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Thermal uncoupling in chloroplasts

During work on carbon assimilation by isolated chloroplasts[1] it was observed that the associated oxygen evolution was appreciably more sensitive to thermal inactivation than earlier studies on the Hill reaction might have suggested[2-4]. Accordingly, spinach or pea chloroplasts were briefly exposed to 50° and their subsequent ability to support oxygen evolution (when illuminated at 15° in the presence of ferricyanide) was tested. When exposure to 50° was limited to 50–60 sec the subsequent rate of oxygen evolution was usually the same or lower than that in the control (containing untreated chloroplasts) and, as anticipated, longer heating led to a progressive decline in activity. Surprisingly, however, chloroplasts subjected to 50° for periods of less than 50–60 sec gave subsequent rates which were substantially increased. The optimal duration (for activation induced by heating at 50°) was about 30 sec and this treatment usually increased the subsequent rate 2–3-fold (Fig. 1, Curve A). It was noted that these heated chloroplasts showed decreased responses to added NH_4Cl and that the rate in its presence was not stimulated by prior heat-treatment but, instead, fell off progressively as the time of heating was extended (Fig. 1, Curve B).

Because of the increased rate, and the decreased response to NH_4Cl we interpret our results as evidence of thermal uncoupling. Conceivably, the physical basis might

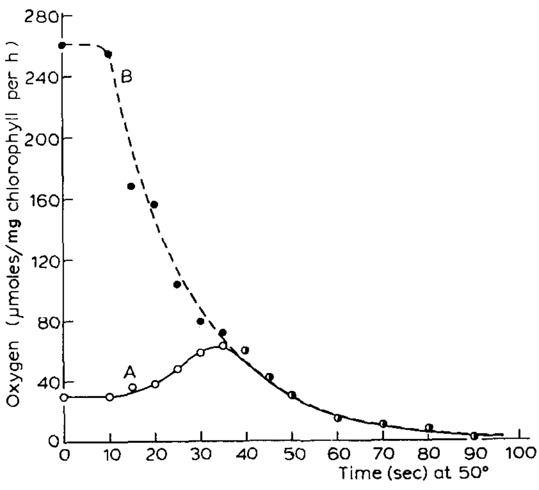

Fig. 1. Chloroplasts were prepared by homogenising spinach leaves in grinding medium containing glucose (0.33 M), orthophosphate (0.01 M, pH 7.5) and $MgCl_2$ (1.0 mM). After centrifugation the chloroplast pellet was resuspended in grinding medium diluted 10-fold and after a second centrifugation the osmotically-shocked chloroplasts were resuspended and assayed in the full strength grinding medium. Oxygen evolution in the presence of ferricyanide was followed simultaneously in two oxygen cathode cells. Prior to assay, 0.2 ml of chloroplast suspension was rapidly rotated in a closed glass tube (carried on a motor driven stirrer) so that it formed a thin film. The tube was then transferred from water at 0° to water at 50° (for the required time interval) and then back to 0°. Control chloroplasts were treated in the same fashion but kept at 0° throughout. Chloroplasts (100 µg chlorophyll in 50–70 µl) were added to assay mixtures (containing 2 µmoles of ferricyanide in 2 ml grinding medium) prior to illumination. The figure shows the rates of O_2 evolution (Curve A) before and (Curve B) after the addition of NH_4Cl (to 10^{-2} M) following pre-heating at 50° for the times indicated.

be a brief liquefaction of a lipid component leading to changes in the permeability of the thylakoid membrane. Heat induced changes in proton effluxes from chloroplasts have been reported by DILLEY AND SHAVIT[5]. Fig. 1 is also remarkably similar to figures published by IZAWA AND GOOD[6] which illustrate the response to methylamine of chloroplasts treated with increasing concentration of triton, digitonin and sodium dodecyl sulphate. This similarity would be explained if the heat treatment, like the detergents, brought about structural changes in the chloroplast membranes.

It seems possible, that in certain circumstances, thermal uncoupling may be of importance even within the temperature range normally used for routine assays. Thus with freshly prepared, well-coupled, spinach chloroplasts we have observed a 14-fold increase in rate following the addition of NH_4Cl at 5° whereas at 25° the stimulation was only 7-fold (in these experiments chloroplasts were added to reaction mixtures at 5° and 25° only one minute prior to illumination and oxygen evolution was measured simultaneously at both temperatures). As the chloroplasts age, following preparation, the degree of coupling decays and this effect becomes progressively less pronounced. With poorly-coupled chloroplasts the response to NH_4Cl (4–5-fold stimulation, or less) was virtually the same at 5° and 25°.

At present much attention is being paid to the stoichiometry of photophosphorylation. If our interpretation is correct and if the effect which we observe applies equally to light driven ATP formation, then (with initially well-coupled chloroplasts) the P/2e ratio could be appreciably affected by the temperature at which it is measured. This would be especially true of assays at relatively high temperatures (20–25°) in which equilibration prior to measurement was prolonged for several minutes.

In photosynthetic carbon assimilation, thermal uncoupling would give rise to an inhibition rather than an acceleration. As such it would contribute to the marked decline in photosynthesis[7] which occurs at temperatures lower than those normally associated with the heat inactivation of respiration and other metabolic processes.

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Received March 17th, 1969