Journal of Plant Physiology **165** (2008) 1238—1247

Available online at www.sciencedirect.com

ScienceDirect

JOURNAL OF PLANT PHYSIOLOGY

www.elsevier.de/jplph

# Tocopherol quinone content of green algae and higher plants revised by a new high-sensitive fluorescence detection method using HPLC – Effects of high light stress and senescence

**Jerzy Kruk[a,*], Renata Szymańska[a], Karin Krupinska[b]**

[a]*Department of Plant Physiology and Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland*
[b]*Institute of Botany, Christian-Albrechts-University of Kiel, Olshausenstr. 40, 24098 Kiel, Germany*

Received 3 March 2008; received in revised form 28 March 2008; accepted 30 March 2008

**KEYWORDS**
HPLC;
Light stress;
Senescence;
Tocopherol;
Tocopherol quinone

## Summary

A rapid, sensitive fluorescence method was applied here for detection of oxidized tocopherol quinones in total plant tissue extracts using HPLC, employing a post-column reduction of these compounds by a Zn column. Using this method, we were able to detect both $\alpha$- and $\gamma$-tocopherol quinones in *Chamydomonas reinhardii* with a very high degree of sensitivity. The levels of both compounds increased under high light stress in the presence of pyrazolate in parallel to a decrease in the content of the corresponding tocopherols. The formation of tocopherol quinones from tocopherols was apparently due to their oxidation by singlet oxygen, which is formed in photosystem II under high light stress.

$\alpha$-Tocopherol quinone was also detected in a variety of higher plants of different age, and its level was found to increase during senescence in leaves grown under natural conditions. In contrast to $\alpha$-tocopherol quinone, $\gamma$-tocopherol quinone was not found in the higher plant species investigated with the exception of young runner bean leaves, where the levels of both compounds increased dramatically during cold and light stress.

Taking advantage of native fluorescence of the reduced $\alpha$-tocopherol quinone ($\alpha$-tocopherol quinol), it can be detected in plant tissue extracts with a high

*Abbreviations*: Chl, chlorophyll; DMPBQ, dimethyphytylbenzoquinol; HPLC, high-performance liquid chromatography; $\alpha$-Toc, $\alpha$-tocopherol; TQ, tocopherol quinone; $TQH_2$, tocopherol quinol.

*Corresponding author. Tel.: +48 12 664 6361; fax: +48 12 664 6902.
*E-mail address:* jkruk@mol.uj.edu.pl (J. Kruk).

0176-1617/$ - see front matter 
doi:10.1016/j.jplph.2008.03.006

sensitivity. In young runner bean leaves, α-tocopherol quinol was found at a level similar to α-tocopherol.


## Introduction

α-Tocopherol quinone (α-TQ) has been known for years as a minor constituent of all oxygenic photosynthetic organisms that are able to perform biosynthesis of α-tocopherol (α-Toc). It has been identified in green plant tissue from various plant species and taxonomic groups, usually at a proportion between 1 and 2 moles per 100 moles of chlorophyll (Chl) (Lichtenthaler and Park, 1963; Lichtenthaler and Calvin, 1964; Bucke et al., 1966; Lichtenthaler 1968a; Kruk and Strzałka, 1995). The presence of α-TQ, in addition to α-Toc, has also been reported in non-green plant tissue (Lichtenthaler, 1968b, 1969a), as has their accumulation during chromoplast development (Lichtenthaler, 1969b) and during senescence of leaves (Lichtenthaler, 1971). Within the chloroplast, α-TQ was found as an integral component of thylakoids, where it has been recognized as a component of both photosystems (Lichtenthaler, 1969c), as well as in osmophilic plastoglobuli of the plastid stroma (Lichtenthaler and Sprey, 1966) and in chloroplast envelopes (Lichtenthaler et al., 1981). α-TQ has also been found to be an insect-feeding stimulant on the leaf surface of *Populus* (Lin et al., 1999).

The presence of α-TQ in plants and cyanobacteria has been primarily attributed to the antioxidant action of α-Toc, whereby α-TQ is formed as a result of α-Toc oxidation by reactive oxygen forms. Such a reaction was shown to occur both *in vitro* (Neely et al., 1988; Kaiser et al., 1990; Yamauchi et al., 1996) and *in vivo* (Ruggeri et al., 1985; Yamauchi, 2003; Kruk and Trebst, 2008). While in most studies α-TQ is regarded just as a product of tocopherol oxidation without any specific function, several other studies have indicated that this prenylquinone interacts specifically with photosystem II components: cytochrome $b_{559}$ (Kruk et al., 2000; Burda et al., 2003) and non-heme iron (Burda et al., 2003). It has been shown in these studies that α-TQ shows a photoprotective effect on photosystem II by dissipation of excess absorbed light energy due to stimulation of cyclic electron transport around PSII (Kruk et al., 1997a, b) and due to excitation energy quenching (Kruk et al., 2000). In accordance with this view, increased α-TQ accumulation was observed in water-stressed plants associated with decreased PSII efficiency (Munné-Bosch et al., 2005; Müller et al., 2006).

In addition to oxidized α-TQ, the reduced form, α-tocopherol quinol (α-$TQH_2$), has also been found in young broad bean leaves (Bucke et al., 1966). In many studies, α-$TQH_2$ has been shown to scavenge reactive oxygen forms and to inhibit lipid peroxidation (Mukai et al., 1993; Kruk et al., 1994, 1997, 2003; Neužil et al., 1997; Shi et al., 1999). It has an even stronger antioxidant action when compared with tocopherols and ubiquinol. It has also been shown that α-TQ may form charge-transfer complexes with its reduced form *in vitro* and likely also with other phenolic prenyllipids (reduced plastoquinone, α-Toc) present in thylakoid membranes (Kruk, 1988a, b). Moreover, it has been suggested that α-TQ participates as hydrogen acceptor in fatty acid desaturation in human (Infante and Huszagh, 2001).

It is still an open question whether, in some plants, α-TQ can be synthesized *de novo* independently of tocopherol biosynthesis. This seems to be the case for many bacteria and yeast strains where α-TQ and its reduced form were identified (Hughes and Tove, 1982), and which are not able to synthesize tocopherols. In the rumen bacterium *Butyrvibrio fibrisolvens*, α-$TQH_2$ was suggested to take part in the unique reaction of biohydrogenation of linoleic acid (Hughes and Tove, 1980).

Most studies of α-TQ/α-$TQH_2$ content in leaves were performed years ago using thin-layer chromatography, when sensitive and more reliable high-performance liquid chromatography (HPLC) analysis methods were not available. Therefore, the data concerning TQ content in plants tissues and microorganisms require verification with state of the art HPLC methods. However, due to its very low content in plant tissues and possible interference with other compounds, a precise determination of TQs content by HPLC in combination with UV-absorption detection is still difficult. Sensitive florescence detection methods cannot be applied directly since TQs, unlike tocopherols and reduced prenylquinones (Kruk et al., 1993, 2006), are not fluorescent.

In order to enhance sensitivity of TQs determination in plants, we applied a HPLC method where these compounds, after chromatographic separation, were reduced to fluorescing TQHs on a Zn column according to the method used for TQs determination in human serum (Pollok and Melchert, 2004). Moreover, a system was developed for direct determination of α-$TQH_2$ in leaves