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Light-induced changes of α-tocopherylquinone in spinach chloroplasts

The presence of both α-tocopherol and α-tocopherylquinone in chloroplasts from spinach (*Spinacea oleracea*) and lilac (*Syringa vulgaris*)[1,2] implies that these compounds may function as a redox system in photosynthetic electron transport. In order to investigate this possibility, we have studied the effect of light and certain electron acceptors on the steady-state levels of α-tocopherol and α-tocopherylquinone in spinach chloroplasts. We have not yet found significant changes in the levels of α-tocopherol, but the level of quinone undergoes significant changes.

In order to study these changes, chloroplasts prepared by the method of JAGENDORF AND AVRON[3] are extracted with 80 % acetone after exposure to the experimental conditions. The lipids from the acetone extract are then chromatographed on thin layers of silica gel G using 1 % ethyl ethyl ether in chloroform for development. The region of the chromatogram containing α-tocopherylquinone is scraped off and eluted with ethanol. The change in absorbance of this solution at 262 mμ when treated with potassium borohydride shows the amount of quinone present. A similar procedure has been used to determine changes in plastoquinone A plus B after they are eluted from a different portion of the chromatogram[4]. In order to determine the recovery of total tocopherylquinone, the reduced form (presumed to be the hydroquinone) is converted to quinone by heating a sample of the lipid in isooctane to 60° and bubbling air through the solution for 2 min. This treatment will convert all hydroquinone to quinone, but does not oxidize α-tocopherol to a significant extent.

TABLE I

α-TOCOPHERYLQUINONE IN CHLOROPLASTS

Reaction mixture: 60 mg chlorophyll in fresh chloroplasts, 0.05 M Tris–HCl (pH 8.0), 0.01 M NaCl. Total volume 200 ml in a 2000-ml erlenmeyer flask. Light intensity 1600 ft candles from a 250-W Tungsten bulb at the bottom of the flask shining through 5 cm of water. 7.5 μmoles NADP added as indicated.

Treatment	*α-Tocopherylquinone (no oxidation) (mg)*	*α-Tocopherylquinone (oxidized extract) (mg)*	*Plastoquinones A + B (no oxidation) (mg)*
5 min in the dark	0.025	0.050	1.6
5 min in the light	0.056	0.048	2.2
5 min in the light + NADP	0.015	0.083	1.2
5 min in the light + NADP followed by 5 min in the dark	0.040	0.048	1.5

In the original chloroplast preparation α-tocopherylquinone is partially in the reduced form and this relationship does not change during 5 min incubation in darkness with or without added NADP. Exposure of chloroplasts to light causes oxidation to the extent that all available α-tocopherylquinone appears in the extract. Addition of NADP during incubation in dark does not change the level of α-tocophorylquinone, but incubation in light with NADP causes a decrease. Oxidation of the extract incubated with NADP plus light shows more α-tocopherylquinone than was originally present in the chloroplasts indicating the conversion of a precursor such as α-tocopherol into hydroquinone which then appears as quinone when the extract is oxidized. When chloroplasts are put in the dark after these treatments there is a slow return to the original levels of apparent and total α-tocopherylquinone. An example of these effects is shown in Table I.

These results indicate that the steady-state level of α-tocopherylquinone is increased in light by oxidation of the hydroquinone. In the presence of excess NADP the conversion from quinone to hydroquinone is favored and there is mobilization of additional hydroquinone possibly derived from α-tocopherol In the dark there is a reversal of these reactions in the direction of the original steady-state relationship.

The effective light intensity in these experiments is low because of the large amounts of chloroplasts required to make measurements of α-tocopherylquinone The increase of plastoquinones A and B in dim light is consistant with the previously observed changes which contrast to the reduction of plastoquinones A and B in bright light.

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