RESTORATION OF FERRICYANIDE REDUCTION IN ACETONE-EXTRACTED CHLOROPLASTS BY β AND γ TOCOPHEROL QUINONES

M. D. Henninger, R. A. Dilley and F. L. Crane
Department of Biological Sciences, Purdue University
Lafayette, Indiana

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During our studies of the lipid components of chloroplasts we have found relatively large quantities of α tocopherol as well as smaller amount of β and γ tocopherol (Dilley et al. 1962). We have also found in this laboratory that α tocopherol quinone is present in spinach chloroplasts and that it occurs in significant amounts in leaves of several other species (M. Ashley unpublished). In addition we have recently found in spinach chloroplasts small amounts of two quinones which appear to be identical to β and γ tocopherol quinones. Thus it now appears that spinach chloroplasts contain seven quinones including vitamin K_1 (Kegel and Crane 1962), plastoquinones A, B, and C (Kegel et al. 1962) and α, β, and γ tocopherol quinones.

The functional significance of plastoquinone A (PQA) has already been clearly indicated by the studies of Bishop (1959) and Krogmann (1961). Krogmann and Olivero (1962) have also shown that γ tocopherol quinone (phytyl PQ) will partially replace PQA in restoration of the Hill reaction in heptane extracted chloroplasts. In addition we have preliminary

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evidence that plastoquinones B and C stimulate electron transport in chloroplasts after solvent extraction although their effects vary with the assay system employed. In this note we would like to present evidence that β and γ tocopherol quinones are also of functional significance in the electron transport system of chloroplasts.

Extraction of β and γ tocopherol quinones from chloroplasts was carried out with the propanol heptane solvent mixture previously described (Kegel et al. 1962). The quinones were separated from other lipids by preparative chromatography on thin layer silica gel plates using 15% ethyl acetate in benzene as the developing solvent. In this system the β and γ tocopherol quinones have an R_f slightly lower than plastoquinone C. After extraction of the quinone positive spot from the plate the mixture of β and γ tocopherol quinone can be resolved by re-chromatography on the same thin layer system. The amount of γ tocopherol quinone present is considerably less than the β tocopherol quinone. The purified β tocopherol quinone has a spectrum with a single maximum at 261 mμ and shows the same R_f as pure β tocopherol quinone prepared from β tocopherol. The mixture of quinones shows a similar R_f with a slight separation of the γ tocopherol quinone as is shown by a mixture of the two authentic quinones. The relative amounts of these quinones as compared to other chloroplast quinones is shown in Table I.

Assay for activity in electron transport was carried out by measuring the rate of reduction of ferricyanide in light in the presence of acetone-extracted chloroplasts. Ferricyanide reduction was measured by decrease in absorbancy at 420 mμ. The chloroplasts were lyophylized in absence of sucrose and then

Table I

Relative Amounts of Plastoquinones and Tocopherol Quinones in Spinach Chloroplasts

Quinone	Amount mg/mg chlorophyll
Plastoquinone A	0.079
Plastoquinone B	0.015
Plastoquinone C	0.016
α tocopherol quinone	0.005
β tocopherol quinone	0.0002
γ tocopherol quinone	trace
vitamin K_1	0.008

*Chloroplasts extracted as described (Kegel et al. 1962) it should be noted that the values of plastoquinone C vary considerably depending on the chloroplast preparation. Values represent actual amounts recovered after thin layer chromatography and are somewhat low.

the dry chloroplasts were extracted by shaking with 100% acetone for 90 min. at room temperature. This procedure removes considerable lipid including chlorophyll from the chloroplasts, but sufficient chlorophyll remains to provide a system with a potential activity of 0.03 of the original chloroplasts on a weight basis and as shown in the restored activity more than equal to the original on a chlorophyll basis. When the acetone extract is chromatographed on the thin layer system β and γ tocopherol quinones can be shown to be present.

Restoration of ferricyanide reduction in acetone-extracted chloroplasts by β tocopherol quinone is shown in Table II. The reaction is light dependent and does not occur when the extracted chloroplasts are heated to 100°C for 2 min.

