

## Enzyme Regulation during Plant Stress

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Regulation of enzyme activities is of key importance in all living cells and especially during stress conditions enzyme regulation is crucial for plant's survival. Due to their immobility plants cannot escape from unfavorable extrinsic environment. Contrarily plants have to withstand stress by extensive intrinsic rearrangements of primary and secondary metabolism [1]. Metabolic flow through some pathways under stress is decreased (e.g. photosynthetic production of ATP, Calvin cycle, nitrogen assimilation) while the other metabolic pathways become more intensive or bypassed by additional ways [2]. Examples of more intensive pathways can be increased respiration and activated antioxidant system accompanying majority of plant defense responses and of bypassed alternative reactions glycolytic bypass formed by phosphoenolpyruvate carboxylase (PEPC, EC 4.1.1.31) under stress caused by phosphate deficiency, when ADP and inorganic phosphate for cytosolic pyruvate kinase (EC 2.7.1.40) is limited [3].

In enzyme regulation pH, concentration of substrates, coenzymes, and cofactors and the presence of activators and inhibitors play a pivotal role. Many enzymes are pH sensitive, which e.g. ensure their regulation between night and day. This is the case of Calvin cycle enzymes fructose-1,6-bisphosphatase (EC 3.1.3.11) and sedoheptulose-1,7-bisphosphatase (EC 3.1.3.37) and their activation during the dark/light transition, when the pH can change from 7.2 to 8.0, which corresponds to pH optimum of these enzymes [4]. Some enzymes directly function as regulators of pH; well known is the Davies-Roberts lactate dehydrogenase (LDH, EC 1.1.1.27)/pyruvate decarboxylase (PDC, EC 4.1.1.1) pH stat, which operates under the onset of oxygen deprivation. This system regulates anaerobic metabolism between lactic and ethanolic fermentation. Initially pyruvate produced by glycolysis is converted by enzyme with pH optimum at physiological range: LDH, but after acidification of cytosol with lactate, LDH is inhibited and on the contrary PDC is activated by lower pH and fermentation via PDC and alcohol dehydrogenase (EC 1.1.1.1) leading to ethanol is preferred [1]. Also PEPC together with NADP-malic enzyme (NADP-ME, EC 1.1.1.40) regulates pH. Alkalinization activates PEPC, oxaloacetate is converted to relatively acidic malate. On the other hand NADP-ME catalyzes reaction, in which malate is decarboxylated to less acidic pyruvate [2,5].

Besides the availability of substrates and coenzymes the presence of cofactors affects the activity of many enzymes. The activity of NADP-ME from *Nicotiana tabacum* L. is regulated by following metal ions: Mg<sup>2+</sup> (100% of activity), Mn<sup>2+</sup> (140%), Co<sup>2+</sup> (59%), Ni<sup>2+</sup> (54%), and Zn<sup>2+</sup> (20% activity compared to Mg<sup>2+</sup> ions). Moreover the type of cofactor affects the affinity of NADP-ME to substrate malate, while the affinity of NADP-ME to NADP<sup>+</sup> was not influenced [6]. Enzyme activity is substantially affected by concentration of substrate. Many deviations from hyperbolic Michaelis-Menten dependence of reaction rate on substrate concentration are known. Namely, the sigmoidal kinetics cause fast increase of reaction rate. The dependence of NADP-ME reaction rate on concentration of Mn<sup>2+</sup>, Co<sup>2+</sup> and Ni<sup>2+</sup> was sigmoidal with the highest positive cooperative for Mn<sup>2+</sup> ions (the value of Hill coefficient 8 indicates 8 binding sites for Mn<sup>2+</sup> per tetramer). On the other hand dependence of NADP-ME reaction rate on Mg<sup>2+</sup> was non-linear and corresponded to equation for 2 binding sites per subunit [6]. The inhibition by access of substrate, which participates in enzyme

regulation, was found for photosynthetic NADP-ME from maize plants [7].  $\beta$ -Hexosaminidase from *Penicillium oxalicum* was also inhibited by access of substrate p-nitrophenyl-N-acetyl-D-glucosamine but not p-nitrophenyl-N-acetyl-D-galactosamine [8].

