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New insights into redox control of starch degradation

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Starch is one of the major sinks of fixed carbon in photosynthetic tissues of higher plants. Carbon fixation and the synthesis of primary starch occur during the day in the chloroplast stroma, whereas starch degradation typically occurs during the following night to fuel the whole plant with energy and carbon in the absence of photosynthesis. Redox-based regulatory systems play a central role in the modulation of several chloroplastic pathways. Reversible oxidations of cysteine residues are post-translational modifications that orchestrate the precise functioning of chloroplast pathways together with changes in pH, Mg^{2+} and concentrations of metabolic intermediates. Leaf starch metabolism has been intensively studied. The enzymes involved in starch synthesis and degradation have been identified and characterized. However, the redox control of the enzymes responsible for starch degradation at night remains elusive, and their response to redox transitions conflicts with the timing of the physiological events. Most of the enzymes of starch degradation are activated by reducing conditions, characteristic of daytime. Thus, redox control may have only a minor role during starch degradation at night, but could become relevant for daily stomatal opening in guard cells or in the re-allocation of fixed carbon in mesophyll cells in response to stress conditions.

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Introduction

Starch is a complex polymer of glucose synthesized in plastids of higher plants, where it accumulates as insoluble, osmotically inert granules. Starch metabolism has been thoroughly investigated due to the importance of starch for human and animal nutrition as well as raw material for many industrial applications [1]. Primary and secondary starches are structurally indistinguishable, but they have a different metabolism and are stored in different plant organs. Secondary starch is typically found in storage organs, such as seeds or tubers, and it is remobilized in response to internal stimuli to support specific phases of growth, for example, seedling establishment during germination. In contrast, primary starch is found in chloroplasts of autotrophic tissues and shows a rapid turnover [2]. Under normal growth conditions, primary starch is synthesized during the day from carbon assimilated through photosynthesis and is degraded during the following night to sustain plant maintenance and growth in absence of light. However, primary starch can be degraded in the light in response to abiotic and biotic stress to meet the changes in energy and carbon demand that are required to counteract these stress. Primary starch accumulates also in the chloroplasts of guard cells [3]. Guard cells are highly specialized cells of the plant epidermis, which control water and carbon dioxide movements through the stomatal pore, therefore forming a gate between the plant and the environment. In these cells, starch metabolism follows an opposite rhythm compared to mesophyll cells. Starch is mobilized in the light period to produce malate and sucrose that contribute to increasing guard cell osmolarity, turgor and stomatal opening [4•].

Chloroplasts evolved from endosymbiosis, through which plants acquired the capacity for oxygenic photosynthesis. As a consequence of photosynthesis being performed in an oxygen-rich atmosphere, chloroplasts must cope with reactive oxygen species (ROS) derived from the unavoidable one-electron reduction of oxygen by components of the photosynthetic electron transport chain. Because they are so unstable, ROS tend to oxidize different types of cellular components, but under tightly regulated conditions, ROS reactivity is also involved in signaling pathways. Therefore, in chloroplasts, redox homeostasis plays a central role. Due to their physicochemical properties, cysteine residues have been selected by evolution to work as redox switches and/or redox sensors [5••]. In some cases, oxidative modifications of selected protein cysteines can be used in metabolic regulation. Reversible cysteine oxidation can affect single or double thiol groups and takes part in the comprehensive and complex regulation of chloroplast metabolism.

Here we focus on redox modifications that affect the activity of the enzymes involved in starch breakdown of mesophyll cells. We describe the response of each enzyme known to be redox regulated and give a tentative explanation for this regulation in a physiological context.

The birth of redox control: the ferredoxin/thioredoxin system

The redox biology field has its foundation back in 1964, when Peter Reichard and colleagues discovered and coined the name 'thioredoxin' (Trx; for an historical view see [6•]). At the beginning, the term Trx referred to a small dithiol redox protein that in *Escherichia coli* acted as electron donor for ribonucleotide reductase [7]. The subsequent discovery that *E. coli* Trx could also activate fructose 1,6-biphosphatase (FBPase) in chloroplasts — an enzyme of the Calvin–Benson cycle — opened the field of redox regulation and thiol-based redox signaling in plants [8,9]. In photosynthetic organisms, most of the effort on redox regulation was initially focused on the enzymes of the Calvin–Benson cycle. Indeed, it became immediately evident that the Trx system was a good mechanism for regulating enzyme activity as a function of light. Chloroplast Trxs are reduced during photosynthesis by ferredoxin:thioredoxin reductase (FTR), which, in turn is reduced by ferredoxin (Fd), the end product of the photosynthetic electron transport chain (Figure 1) [10]. The Fd:Trx system provided such an excellent link between the two phases of photosynthesis, that, after its discovery, these became known as 'light' and 'carbon' (instead of dark) reactions.

