Thermal properties of the S-layer protein from *Lactobacillus salivarius*

Liliana Lighezan[1, 3], Ralitsa Georgieva[2] and Adrian Neagu[3]

[1] Faculty of Physics West University of Timisoara, Timisoara, Romania;
[2] The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria;
[3] Center for Modeling Biological Systems and Data Analysis, Department of Functional Sciences, Victor Babes University of Medicine and Pharmacy, Timisoara, Romania

llighezan@physics.uvt.ro, ralica_nedkova@mail.ru and neagu@umft.ro

Abstract

Surface layer (S-layer) proteins have been identified in outermost structures of the cell envelope in many organisms, such as bacteria and archaea [1, 2]. They display intrinsic self-assembly property, forming monomolecular crystalline arrays with oblique, square or hexagonal symmetry [3, 4].

The biological functions of S-layer proteins are not completely understood. It is assumed that S-layer proteins act as protective coats, cell shape determinants, molecular and ion traps, adhesion sites for exoenzymes and structures involved in cell adhesion and surface recognition [3, 4].

Isolated S-layer proteins possess the unique ability to recrystallize into regular monomolecular arrays, on solid supports, on liquid surface-interfaces, on lipid films and liposomes, or in suspension. The ability to self-assemble into regular lattices, with pores of identical size and morphology (of about 1 to 10 nm), facilitates the use of S-layer proteins in biotechnological applications, such as the control of the architecture of biomimetic surfaces [5, 6]. S-layer proteins are also used in the production of isoporous ultrafiltration membranes, in the construction of supporting structures for the controlled immobilization or incorporation of functional molecules (antigens, antibodies, ligands, enzymes) required for biosensors [7, 8]. Moreover, S-layer proteins are used to produce matrices for the formation of ordered arrays of metal clusters or nanoparticles, required in molecular electronics and nonlinear optics [9]. Also, they are used for drug targeting, being involved in the formation of supporting and stabilizing matrices for functional lipid membranes, liposomes and emulsomes [10, 11]. In recent applications, S-layer proteins were used to obtain encapsulated drugs [12] and as drug microcarriers [13]. For all these nanobiotechnological purposes, as well as for their biological functions, the thermal stability of the S-layer proteins under high temperature conditions is very important.

In this study, the S-layer protein has been isolated from *Lactobacillus salivarius* 16 strain of human origin, and purified by cation-exchange chromatography. Using circular dichroism (CD) spectroscopy, we have investigated the structure and the thermal properties of the S-layer protein.

The far UV circular dichroism spectra indicate that the secondary structure of the S-layer protein consists mainly of irregular motifs, but it can also contain small fractions of α-helices and β-sheets. The near UV circular dichroism spectra show that the tertiary structure of the S-layer protein is determined by a high content of hydrophobic amino acids, such as Trp, Tyr and Phe, bound into a local chiral environment, which tend to compact the protein's tertiary structure.

According to the far UV CD spectra taken at different temperatures, the thermal denaturation of the secondary structure of the S-layer protein takes place in the temperature range between 40 °C and 80 °C and is partially reversible. The shape of the thermal denaturation ellipticity curve in the far UV domain (at a wavelength of 203 nm) is concentration-dependent and it also depends slightly on the heating rate (figure 1). The curve shows the existence of a metastable intermediate state in the protein denaturation pathway (figure 1 b). The thermal denaturation of the tertiary structure of protein occurs in the same temperature range, between 40 °C and 80 °C, and is partially reversible, too. The temperature dependence of the CD signal in the near UV domain (at a wavelength of 285 nm) reveals the presence of an intermediate state, at about 60 °C, in a good agreement with the temperature dependence of the CD signal in the far UV domain for the protein concentration of 0.25 mg/ml (at a wavelength of 203 nm). So, during thermal denaturation, the protein changes its secondary and tertiary structure simultaneously. After the heating of the protein up to 90 °C and, subsequently, its cooling down to 10 °C, the secondary and tertiary structures of the S-layer protein are partially recovered.

By fitting the thermal denaturation curves with sigmoidal functions, we have determined the temperature dependence of the population fractions of the native, intermediary and denatured states. We have also determined the transition rates and free energy variations.

Taken together, our CD spectroscopy results concerning the thermal behavior of the S-layer protein from *Lactobacillus salivarius* 16 strain could be important for the use of S-layer proteins in nanobiotechnological applications, as well as for a better understanding of the protein's structure and function.

References

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Figures

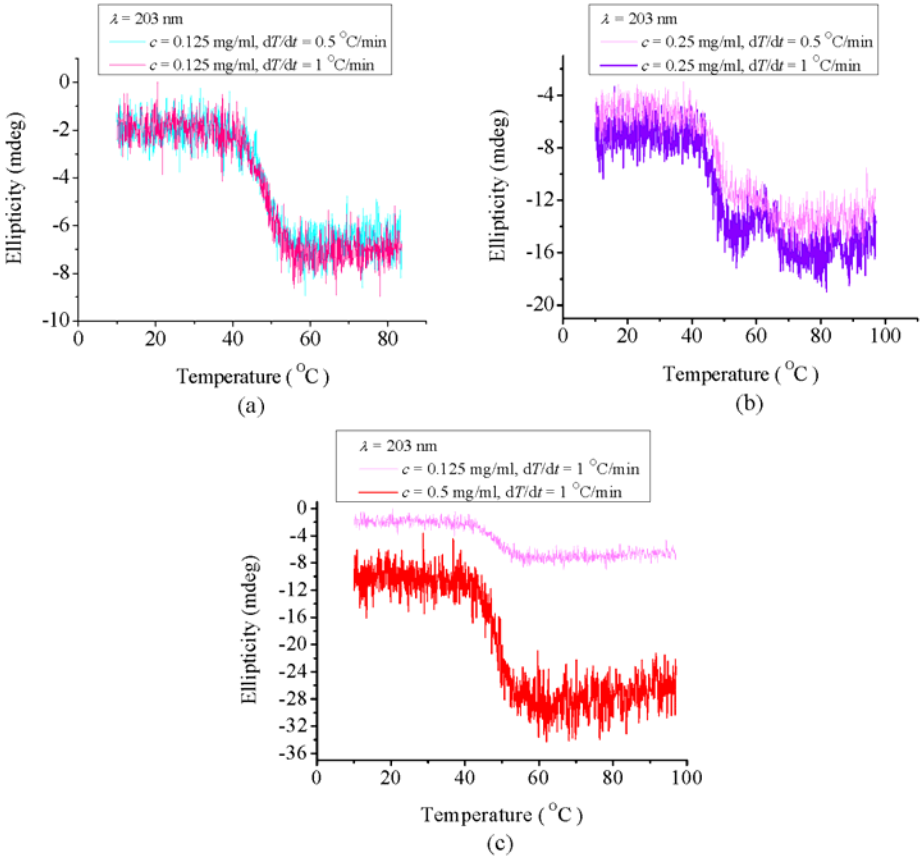

Figure 1. The temperature dependence of the ellipticity of the S-layer protein from *Lactobacillus salivarius* 16 strain, recorded at the fixed wavelength of 203 nm, for samples with different concentrations and different heating rates: (a) for two samples with the same concentration of 0.125 mg/ml and different heating rates, of 0.5 °C/min and 1°C/min; (b) for two samples with the same concentration of 0.25 mg/ml and different heating rates, of 0.5 °C/min and 1°C/min; (c) for two samples with different concentrations of 0.125 mg/ml and 0.5 mg/ml, and the same heating rate, of 1°C/min.