Engineering and characterization of human 14-3-3 zeta with the controlled oligomer dynamics

P-02.5-21

D. Kalacheva[I,II], K. Tugaeva[I,II], N. Sluchanko[I,III]

[I]A.N. Bach Institute of Biochemistry, Federal Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, Russia, [II]Department of Biochemistry, School of Biology, M.V. Lomonosov Moscow State University, Moscow, Russia, [III]Department of Biophysics, School of Biology, M.V. Lomonosov Moscow State University, Moscow, Russia

14-3-3 proteins coordinate numerous intracellular processes via binding to phosphopartners and also show phosphorylation independent chaperone-like activity (CLA) by preventing aggregation of misfolded or denatured proteins. Although 14-3-3 proteins form dimers, phosphorylation of Ser58 in the interface induces partial dimer dissociation. It was proposed that, depending on the oligomeric state, 14-3-3 may play different roles in the cell. Here, we endow human 14-3-3 zeta with the engineered A16C and S58C mutations, which would fix the dimer interface upon oxidation and open new perspectives to study properties of 14-3-3 zeta depending on dimer dissociation. According to size-exclusion chromatography and differential scanning calorimetry, the A16C mutation preserves dimerization of recombinant human 14-3-3 zeta, whereas S58C mutation causes partial dimer destabilization. After finding conditions for an efficient 14-3-3 zeta A16C/S58C (=14-3-3Zcc) oxidation without using specific chemicals, we confirmed that such modification does not disturb 14-3-3Zcc interaction with phosphorylated human HSPB6, as compared with the wildtype 14-3-3 zeta (=14-3-3Zwt). Tryptophan fluorescence at a constant heating rate indicated that the thermal stability of the oxidized 14-3-3Zcc is 15 C° higher than that of the 14-3-3Zwt, confirming fixation of 14-3-3Zcc dimer by disulfide bridges and indirectly supporting the dissociative mechanism of 14-3-3 zeta denaturation. Finally, we assessed the CLA of 14-3-3Zcc on the thermally induced aggregation of myosin subfragment 1 (S1). Our preliminary data suggest that the oxidation of 14-3-3Zcc and fixation of its dimer interface decreases its CLA on aggregating S1, suggesting the functional role of the interface region in the anti-aggregation activity of 14-3-3 and warranting further studies using other protein substrates and different chaperone:substrate ratios. This work was supported by RSF grant no. 19-74-10031