STRUCTURAL AND FUNCTIONAL ANALYSIS OF BIOPOLYMERS AND BIOPOLYMER COMPLEXES

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The Role of Calcium in the Conformational Changes of the Recombinant S100A8/S100A9

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Abstract—Calprotectin is a member of the EF-hand proteins, composed of two subunits, S100A8 (MRP8) and S100A9 (MRP14). These proteins are involved in important processes including cell signaling, regulation of inflammatory responses, cell cycle control, differentiation, regulation of ion channel activity and defense against microbial agents in a calcium dependent manner. In the present study, recombinant S100A8 and S100A9 were expressed in E. coli BL21 and then purified using Ni-NTA affinity chromatography. The structure of the S100A8/A9 complex in the presence and absence of calcium was assessed by circular dichroism and fluorescence spectroscopy. The intrinsic fluorescence emission spectra of the S100A8/A9 complex in the presence of calcium showed a reduction in fluorescence intensity, reflecting conformational changes within the protein with the exposure of aromatic residues to the protein surface. The far ultraviolet-circular dichroism spectra of the complex in the presence of calcium revealed minor changes in the regular secondary structure of the complex. Also, increased thermal stability of the S100A8/A9 complex in the presence of calcium was indicated.

Keywords: calprotectin, S100A8, S100A9, circular dichroism, thermal denaturation

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INTRODUCTION

Changes in the cytosolic calcium concentration regulate a variety of cellular processes [1]. These changes, act as a signal mediator, and the signal is transduced as activation or inactivation of Ca\(^{2+}\) binding proteins including a large family of proteins characterized by the EF-hand structural motif [2, 3]. S100 is a multigenic family of non-ubiquitous Ca\(^{2+}\)-modulated proteins of the EF-hand type expressed exclusively in vertebrates and implicated in intracellular and extracellular regulatory activities [4, 5]. S100A8 and S100A9 are members of the S100 family characterized by two calcium-binding sites with different affinity—a high affinity site at the C terminus (EF-hand II) and a low affinity site at the N terminus (EF-hand I) which are flanked by hydrophobic regions at either terminus and separated by a central hinge region [6]. They are specifically expressed in circulating neutrophils and early differentiation stages of monocytes, as well as in keratinocytes and epithelial cells under inflammatory conditions [7]. The elevated level of intracellular calcium also allows S100A8 and S100A9 to associate into non-covalent heterodimers and translocate from the cytosol to the cytoskeleton and the plasma membrane, thus mediating calcium signals by binding to other intracellular proteins [8]. The heterodimer of S100A8/A9 plays a crucial role in the regulation of inflammatory processes and the immune response, cell cycle control, differentiation, regulation of ion channel activity, apoptosis and defense against microbial agents [9—11]. Also increased serum levels of the heterodimer are observed in several human diseases such as cystic fibrosis, rheumatoid arthritis, acute inflammatory lesions, cardiomyophathy, and formation and deposition of amyloids in the ageing prostate and Alzheimer’s disease [12, 13]. More recently, they were also detected in various human cancers [14, 15]. Calcium induces conformational changes in calprotectin which may allow its interaction with the target proteins [16]. These changes of conformation result in the exposure of the hydrophobic surface for interactions with other proteins [17—19].

The aim of this study focused on structural and thermodynamic characterization of S100A8 and S100A9 subunits and the role of calcium in their complex to form a dimer protein with new conformational changes and thus its different role as calprotectin.

1 The article is published in the original.