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Novel water-soluble silica precursor for silicateins

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Silicateins are the major spicule-forming enzymes of marine sponges. They are members of the cathepsin family of cysteine proteases but are distinguished from cathepsins by Cys to Ser substitution in the catalytic triad (Cys-His-Asn). Silicateins catalyze formation of amorphous SiO2 from the silica precursors at physiological conditions. It is a promising biotechnological tool to obtain bio-silica covers and particles. The most popular silica precursor for silicatein is tetraethyl orthosilicate (TEOS). However, this compound is poorly soluble in water, and thus it is not readily available for the protein. We recently introduced a new substrate, tetrakis-(2-hydroxyethyl)-orthosilicate (THEOS), which is soluble in water, but its hydrolysis rate is very high, leading to a high level of spontaneous polymerization. In addition, TEOS, THEOS and its hydrolysis products are highly toxic for cells. Here we report a novel silica precursor for silicateins tetrakis (glycerol) orthosilicate (TGS). It is soluble in water and has a low spontaneous polymerization rate. Also, we found that TGS is much less toxic for mammalian cultured cells compared to TEOS and THEOS. We have tested TGS as a substrate for silicatein A1 from the marine sponge *Latrunculia oparinae*. It exhibited high activity with TGS and effectively formed silica particles (figure 1). Water-solubility of TGS and appropriate activity level enabled us to measure for the first time silicatein's biochemical properties-activity dependence on pH, temperature and precursor concentration. We also constructed several mutants of the silicatein A1 presumed active site triad (Ser25-His163-Asn187). Surprisingly, even Ala mutants (S25A, H163A, or N187A) retained a high activity in polymerization of TGS and THEOS, but not TEOS. These data call for reevaluation of the suggested earlier mechanisms of action and biochemical properties of silicateins.

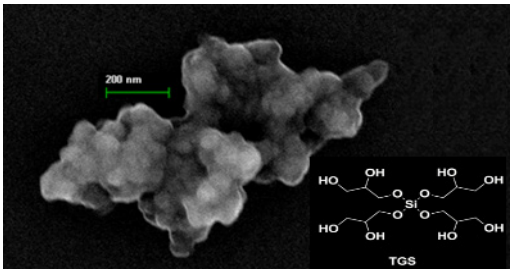

Figure 1: SEM image of the silica particle formed by silicatein A1 from the marine sponge *L. oparinae* with tetrakis (glycerol) orthosilicate (TGS) as a silica precursor. Inset shows TGS structure.

Biography

Natalia V Povarova has got her Master's degree in Biochemistry ("Study on interchangeability of picornaviral security proteins") at Lomonosov Moscow State University, Russia in 2011. Currently, she is doing her PhD degree in the Biophotonics Lab of Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry (Moscow, Russia) on Silicatein Biochemistry and its applications.

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