The enzyme activity strongly influences low-molecular activators and inhibitors. For example PEPC is inhibited by malate and activated by glucose-6-phosphate. Moreover, phosphorylation often means switch on or switch off of some metabolic pathways, but in case of PEPC phosphorylation affects kinetic properties and sensitivity to effectors. Dephosphorylation of maize seeds PEPC results in the changed kinetics from hyperbolic to sigmoidal and increased sensitivity to inhibition by malate [9]. The phosphorylated PEPC from many plant species show decreased sensitivity to inhibition by malate but inhibition of tobacco PEPC is not affected by phosphorylation [10]. Final metabolic products or energetically rich compounds such as ATP and GTP often serve as inhibitors of enzymes. In the case of tobacco NADP-ME was found that this enzyme is inhibited by GTP, ATP, and ADP [11].

Not only phosphorylation but also other posttranslational modifications (PTMs) substantially affect enzyme activity. Secreted proteins are often glycosylated; this modification acts primarily on enzyme stability and resistance to proteolysis, less commonly directly on enzyme activity [8]. The detailed examination of enzyme molecules by mass spectrometry and other techniques continues to identify hundreds of distinct PTMs. Multiple PTMs (both enzymatic such as glycosylation, phosphorylation, ubiquitylation, methylation, prenylation, myristoylation, ADP-ribosylation, -SH group modification etc. and non-enzymatic such as glycation, lipoxidation, oxidation, carbamylation, S-nitrosylation etc.) within a single enzyme molecule and their mutual interplay are critical for the regulation of catalytic activity [12].

Enzymes composed of subunits could be regulated by oligomerization state.  $\beta$ -Glucosidase is able to catalyze hydrolysis of inactive ABA glucoside to active hormone, which plays important role in plant response to abiotic stress. Under dehydration plants enhanced activity of  $\beta$ -glucosidase by aggregation of its molecules [13]. NADP-ME in *Nicotiana benthamiana* plant organs is present in different oligomers [14].

Important regulation of enzyme activity during stress is synthesis *de novo*. Increased mRNA for cytosolic NADP-ME was found during viral infection caused by *Potato virus Y*, strain NTN [15]. Many signal transductions and cascades, which may involve reorganization of cytoskeleton, release of Ca<sup>2+</sup> ions, G-proteins, changes in

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Received July 31, 2013; Accepted August 01, 2013; Published August 03, 2013

Citation: Doubnerova V, Ryslava H (2013) Enzyme Regulation during Plant Stress. *Biochem Anal Biochem* 2: e141. doi: [10.4172/2161-1009.1000e141](https://doi.org/10.4172/2161-1009.1000e141)

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phosphorylation status etc. lead to expression of genes encoding stress-related proteins [16,17]. Under stress conditions plant cell balances between synthesis *de novo* and reparation or degradation of damage proteins e.g. through heat shock proteins (HSPs). For example the constitutively expressed HSPs70 are often involved in assisting the folding of *de novo* synthesized polypeptides and import/translocation of precursor protein, while environmental stress related HSPs70 are more involved in facilitating refolding and proteolytic degradation of non-native proteins [18,19]. Furthermore, HSP70 can increase enzyme activity, such as in the case of NADP-ME [20].

The multiple levels of enzyme regulation and its diversity can be the reason of problems in genetic engineering e.g. during manipulating of enzymes levels or during incorporating of enzyme cycles in transgenic plants, such as in the case of production of C4-like photosynthetic pathway (PEPC, NADP-malate dehydrogenase EC 1.1.1. 82, NADP-ME, and pyruvate, phosphate dikinase EC 2.7.9.1) to C3 plants for example rice or wheat. The photosynthetic productivity in C4 plants is generally about 1.5-2-fold higher than in C3 plants and therefore this manipulation could increase crop productivity [2,21-25]. However the complete transformation of C3 plants to C4 plants with more efficient photosynthesis has not been successfully solved yet, probably due to complex regulation of individual enzymes.

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