Oxidized and poorly glycosylated band 3 is selectively phosphorylated by Syk kinase to form large membrane clusters in normal and G6PD-deficient red blood cells.

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ABSTRACT:
Oxidative events involving band 3 (Anion Exchanger 1) have been associated with RBC (red blood cell) removal through binding of NAbs (naturally occurring antibodies); however, the underlying mechanism has been only partially characterized. In addition to inducing direct membrane protein oxidative modification, oxidative treatment specifically triggers the phosphorylation of band 3 tyrosine residues.
The present study reports that diamide, a thiol group oxidant, induces disulfide cross-linking of poorly glycosylated band 3 and that the oligomerized band 3 fraction is selectively tyrosine phosphorylated both in G6PD (glucose-6-phosphate dehydrogenase)-deficient and control RBCs. This phenomenon is irreversible in G6PD-deficient RBCs, whereas it is temporarily limited in control RBCs. Diamide treatment caused p72 Syk phosphorylation and translocation to the membrane. Diamide also induced p72 Syk co-immunoprecipitation with aggregated band 3. Moreover, following size-exclusion separation of Triton X-100-extracted membrane proteins, Syk was found only in the high-molecular-mass fraction containing oligomerized/phosphorylated band 3. Src family inhibitors efficiently abrogated band 3 tyrosine phosphorylation, band 3 clustering and NAbs binding to the RBC surface, suggesting a causal relationship between these events. Experiments performed with the non-permeant cross-linker BS(3) (bis-sulfosuccinimidyl-suberate) showed that band 3 tyrosine phosphorylation enhances its capability to form large aggregates.
The results of the present study suggest that selective tyrosine phosphorylation of oxidized band 3 by Syk may play a role in the recruitment of oxidized band 3 in large membrane aggregates that show a high affinity to NAbs, leading to RBC removal from the circulation.

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