Abstract

Glycosylphosphatidylinositol (GPI)-specific phospholipases are highly valuable for studying the structure and function of GPIs. GPI-specific phospholipase C (GPI-PLC) from Trypanosoma brucei and phosphatidylinositol-specific phospholipase C (PI-PLC) from Bacillus cereus are the most widely studied of this class of phospholipases C. Inhibition of protein activity by thiol reagents is indicative of the participation of cysteine residues in biochemical events. The thiol reagent p-chloromercuriphenylsulphonate (pCMPS) inhibits T. brucei GPI-PLC, which has eight cysteine residues. Surprisingly, we found that the activity of B. cereus PI-PLC is also blocked by pCMPS, although the protein does not contain cysteine residues. Inhibition of B. cereus PI-PLC was reversed when pCMPS was size-separated from a preformed pCMPS·PI-PLC complex. In contrast, no activity was recovered when T. brucei GPI-PLC was subjected to a similar protocol. Equimolar β-mercaptoethanol (β-ME) reversed the inhibition of PI-PLC activity in a pCMPS·PI-PLC complex. For T. brucei GPI-PLC, however, ultrafiltration of the pCMPS·GI-PLC complex and addition of a large excess of β-ME was necessary for partial recovery of enzyme activity. Thus T. brucei GPI-PLC is susceptible to inactivation by covalent modification with pCMPS, whereas PI-PLC is not. Kinetic analysis indicated that pCMPS was a competitive inhibitor of PI-PLC when a GPI was a substrate. Curiously, with phosphatidylinositol as substrate, inhibition was no longer competitive. These data suggest that pCMPS is a glyco-mimetic that occupies the glycan binding site of PI-PLC, from where, depending on the substrate, it inhibits catalysis allosterically or competitively.