

# Random Sequence Phage Display Analysis of Neutrophil Flavo-cytochrome B Structure

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Flavocytochrome b (Cyt b) of human neutrophils is the central component of the microbicidal NADPH-oxidase system. However, the membrane topology of this heterodimeric integral membrane protein remains to be determined. Random-sequence bacteriophage peptide library analysis was used to map structural features of Cyt b by determining the target epitopes of monoclonal antibodies (mAbs) specific for native and denatured Cyt b. MAbs 13.16 and 44.1 were produced against the small (p22-phox) Cyt b subunit, and mAb 54.1 was produced against the large (gp91-phox) Cyt b subunit. A nonapeptide phage display library was probed by each mAb to select phage bearing hexapeptides that mimic the Cyt b epitopes. The peptide sequences were determined by sequencing the phage DNAs coding for the peptides. The peptide sequences were then compared to the Cyt b protein for similarity. The nonapeptide on phage selected by mAb 54.1 contained the sequence PKXAVDGP. This sequence is also present in gp91-phox. The nonapeptide expressed by phage clones selected by mAb 13.16 and 44.1 was GGPQVXPIX. This peptide closely resembles the sequence GGPQVNPI, found on p22-phox. Western blotting was used to show the specificity of each mAb for the Cyt b subunit and the selected phage peptide. In FACScan analysis of intact and saponin permeabilized neutrophils, mAb 44.1 bound only saponin treated cells, but 54.1 did not bind to intact or permeabilized cells. Binding of mAb 44.1 to the cytosolic epitope can be blocked by pretreating the mAb with the phage expressing the PQVRPI peptide. These results suggest the GGPQVRPI segment of p22-phox is accessible on its intracellular surface in neutrophils, but the PKIAVDGP region on gp91-phox is not accessible to antibody, and is probably sequestered. This method should be applicable to other mAbs to help define the molecular topology of Cyt b.