Abstract

Glycosylphosphatidylinositol-specific phospholipase C (GPtdIns-PLC) is found in the protozoan parasite Trypanosoma brucei. A region of protein sequence similarity exists between the protozoan enzyme and eubacterial phosphatidylinositol-phospholipases C. The functional relevance of Cys80 and Gln81 of GPtdIns-PLC, both in this region, was tested with a panel of mutations at each position. Gln81Glu, Gln81Ala, Gln81Gly, Gln81Lys and Gln81Leu mutants were inactive. Cleavage of GPtdIns was detectable in Gln81Asn, although the specific activity decreased 500-fold, and kcat was reduced 50-fold. Thus an amide side-chain at residue 81 is essential for catalysis by GPtdIns-PLC. Sulfhydryl reagents inactivate GPtdIns-PLC, suggesting that a Cys could be close to the enzyme active site. Surprisingly, p-chloromercuriphenyl sulfonate (p-CMPS) is significantly more potent than N-ethylmaleimide, the less bulky compound. This knowledge prompted us to test whether replacement of Cys80 with an amino acid possessing a bulky side-chain would inactivate GPtdIns-PLC: Cys80Ala, Cys80Thr, Cys80Phe, Cys184Ala, and Cys269-270-273Ser were constructed for that purpose. Cys80Phe lacked enzyme activity, while Cys80Ala, Cys80Thr and Cys269-270-273Ser retained 33 to 100% of wild-type activity. Interestingly, the Cys80Ala and Cys80Thr mutants became resistant to p-CMPS, as predicted if the sulfhydryl reagent reacted with Cys80 in the wild-type enzyme to form a cysteinyl mercurylphenylsulfonate moiety, a bulky adduct that inactivated GPtdIns-PLC, similar to the Cys80Phe mutation. We conclude that a bulky side-chain (or adduct) at position 80 of GPtdIns-PLC abolishes enzyme activity. Together, these observations place Cys80 and Gln81 at, or close to, the active site of GPtdIns-PLC from T. brucei.