Table II

Restoration of Ferricyanide Reduction in Acetone-Extracted Chloroplasts by β Tocopherol Quinone

Chloroplast preparation	Addition	Ferricyanide reduction μ moles/min./mg chloroph. light	dark
Dried	none	52.9	0.0
Dried	β tocopherol quinone .02 mg	52.0	0.0
Extracted	none	24.5	0.0
Extracted	β tocopherol quinone .02 mg	69.8	0.0
Boiled	β tocopherol quinone .02 mg	0.0	0.0

Assay system: 0.75 μ moles potassium ferricyanide, 0.20 μ moles potassium phosphate buffer pH 7.5 and chloroplasts containing 0.0092 mg chlorophyll in a total volume of 3.0 ml. Additions in 0.04 ml ethanol or less.

Other quinones from chloroplasts can restore activity in the extracted chloroplasts. Plastoquinone A and B and γ tocopherol quinone will restore activity but none are as effective as β tocopherol quinone. It appears that the effects of γ tocopherol quinone and β tocopherol quinone are additive at low levels. At high levels β tocopherol quinone alone produces maximum activity, but γ tocopherol quinone alone (which is the phytyl analog of PQA) will not produce maximum activity. The mixture of β tocopherol quinone with a trace of γ tocopherol quinone produces maximum activity at a low concentration. In addition to the above quinones, coenzyme Q_{10} also shows partial stimulation. α tocopherol quinone and δ tocopherol quinone are inactive. Plastoquinone C and vitamin K_1 are not only inactive but inhibit the residual ferricyanide reduction rate. Other materials which have been tested and found to be inactive are: α, β, γ and δ tocopherols and a carotene preparation from chloroplasts. The activity

of other chloroplast quinones in relation to the activity of β tocopherol quinone is shown in Table III.

Table III

Specificity of Chloroplast Quinones for Restoration of Ferricyanide Reduction in Acetone-Extracted Chloroplasts

Quinone	mg added	Ferricyanide reduction μ moles/ 5 min./mg chloroph.
β tocopherol quinone	0.022	69.8
β tocopherol quinone	0.041	81.5
γ tocopherol quinone	0.024	24.8
γ tocopherol quinone	0.042	53.8
γ tocopherol quinone	0.048	62.1
βTQ + γTQ	0.011 + 0.024	88.6
βTQ + γTQ	0.022 + 0.024	86.1
α tocopherol quinone	0.024	27.7
α tocopherol quinone	0.040	27.8
αTQ + γTQ	0.024 + 0.024	29.4
δ tocopherol quinone	0.040	27.7
plastoquinone A	0.020	50.0
plastoquinone B	0.020	44.0
plastoquinone C	0.020	19.6
coenzyme Q_{10}	0.020	40.8
vitamin K_1	0.020	6.0
PQA + βTQ	0.015 + 0.020	82.8
original dried chloroplasts	no addition	52.9
acetone-extracted chloroplasts	no addition	24.5

Assay as described for Table II using 0.0092 mg chlorophyll. All quinones added in alcohol solution.

From this data it is apparent that the small quantities of β and γ tocopherol quinone found in chloroplasts must be considered as possible functional elements. The additive effects of β tocopherol quinone and γ tocopherol quinone (analogous to PQA) suggest that there are separate sites for function of quinones of the plastoquinone A type and β tocopherol quinone in transfer of electrons to ferricyanide. β tocopherol quinone at high concentration can fulfill the requirement for both types of quinone.

References

Bishop, N.I., Proc. Nat. Acad. Sci. (U.S.), 45, 1696 (1959)

Dilley, R.A., Kegel, L.P. and Crane, F.L., Plant Physiol. 37 suppl. XI (1962)

Kegel, L.P. and Crane, F.L., Nature 194, 1282 (1962)

Kegel, L.P., Henninger, M.D. and Crane, F.L., Biochem. and Biophys. Res. Communs. 8, 294 (1962)

Krogmann, D.W., Biochem and Biophys. Res. Communs. 4, 275 (1961)

Krogmann, D.W., and Olivero, E., Jour. Biol. Chem., 237, 3292 (1962)