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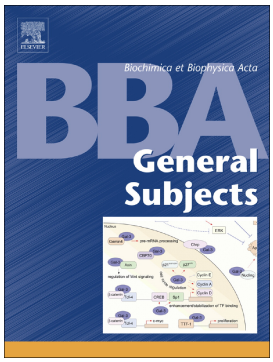

High-resolution atomic force microscopy visualization of metalloproteins and their complexes

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High-resolution atomic force microscopy visualization of metalloproteins and their complexes

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Abstract

Background. Metalloproteins myeloperoxidase (MPO), ceruloplasmin (CP) and lactoferrin (LF) play an important role in regulation of inflammation and oxidative stress in vertebrates. It was previously shown that these proteins may work synergetically as antimicrobial and anti-inflammatory agents by forming complexes, such as MPO-CP and LF-CP. However, interaction of metalloprotein molecules with each other has never been characterized at a single-molecule level.

Methods. In this study, the pairwise interactions of MPO, CP and LF molecules were investigated at a single-molecule level using high-resolution atomic force microscopy (AFM). Highly oriented pyrolytic graphite surface (HOPG) modified with oligoglycine-hydrocarbon graphite modifier (GM) was used as a substrate for protein deposition.

Results. The procedure for reliable AFM investigation of metalloproteins and their complexes has been developed. Using this procedure, we have visualized, for the first time, single MPO, CP and LF molecules, characterized the morphology of MPO-CP and LF-CP complexes and confirmed the absence of direct contacts between MPO and LF molecules. Moreover, we have revealed the novel chainlike shape of MPO-CP conjugates.

Conclusions. GM-HOPG was shown to be a convenient substrate for AFM investigation of metalloproteins and their complexes. Direct AFM visualization of MPO-CP and LF-CP complexes, on the one hand, complements previous data obtained from the "bulk techniques" and, on the other hand, provides new insight into the ultrastructure of MPO-CP complexes.

General Significance. The obtained results contribute to the better understanding of regulation of inflammation and oxidation stress mediated by collaborative action of the metalloproteins such as MPO, CP and LF.

Keywords

High-resolution atomic force microscopy; myeloperoxidase; ceruloplasmin; lactoferrin; metalloprotein complexes; oxidation stress.

Introduction