FEBS Letters 425 (1998) 530–531 FEBS 20049

Dehydroascorbate reduction: the phantom remaining

Susanne Morell[a], Hartmut Follmann[a], Mario de Tullio[b], Ingo Häberlein[a,*]

[a] *Department of Biochemistry, University of Kassel, Heinrich-Plett-Str. 40, D-34109 Kassel, Germany*
[b] *Institute of Botany, University of Bari, Via E. Orabona, I-70125 Bari, Italy*

Received 23 February 1998

In our recent contribution [1] we questioned the role of dehydroascorbate (DHA) and DHA reductase as reliable indicators of oxidative stress in plants. Several lines of evidence were presented to substantiate that the measurement of DHA pools and DHA reductase activity in crude plant extracts is falsified by technical problems and by the fact that plant cells contain several redox-active proteins with unspecific DHA-reducing activity. We discussed whether the disproportionation of monodehydroascorbate (MDHA) leads to an appreciable formation of DHA in plant cells considering the known chemical and biochemical data of MDHA reductase and other MDHA-reducing components. Moreover, we questioned whether plant cells necessarily require a specific DHA reductase and concluded that they probably do not possess a specific DHA reductase at all. It is not surprising that these statements raise a dispute. Unfortunately, Foyer and Mullineaux [2] in their commentary try to counter our conclusions not by new facts but by remote arguments.

First of all we did not suggest that DHA will not exist in plant cells at all. We outlined that *it is improbable that significant amounts of DHA will accumulate*, a conclusion drawn not only from ample biochemical evidence found in the literature [1] but from data showing that a steady-state concentration of 50 μM will substantially inhibit the regulatory action of plant thioredoxins essential for light-dependent regulation of processes such as CO_2 fixation, sulfate assimilation, and nitrate assimilation. Foyer and Mullineaux argue [2] that this observation was derived from assays not describing the situation in vivo because thioredoxin reductase was not added to regenerate reduced thioredoxin. They assume that *the stromal enzymes regulated by the thioredoxin system require ongoing reduction to remain active*. This would imply that in light the reductively activated target enzymes are permanently deactivated by oxidants and require repeated activation by thioredoxins in vivo. In the last 10 years knowledge about the action of thioredoxin has improved substantially enough (e.g. by demonstration of complex formation between thioredoxin and target enzymes) to indicate that the very opposite is the case. A detailed explanation is beyond the scope of this comment but the reader's attention is drawn to a recent review summarizing the biochemical action of thioredoxins [3].

Among the known proteins possessing DHA reductase activity are thioredoxins, a Kunitz-type trypsin inhibitor, glutaredoxins (thiol transferases), and protein disulfide isomerases. All these proteins are characterized by a very similar redox-active site of the general amino acid sequence Cys-X-X-Cys. Reversible redox changes between the dithiol form and disulfide form supply the reducing equivalents for DHA reduction. If we isolated one of these proteins using the common DHA reductase assay and did not know their original function in plant metabolism we, too, would regard it as DHA reductase. Many more enzymes containing Cys-X-X-Cys motifs might be able to reduce DHA but in the absence of pertinent experiments have not been recognized. This offers an explanation of why DHA-reducing proteins have been found in unpredictable numbers in plant tissues [4]. In line with these arguments we suggested further experiments to establish unambiguously the identity of the DHA-reducing protein isolated by Kato et al. [5]. We point, for example, to the identification of a putative DHA reductase as a 3α-hydroxysteroid dehydrogenase [5,6]. In our contribution, we may not have been precise enough in discussing this point. We never stated that we consider the activity described by Kato et al. to be artifactual but do maintain that it could be the side activity of some different enzyme. The sequence similarity mentioned by Foyer and Mullineaux does not prove a DHA reductase proper.

Extraction of plant samples with a phosphate buffer at physiological pH in a Waring blender is certainly not a procedure to denature proteins. One of the most gentle methods to observe different isoforms of an enzyme is non-denaturing gel electrophoresis when the protein extracts are applied without delay. Thus, a specific and original DHA reductase should be measurable by activity staining. That we did observe the side activities of unrelated proteins obviously repudiates the argument that the investigated protein samples were too diluted. Moreover, different chromatographic methods have been tried to isolate a specific DHA reductase but all these efforts failed.

The link between DHA formation, recycling of DHA to ascorbate by glutathione-dependent DHA reductase, and reduction of GSSG to GSH by glutathione reductase is commonly regarded as essential for the ascorbate regeneration system in plants. Because of the questionable existence of an original DHA reductase in plants we discussed whether the ascorbate-glutathione cycle is overestimated among processes constituting the defense system against oxidative stress in plants. In this context it is thinkable that the role of glutathione reductase to avoid oxidative stress is overestimated, too, but this in no way downplays the central role of glutathione reductase to maintain a reducing environment and it does not exclude that glutathione is required in other processes in plant cells.

These considerations support our view that one has to be careful in accepting either changing DHA pools or DHA reductase activities as indicators of oxidative stress in plants. This is, in fact, in agreement with the statement of Foyer and Mullineaux [2] who remark: "that DHA reductase activity is often found not to increase in stressed plants is evidence that only incidental activities from other proteins are being measured". The remaining problem is to distinguish between incidental and specific DHA reductase activities. A postulated

*Corresponding author. Fax: (49) (561) 804 4466.
E-mail: haeber@hrz.uni-kassel.de

PII S0014-5793(98)00282-8

specific, yet elusive and unmeasurable chloroplast enzyme is in our opinion not reliable enough as indicator of oxidative stress in plants.

References

[1] Morell, S., Follmann, H., De Tullio, M. and Häberlein, I. (1997) FEBS Lett. 414, 567–570.
[2] Foyer, Ch.H. and Mullineaux, P.M. (1998) FEBS Lett. 425, 528–529.
[3] Follmann, H. and Häberlein, I. (1996) BioFactors 5, 147–156.
[4] Hossain, M.A. and Asada, K. (1984) Plant Cell Physiol. 25, 85–92.
[5] Maellaro, E., Del Bello, B., Sugherini, L., Comporti, M. and Casini, A.F. (1994) Biochem. J. 301, 471–476.
[6] Del Bello, B., Maellaro, E., Sugherini, L., Santucci, A., Comporti, M. and Casini, A.F. (1994) Biochem. J. 304, 385–390.