PN 1267

The role of α-tocopherylquinone in the electron-transport system of chloroplasts

We have previously demonstrated the presence of α-tocopherylquinone in spinach chloroplasts[1]. It has also been possible to show an increase in the amount of α-tocopherylquinone when chloroplasts are exposed to light and a return to the original level when they are returned to darkness[2]. In order to further demonstrate the role of α-tocopherylquinone in the electron-transport chain of chloroplasts, we have used various solvent extraction procedures to remove α-tocopherylquinone from the chloroplasts. When spinach chloroplasts are lyophylized to a dry powder and extracted with 10 ml of acetone per gram of chloroplasts, α-tocopherylquinone is extracted together with β- and γ-tocopherylquinone and the plastoquinones[1]. After extraction the chloroplasts have lost most of their ability to catalyse the photoreduction of ferricyanide, cytochrome *c* and NADP. When small amounts of α-tocopherylquinone are added back, the photoreduction of cytochrome *c* by the extracted chloroplasts is restored. The restored activity is light dependent, requires the addition of photosynthetic pyridine nucleotide reductase[3] and does not occur if the extracted chloroplasts are heated for 2 min at 100°.

The restoration of cytochrome *c* photoreductase by chloroplast quinones is shown in Table I. The tocopherol quinones are effective at low levels in the range found in the original chloroplasts. We have previously shown that β- and γ-tocopherylquinones are effective for restoration of ferricyanide photoreductase activity and that α-tocopherylquinone is inactive[1]. The cytocnrome *c* reduction is distinguished by the

TABLE I

RESTORATION OF CYTOCHROME *c* PHOTOREDUCTASE ACTIVITY BY CHLOROPLAST QUINONES

Quinone	*Amount added (μmoles)*	*Cytochrome c reduction* μmoles/2 min/mg chlorophyll*
α-Tocopherylquinone	0.00014	1.7
	0.00046	2.7
	0.001	2.8
	0.004	2.7
	0.04	3.5
β-Tocopherylquinone	0.0002	1.2
	0.001	1.8
	0.02	3.2
γ-Tocopherylquinone	0.0002	0.8
	0.0005	2.6
	0.0[illegible]	3.3
Plastoquinone A	0.026	—0.2
Plastoquinone B	0.026	0.4
Plastoquinone C	0.013	1.0
Plastoquinone D	0.013	—0.5
Extracted chloroplasts	no addition	0.8
Untreated dried chloroplasts	no addition	7.9

* Assay as described by KIESTER *et al.*[3]. Quinones added in 0.02 ml ethanol. Chloroplasts containing 0.08 mg chlorophyll used for each assay. Light intensity 4000 ft. candles from a tungsten bulb.

effectiveness of the α-tocopherylquinone which indicates that its site of function is closely related to the transfer of electrons to cytochrome *c* through photosynthetic pyridine nucleotide reductase. Added β- and γ-tocopherylquinone can apparently substitute for α-tocopherylquinone at this site.

Supported in part by a grant number GM-10741 from the National Institutes of Health.

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Received April 6th, 1963

Biochim. Biophys. Acta, 75 (1963) 144–145

PN 1270

Appearance of fluorescence on treatment of histidine residues with *N*-bromosuccinimide

It has been observed that treatment of proteins with *N*-bromosuccinimide (NBS) in phosphate buffer, gives rise to visible fluorescence. The proteins that were tested in this way include horse liver alcohol dehydrogenase (EC 1.1.1.1), bovine serum albumin, ovalbumin, chymotrypsin (EC 3.4.4.5) and RNAase (EC 2.7.7.16)*.

In an effort to find which amino residues are involved in this phenomenon we followed the reaction between all the amino acids present in the above proteins and NBS fluorometrically. NBS was added to each of the amino acids in various mole ratios (from 1 to 12 moles/mole). The reactions were carried out in 0.1 M phosphate buffer (pH 7.4). Under these conditions visible fluorescence was observed only with histidine and tryptophan. Tyrosine and tryptophan lost their characteristic ultraviolet fluorescence after being reacted with NBS.

The reaction between NBS and histidine has been studied in detail and some of the results are presented here. Two distinct fluorescent products are formed with different fluorescence excitation and emission spectra. The optimum mole ratio of NBS to histidine for fluorescence appearance is different for these two products. One type (emission maximum at 540 mμ) is formed predominantly when four moles NBS are used. The fluorescence appears within 1–2 min. The other type (emission maximum at 460 mμ) predominates when two moles NBS are used and over 20 min elapse before the maximum fluorescence is formed. The fluorescence excitation and emission spectra of these two products are shown in Fig. 1. It is the second of these products (emission maximum near 460 mμ) which is seen when proteins are reacted with NBS.

Abbreviation: NBS, *N*-bromosuccinimide.

* The finding of visible fluorescence with horse liver alcohol dehydrogenase and NBS confirms unpublished results of L. BRAND AND N. O. KAPLAN.