macromolecules are complex structures with numerous groups, which may be modified under the influence of ionizing radiation, and are among the biological objects with high radiation sensitivity (Davies, 2005). Since the irradiation of protein solution leads to changes in the structure of protein molecules (Gaber, 2005; Pomeraia et al., 2003; Foley et al., 2011), it should affect the formation of the film texture. For example, in (Lee et al., 2005) it was shown that γ-irradiation of soy protein isolate solutions alters the properties of the films obtained from those solutions.

The influence of ionizing radiation on biopolymers is the subject of numerous papers (Gaber, 2005; Oliveira et al., 2007; Mishra et al., 2014; Saad-El-Din et al., 2014). Radiolysis of water produces $H_2O^{\bullet}$, H_2O^+, OH^+, H^-, H^+, O^+, O^- radicals (Henley and Johnson, 1969), as well as biologically relevant oxygen radicals $^{\bullet}OH$ (hydroxyl radical), O_2^- (superoxide anion radical) and $HO_2^{\bullet}$ (hydrodioxyl radical, which may have a limited biological significance) (Davies, 1987). According to Davies (1987), $^{\bullet}OH$ causes protein aggregation; O_2^- on its own has no apparent effects, but $^{\bullet}OH + O_2^- (+O_2)$ causes protein fragmentation. Both $^{\bullet}OH$ and, to a lesser extent, $^{\bullet}OH + O_2^- (+O_2)$ result in tryptophan loss, bityrosine production and electrical charge change.

Protein aggregation is also affected by the environment. For example, ethanol, methanol and other polar water-soluble solvents reduce the water shell around the protein and the dielectric constant of the solution, which increases the electrostatic interaction between BSA molecules (Achilli et al., 2015). Saturation of BSA solution with radiation active gases (e.g. O_2, C_2H_2) previous to irradiation also has a minor effect on aggregation (Achilli et al., 2015). Inorganic salts can also play a role, since ions in solution may generate osmotic forces which destroy the hydration barrier that prevents protein aggregation (Rozhkov and Goryunov, 2000).

The effects of ionizing radiation depend on the dose. Typically, the change in the local environment of polypeptide chains is observed below 0.5 kGy (Gaber, 2005), protein fragmentation (cleavage of the peptide bonds) above 1 kGy (Gaber, 2005; Cho and Song, 2000; Kar et al., 2017), and aggregation (due to covalent cross-linking, hydrophobic and electrostatic interactions, and formation of bityrosines and cysteine linkages) above 5 kGy (Gaber, 2005; Davies, 1987; Queiroz et al., 2016; Cho and Song, 2000; Kar et al., 2017).

According to SDS-PAGE (sodium dodecyl sulfate – polyacrylamide gel electrophoresis) studies, BSA damage depends logarithmically on the dose (10–20% at 0.2 kGy, 50–60% at 1 kGy) (Mishra et al., 2014), and even at 2.5 kGy some amount of undamaged BSA still remains (Akhavan et al., 2010). On the other hand, angular light scattering shows a sharp decrease in BSA molecular weight between 0.5 and 1 kGy (Gaber, 2005): 73 kDa at 0 and 0.5 kGy, 45 kDa at 1 and 5 kGy. The size of BSA particles (as measured by dynamic light scattering) is 6.6 nm at 0 kGy (Queiroz et al., 2016; Varca et al., 2016), does not change considerably at 2.5 kGy (Varca et al., 2016), 8.8 nm at 5 kGy (Varca et al., 2016), 12.4 nm at 7.5 kGy (Varca et al., 2016) and 16.6 nm at 10 kGy (Queiroz et al., 2016; Varca et al., 2016).

In addition to fragmentation and aggregation, there are also changes in the secondary structure of BSA. Decrease in α-helical content (Geng et al., 2016; Cho and Song, 2000; Hu et al., 2016), increase in β-fold (Geng et al., 2016; Cho and Song, 2000) and random coil (Cho and Song, 2000) content, and transformation of β-turns into β-sheets (Gaber, 2005) typically become noticeable at 1 kGy, although some authors report changes in the secondary structure, surface charge and hydrodynamic size (from 36.74 nm to 47.89 nm) even at 5 Gy (Zarei et al., 2017).

In our previous experiments, we have observed that the structural state of DNA or BSA in solution affects the texture of the resulting films (Glibitskiy et al., 2012a, 2012b, 2015, 2016; Glibitskiy, 2014; Glibitskiy and Glibitskiy, 2016). In particular, zigzag patterns (Z-structures) are typical for native biopolymer solutions, but the formation of these structures becomes less likely or even impossible after the addition of biologically active substances (Glibitskiy et al., 2016) or thermal treatment of the solution (Glibitskiy et al., 2015). In the present study, we investigate how a different physical factor (ionizing radiation) affects the formation of zigzag structures, as part of our ongoing effort to determine the range of applicability of this biosensor technique.

2. Materials and methods

2.1. Sample preparation

In the experiment, the reagents BSA ("DiaM", USA) and sodium chloride (NaCl, "reagent grade") were used. Aqueous solutions of salts and protein were prepared in distilled water. Films were prepared from solutions containing BSA (0.5 mg/ml) and sodium chloride (20 mmol/l). The prepared solutions were stirred for 2 h with a mechanical stirrer at room temperature.

2.2. Sample irradiation

BSA solutions were irradiated with gamma-rays from a ^{60}Co source (self-shielding facility MRX-γ-100 "Researcher" at V.N. Karazin Kharkiv National University) at a room temperature. For each of the target doses (1, 100, 200, 2000 or 12,000 Gy), a vial with the solution was placed inside the irradiation facility for a duration of time proportional to the dose. The absorbed dose was estimated via ferrous sulfate (Fricke) dosimetry (Henley and Johnson, 1969) – a method based on the change of optical density of a ferrous sulfate solution due to the irradiation-induced transformation of Fe^{2+} into Fe^{3+}.

2.3. Film preparation and analysis

The films were obtained via vacuum drying of 0.5 ml of each solution in 20 × 20 × 1 mm^3 glass cells under 40 °C thermostatically controlled conditions; the specific experimental setup is described in more detail in (Glibitskiy et al., 2012a, 2012b). For uniform drying, the solution in each cell was stretched to the cell borders (so that its whole volume is filled). After the drying, each film was photographed in 100 locations (arranged in a 10 × 10 grid) using a web camera coupled