ELECTRON TRANSPORT BETWEEN PHOTOSYSTEM 1 AND PHOTOSYSTEM 2 AND ESTABLISHMENT OF A TRANS-THYLAKOID ENERGY GRADIENT DURING CHLOROPLAST BIOGENESIS IN THE WHEAT LEAF.

A.N.Webber, N.R.Baker, M.F.Hipkins and C.D.Paige.

INTRODUCTION.

Monocotyledons such as wheat have a basal intercalary meristem and as a result exhibit a progressive series of cell development along the leaf and provide a convenient experimental system with which to examine chloroplast development (Boffey et.al. 1979). When wheat leaves are grown under a diurnal light regime chlorophyll accumulates extremely rapidly in the newly formed cells (Boffey et.al.1980) and primary photochemical activities associated with the two photosystems rapidly appears (Webber et.al. unpublished). Although photochemically active reaction centres, primary chlorophyll antennae of both PS1 an PS2 and excitation energy transfer between the chlorophyll antennae complexes of PS1 and PS2 are evident at early stages of development, it is not known whether electron transfer occurs from water to a terminal electron acceptor via PS2 to PS1 with a concomitant proton pumping and establishment of a proton motive force across the thylakoids. Clearly the ability of the thylakoid to facilitate electron transport from water to NADP with associated proton pumping and an ability to maintain the proton electro-chemical gradient across the thylakoid are essential prerequisites, and possibly limiting factors, for ATP synthesis.

In this paper we examine the ability of developing thylakoids in the wheat leaf to efficiently photo-oxidise water and transfer electrons via PS2 and PS1 to a terminal electron acceptor using oxygen polarography and analysis of chlorophyll fluorescence induction kinetics. Analyses of the flash induced absorption changes of the thylakoids at 520nm are presented to determine the ability of the developing thylakoids to maintain a light-induced electrochemical gradient across their membranes.

MATERIALS AND METHODS.

Seeds of Triticum aestivum var. Maris Dove were washed in running tap water for 17hrs and sown in John Innes No.2 potting compost. Plants were grown at 23^{o}C with a photosynthetically active photon flux density of 250 µmol m^{-2} s^{-1}. First leaves were harvested 8 days after sowing the seed.

Leaf segments, 1cm in length, were homogenised in isolation medium (50mM Hepes,400mM sucrose, 10mM NaCl, 5mM MgCl, 0.2% BSA(w/v), 2% PVP(w/v), pH 7.6) at 4^{o}C, filtered through 5 layers of cheese cloth and 5 layers of 25um nylon mesh prior to centrifugation at 3000g for 2 minutes. The pellet was resuspended in resuspension medium (50mM Hepes, 400mM sucrose, 10mM NaCl, 5mM MgCl, pH 7.6) and stored in the dark at 4^{o}C.

Rates of light-induced oxygen evolution from 1cm leaf segments were determined under a saturating carbon dioxide atmosphere (1200ppm) and

Sybesma, C. (ed.), Advances in Photosynthesis Research, Vol. IV. ISBN 978-90-247-2945-6.

a continuous photon flux density of 1500μmol m^{-2} s^{-1} using a leaf disc polarographic electrode as previously described by Delieu and Walker (1981).

Oxygen evolved from isolated chloroplasts on excitation with a train of saturating flashes, each of 5μs duration, was measured using a method modified from Joliot and Joliot (1968).

Kinetics of chlorophyll fluoresence emission from PS2 were measured as previously described (Dominy, Baker 1980) except that tissue was excited with 440nm light (bandwidth 20nm) produced from a xenon source through a high irradiance monochromator. Light intensities used were chosen to generate maximal variable fluorescence, e.g. 100 and 20μmol photons m^{-2} s^{-1} for the leaf tip and base respectively. Where samples were pretreated with light prior to measurement, leaf segments were either preirradiated for 3 seconds followed by 3 seconds dark or preirradiated for 3 seconds followed by 3 seconds dark and followed by a further 2 second preirradiation with far-red light (710nm).

Kinetics of 520nm absorption changes were measured for isolated thylakoids using the apparatus and method previously described (Musto, Hipkins 1980).

Chloropyhll concentrations were determined by the method of Arnon (1949).

RESULTS.

Oxygen evolution measured from intact leaf segments taken at various distances from the leaf base demonstrated that all developmental stages could sustain light-stimulated oxygen evolution for at least two minutes, after which oxygen evolution decreased in the younger tissue due to lack of terminal acceptors. The oscillations observed in the older tissue were characteristic of oscillations in carbon metabolism (Walker 1981).

Flash-induced oxygen evolution from isolated thylakoids showed the expected result of maximum yield every fourth flash for all stages of thylakoid development that could be measured by this technique. The yields of oxygen evolved per flash decreased markedly towards the base. It should be noted that the flash train technique was not sensitive enough to detect oxygen from thylakoids isolated from the leaf base. However, oxygen could be detected under steady state light conditions with ferricyanide as the terminal electron acceptor from these membranes, thus there is no reason to suspect differences in the patterns of oxygen oscillations upon excitation with the flash train at these early developmental stages.

The effects of pre-irradiation or pre-irradiation followed by an additional far-red irradiation on the kinetics of the fluorescence induction at different stages of development were investigated. The I to D dip in the fluorescence induction curve could be seen at all developmental stages, pre-irradiation enhanced the I to D transition but far-red light was seen to decrease the I to D transition.