EFFECT OF REACTIVE OXYGEN SPECIES ON VIRAL PROTEIN ASSEMBLIES

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Introduction. ROS are highly reactive molecules responsible for the oxidation of macrostructures, such as lipids, DNA and proteins. Oxidation of proteins results in cross-linking, misfolding, aggregation, cleavage and structural changes. Viral protein assemblies (VPA) have applications as vaccines, bio-nanotemplates and drug and gene delivery systems (1). Oxidation of VPA has been scarcely studied. In addition to the effects of oxidation in monomeric proteins, oxidation of VPA can cause disassembly, reduce assembly efficiencies and destabilize the macrostructure, affecting VPA function. In this work, the impact of oxidation on VPA structure, assembly efficiency of monomers and quality of VPA was studied. The polymorphic protein rotavirus VP6 was used as model. Knowledge about how ROS affects VPA is useful to develop integrated production processes that result in VPA with high quality and stability.

Methods. VP6 nanotubes were produced in the insect cell-baculovirus system as described previously (1,2). VP6 was disassembled and assembled by calcium addition or removal, respectively. The monomeric protein or its assemblies were oxidized with H_2O_2 or by the Fenton reaction (FR, •OH). H_2O_2 concentration was varied between 0.05 to 10 mM were tested. For the FR, the iron concentration was maintained at 100μM. Oxidized and control untreated samples were tested for carbonylation (resulting from oxidation) by a colorimetric assay. Protein assemblies and monomers were characterized by SDS-PAGE, dynamic light scattering (DLS), fluorescence spectroscopy, gel permeation (GP) HPLC and transmission electron microscopy.

Results. Oxidation of VP6 monomers ($VP6_U$) or nanotubes ($VP6_{NT}$) with H_2O_2 did not modify the protein structure, as no change of the center of spectral mass (CSM), fluorescence intensity or size (measured by DLS) were observed. However, oxidation through the FR ($^{\bullet}OH$) resulted in a decrease in protein detection by GP-HPLC or SDS-PAGE, probably caused by aromatic amino acid oxidation and cleavage of peptide bonds. Higher H_2O_2 concentrations were needed to oxidize $VP6_{NT}$ than for $VP6_U$, indicating that assembled VP6 is more resistant to oxidation. Carbonyl content in $VP6_U$ or $VP6_{NT}$ exponentially increased with the H_2O_2 concentration, until a maximum content at 5 mM H_2O_2 was reached. Oxidation decreased $VP6_{NT}$ length and caused aggregation of $VP6_U$, as determined by DLS. Oxidation changed the fluorescence intensity and CSM of both assemblies, indicating changes in the protein tertiary structure. Oxidized $VP6_U$ could still assemble into $VP6_{NT}$. The quality of nanotubes (Figure 1) and the assembly efficiency decreased with higher H_2O_2 concentration. To our knowledge, this is the first study of the effect of ROS in viral protein assemblies.

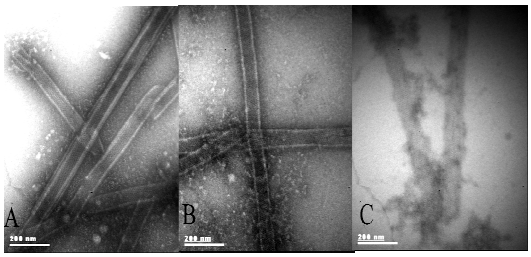

Figure 1. Transmission electron microscopy of re-assembled oxidized $VP6_U$. A) 0 mM, B) 0.5 mM and C) 5 mM of H_2O_2.

Conclusions. VP6 was resistant to oxidation by H_2O_2, in contrast with other proteins. Assembled VP6 was less susceptible to oxidation than $VP6_U$. Oxidation by •OH reduced the $VP6_{NT}$ length, affected VP6 tertiary structure, decreased the assembly efficiency of $VP6_U$ and provoked aggregation. $VP6_{NT}$ obtained from the assembly of oxidized $VP6_U$ had low quality and yields.

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