Alpha-glucan phosphorylases (α-GPs) are a class of glycosyltransferases that play a critical role in carbon storage and mobilization, regulating mammalian glycogen metabolism and plant starch metabolism. α-GPs can degrade α-1,4-polyglucans chains by using orthophosphate to release glucose-1-phosphate or synthesize α-1,4-polyglucans by removing the orthophosphate and adding a glucose residue. In mammals, this is the rate-limiting step in glycogen mobilization. In plants, this reaction is proposed as an alternative pathway of starch synthesis and nocturnal degradation. Impaired function or improper regulation of α-GPs and carbon mobilization can lead to neurodegenerative diseases in humans, and a poor response to environmental stress tolerance in plants. Our results demonstrate that the plastidal α-GP isoform in plants (Pho1) shows increased activity when oxidized in vivo. Additionally, two of the three mammalian isoforms – brain and liver – also behave similarly in vitro. Notably, this oxidation-induced behaviour is not shared by specific α-GP isoforms such as plant isoform Pho2 and other plant cytosolic isoforms. Sequence alignment analysis indicates that α-GPs activated by oxidation share a common C-terminal cysteine, while those lacking this cysteine do not exhibit such behaviour. Our research demonstrates that α-GPs are likely regulated by glutathionylation under oxidative conditions. My research investigates the role of C-terminal cysteines of α-GPs and glutathionylation in plants and animals, potentially identifying a universal form of post-translational regulation of α-GPs by cellular oxidation. This research will increase our understanding of cellular oxidative stress in relation to its involvement in regulating carbon storage within eukaryotic cells.