CHARACTERISATION OF A CHLORORESPIRATORY PATHWAY IN *BETA VULGARIS* AND *TRIFOLIUM REPENS*

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ABSTRACT

The chloroplast respiratory pathway (chlororespiration) is postulated to interact with the photosynthetic pathway through the plastoquinone (PQ) pool. Two enzymes are proposed to operate in the pathway: an NAD(P)H dehydrogenase that is homologous to the mitochondrial NADH dehydrogenase, and a putative terminal oxidase. To study the operation and regulation of the chlororespiratory pathway in two higher plant species, silverbeet (*Beta vulgaris* L.) and white clover (*Trifolium repens* L.), two approaches have been used. The first uses salicylhydroxamic acid (SHAM), an inhibitor of the mitochondrial quinol-oxidising alternative oxidase, to identify the site of inhibition during electron transfer through the photosynthetic electron transfer chain. The second uses antibodies to two subunits of the NAD(P)H dehydrogenase complex, and examines changes in accumulation of these proteins during physiological conditions proposed to regulate the operation of the NAD(P)H dehydrogenase.

For the first part, inhibition by SHAM on the photosynthetic electron transfer chain was shown to be in the vicinity of Q_A using oxygen electrode and fluorescence analysis, and a number of specific photosynthetic electron acceptors, donors and inhibitors in order to isolate specific parts of the photosynthetic electron transfer chain. By the analysis of electron transfer through the whole chain, PS I, PS II and electron transfer from P680 through to Q_A, inhibition by SHAM was shown to be in the vicinity of Q_A. These observations are consistent with the reported effects on chlorophyll fluorescence, but do not exclude the existence of an alternative oxidase in the thylakoid membrane.

To examine the accumulation of the NAD(P)H dehydrogenase complex, antibodies to the NDH-F subunit from barely and the NDH-K subunit from pea were used. Preliminary experiments revealed that the NDH-F antibodies recognised a protein of 79 kDa in isolated silverbeet thylakoid membranes, and the NDH-K antibody recognised a 28 kDa in a preparation of extracted thylakoid membrane proteins from silverbeet. In etiolated silverbeet seedlings, a protein of 31 kDa was recognised by the NDH-K antibody. This recognition was lost in the seedlings following exposure to 12 h and

24 h of light. A protein of 33.1 kDa was recognised by the NDH-K antibody in leaves harvested over a 24 hour period, with no notable difference in level of accumulation throughout the day/night periods. During leaf development in white clover, a protein of 69 kDa, which was more prevalent in senescent leaves, was recognised by the NDH-F antibody, while the NDH-K antibody recognised a protein of 35 kDa that was more prevalent in mature, photosynthetically competent leaves. These results are evaluated in terms of providing evidence for the developmental regulation of chlororespiration in chloroplasts of silverbeet and white clover.

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