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Localization of an Antimicrobial Peptide in *Staphylococcus aureus* by Eosin-Induced Photo-Oxidation of Diaminobenzidine and Visualization with STEM-EDS

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Photo-oxidation of diaminobenzidine (DAB) has been used with a number of systems to localize sites of activity of drugs at the ultrastructural level. Use of colloidal gold labeled immunocytochemistry does not always provide the specificity of localization that can be attained with DAB photo-oxidation methods. We used the method of Deerinck *et al,* [1] to localize in *Staphylococcus aureus* the antimicrobial peptide $(KLAKLAK)_2$ labeled with the photosensitizer eosin Y [2].

Staphylococcus aureus was grown in liquid broth, pelleted and washed with 10mM phosphate, 150mM NaCl buffer, pH 7.4, then re-suspended to 10^8 CFU/ml in fresh buffer plus eosin-$(KLAKLAK)_2$. After a 10 min incubation, the cells and bound peptide were fixed with 1% acrolein in cacodylate buffer, pH 7.2, and washed in cold cacodylate buffer containing DAB (1mg/ml) (purchased from Polysciences, Inc., Warrington, PA). To observe the precise localization of eosin-$(KLAKLAK)_2$, DAB was then photo-oxidized by light excitation of the eosin group to yield the electron dense areas around the plasma membrane and cell wall after osmium staining. Cells were fixed with 1% acrolein in cacodylate buffer, pH 7.2, washed with cold cacodylate buffer, treated with DAB (1 mg/ml) (purchased from Polysciences, Inc., Warrington, PA) and osmicated with 1% (wt/vol) osmium tetroxide, dehydrated with 10% steps of methanol to (10%-100%), infiltrated overnight, and embedded in Quetol 651-Spurr epoxy resin [3] and polymerized overnight. Thin sections (200-250 nm) were cut with a Microstar diamond knife, (Huntsville, TX) using an AO Ultracut ultramicrotome picked up on grids and examined in a FEI Tecnai Field emission electron microscope at 200 kV accelerating voltage after carbon stabilizing the grids with approximately 10 nm of carbon using a Cressington 308 evaporative coater. EDS analysis was done with an Oxford EDS System.

Electron dense areas (Figs. 1 & 2) are indicative of photo-oxidized and thus polymerized DAB, which significantly enhances the staining by osmium tetroxide. There are four atoms of bromine per molecule of eosin Y. This accounts for the bromine peak detected by EDS in Fig. 3, and serves as a marker for the eosin-peptide conjugate. Photo-oxidation of DAB, combined with EDS allows for an improved determination of peptide location.