The role of redox regulation in chloroplast spread beyond the mere adjustment of the Calvin–Benson cycle and other metabolic pathways in response to light/dark cycle when the NADPH-dependent thioredoxin reductase (NTRc) was found in plastids [11,12]. The special feature of NTRc is the combination of a NADP-dependent flavo-domain with a thioredoxin domain, making possible the direct NADPH-dependent reduction of target enzymes also in the absence of light. With the advent of the 'omics-era', the number and type of Trxs increased [13,14], and the number of Trx targets increased to about 300 [15,16].

The evolution of redox control: S-glutathionylation and other mono-thiol modifications

Trxs directly interact and reduce their target enzymes by performing dithiol/disulfide exchange reactions between two cysteine residues of the same protein subunit (intramolecular disulfide bridge) or two different protein subunits (intermolecular disulfide bridge) (Figure 1). With time, such thiol-based protein regulation has widened. Along with the regulation mediated by Trxs, it emerged the regulation system based on a single cysteine residue (Figure 1). A major mono-thiol regulation depends upon the capacity of a cysteine to form a mixed disulfide bond with a non-protein thiol, such as the small redox molecule glutathione (GSH) (Figure 1) [17]. This modification, known as S-glutathionylation, entered the field of redox-regulation after the discovery that glutaredoxin (Grx), initially considered as hydrogen donor of the ribonucleotide reductase [18], was able to remove S-glutathionylation from target enzymes, restoring their activity [5••].

Not all cysteines are equally susceptible to be redox modified. Their susceptibility mainly depends on the first, accessibility; second, local environment; and third, propensity to be deprotonated under physiological pH condition. Typically, the pK_a value of reactive cysteines is markedly lower in comparison to non-reactive ones (~6.5 versus ~8.5, respectively) [19•]. An intrinsic feature of a life in an aerobic environment is the inevitable exposure of the thiolate anion (S^-) to oxidants such as hydrogen peroxide, which leads to the conversion of S^- in sulfenic acid (SOH), a short-lived oxidized form of cysteine (Figure 1). The fate of SOH depends on several conditions. Due to its high reactivity toward thiol groups, SOH could lead to the formation of disulfide bond. Alternatively, it could form a mixed disulfide, or it could be further and irreversibly oxidized to sulfinic (SO_2H) and sulfonic (SO_3H) acids (Figure 1). Oxidation to SO_2H and SO_3H irreversibly damage proteins. In contrast, disulfide bond, S-glutathionylation, S-nitrosylation and S-sulfhydration are considered as post-translational modifications (PTMs). The formation of disulfide bonds is mainly involved in the regulation of specific target enzymes activity under physiological conditions, whereas the other PTMs constitute a protecting network against oxidative damage (Figure 1).

Redox control of primary starch metabolism in chloroplast

Due to their characteristics, metabolic pathways that occur in chloroplast are particularly prone to redox regulation. In chloroplasts of eukaryotic phototrophs, photosynthesis converts light energy into chemical energy, which is in part consumed to keep the pool of Trxs reduced [10]. Inevitably, photosynthesis leads to the simultaneous accumulation of ROS, which can cause oxidative damage of redox sensitive enzymes [20]. ROS production and ROS-induced damage increase under stress conditions because the photosynthetic carbon reactions are directly or indirectly down regulated resulting in over-reduction of the photosynthetic electron transport chain and photoreduction of dioxygen (O_2). For these reasons, redox homeostasis is crucial for chloroplast metabolism. Redox control based on thiol-reactivity of the Calvin–Benson cycle enzymes is well characterized [21], whereas the knowledge about redox regulation of enzymes involved in the primary starch metabolism is still incomplete.

In most plant species, including *Arabidopsis thaliana*, 40–50% of the photoassimilates produced during the day by the Calvin–Benson cycle are transiently stored in chloroplasts as primary starch [22]. During the following night, primary starch is degraded, and the resulting