References

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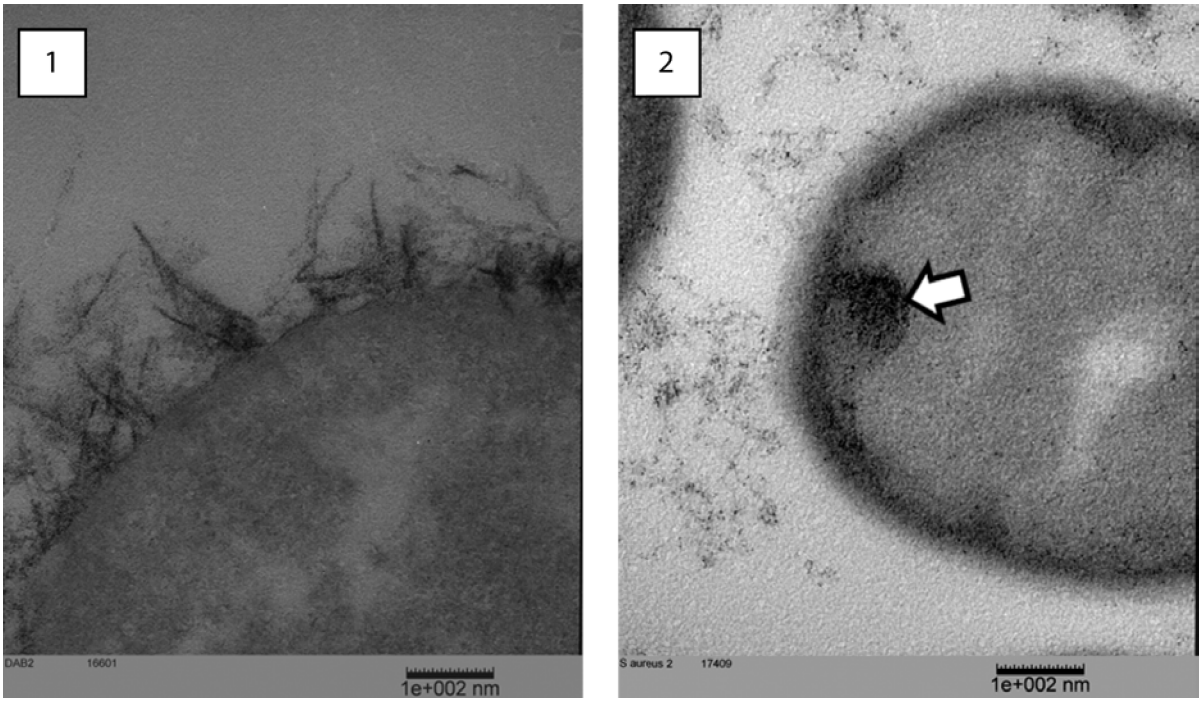

Fig. 1. *S. aureus* treated with the antimicrobial peptide $(KLAKLAK)_2$ labeled with the photosensitizer eosin Y. Note the electron dense areas on the surface of the cell as well as the plasma membrane.

Fig. 2. *S. aureus* treated with eosin-$(KLAKLAK)_2$. Note the dispersal of the osmium into the cytoplasm just beyond the surface of the cell wall.

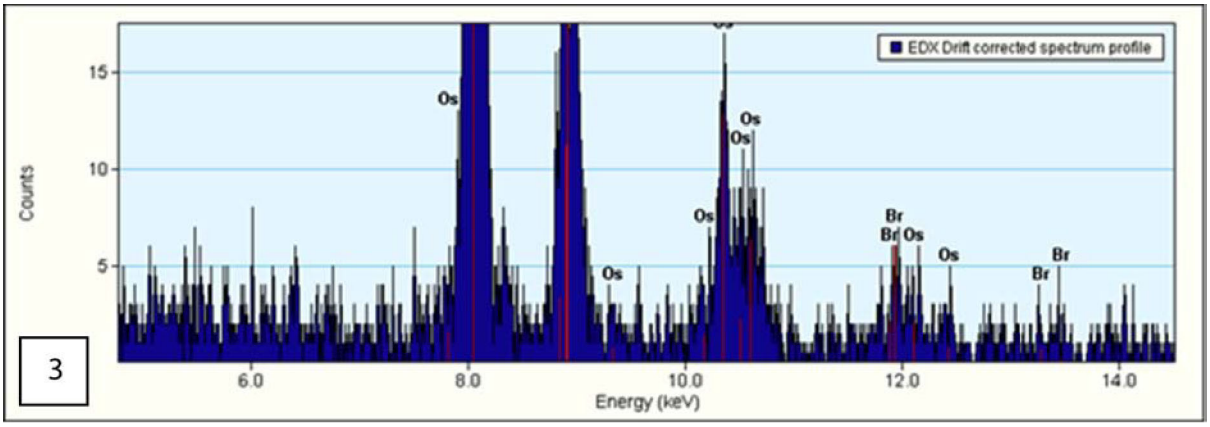

Fig. 3. EDS spectra of cells treated with DAB and eosin-$(KLAKKLAK)_2$. The highest Br peak is a Kα orbital.

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