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# REDOX POTENTIAL OF PLASTOQUINONE A IN SPINACH CHLOROPLASTS

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## SUMMARY

The redox potential of plastoquinone A in spinach chloroplasts was determined. The midpoint potential of the quinone is about +80 mV at pH 7.0 with an *n* value of 2. The pH-dependence of the potential is −30 mV per pH between pH 4.0 and 5.7, and −60 mV per pH between pH 5.7 and 8.0. The change of the slope at pH 5.7 is interpreted as the protonation of the oxidized plastoquinone A.

## INTRODUCTION

Plastoquinone is known to participate in the electron transport between Photosystems I and II, and in the proton transport across the thylakoid membrane (for a review, see ref. 1). Our previous study indicated that the quinone was also an indispensable component of the oxidizing side of Photosystem II [2].

The redox potentials of plastoquinones A and C dissolved in a mixture of light petroleum and ethanol were reported to be +115 mV and +55 mV, respectively [3]. The present study shows that the redox potential of plastoquinone A in the thylakoid membranes of chloroplasts was about +80 mV at pH 7.0.

## MATERIALS AND METHODS

Chloroplasts were isolated from spinach leaves. Depetioled leaves were ground in 0.05 M Tris buffer (pH 7.6) containing 0.2 M sucrose and 0.01 M $MgCl_2$ in a blender. The homogenate was filtered through two layers of gauze and was centrifuged at $100 \times g$ for 1 min. The supernatant was centrifuged at $1000 \times g$ for 7 min. The pellet obtained was suspended in the isolation buffer, and the low-high speed centrifugation cycle was repeated. The pellet was suspended in the buffer used for the redox titration, and centrifuged at $10\,000 \times g$ for 10 min. The final pellet was resuspended in the same buffer.

Abbreviation: $E_{m7}$, midpoint potential at pH 7.0.

A combination Pt/Ag-AgCl electrode (Horiba, No. 6810-05T) was used to measure the redox potential. The electrode was calibrated with quinhydrone [4]. All redox potentials are reported relative to the standard hydrogen electrode.

The anaerobic titrations of the redox potential were performed at 25 °C in a pear-shaped 50 ml conical vessel with a silicone rubber stopper, which accommodated the electrode, and a gas inlet and outlet. The side-arm was sealed with a septum through which additions could be made and samples withdrawn. Anaerobic conditions were established by bubbling $N_2$ gas continuously through the vessel.

The reaction mixture contained, in 30 ml of 0.05 M potassium phosphate/citrate buffer (pH 4.0 and 5.0) or 0.05 M potassium phosphate buffer (pH 6.0, 7.0 and 8.0), a small amount of antifoam (Dow Corning AF), chloroplasts equivalent to 30 mg of chlorophyll, and the following redox mediators; 0.75 $\mu$mol of 2,6-dichlorophenol-indophenol ($E_{m7}$, +217 mV), 1.5 $\mu$mol of 1,2-naphthoquinone ($E_{m7}$, +135 mV), 1.5 $\mu$mol of 1,4-naphthoquinone ($E_{m7}$, +60 mV), 0.75 $\mu$mol of methylene blue ($E_{m7}$, +11 mV), and 1.5 $\mu$mol of 2,5-dihydroxy-1,4-benzoquinone ($E_{m7}$, −60 mV). The reductant and oxidant were 0.01 M $Na_2S_2O_4$ (in 0.01 M NaOH) and 0.1 M $K_3Fe(CN)_6$, respectively. Aliquots of 1–10 $\mu$l of the oxidant and reductant were added with a microsyringe through a septum on a side-arm. The redox titrations were generally started at the high potential end. 1 ml of chloroplasts suspension poised at a desired potential was withdrawn with a syringe and was injected into a lyophilizing flask immersed in a mixture of solid $CO_2$ and isopropanol (−80 °C). The frozen sample was lyophilized for 4 h, and was extracted three times with light petroleum (30–70 °C), essentially as described in the previous paper [2]. The redox mediators used in the present study were practically insoluble in light petroleum. The combined light petroleum-extract was washed twice with an equal volume of 95 % methanol to remove lipids which interfered the spectrophotometric determination of plastoquinone, and was dried under vacuum at 30 °C in a rotary evaporator. The dried extract was dissolved in 4 ml ethanol. From the difference in absorbance at 255 nm of the ethanol solution before and after reduction with $NaBH_4$, the amount of oxidized plastoquinone was calculated using the oxidized-minus-reduced difference molecular extinction coefficient for plastoquinone [5]. Plastoquinone was assumed to be fully oxidized at the highest potential in one sequence of the titration. Absorption spectra were determined with a Shimadzu spectrophotometer MPS 5000.

## RESULTS

The amount of plastoquinone in the oxidized form in spinach chloroplasts measured as a function of redox potential under anaerobic condition at pH 7.0 is shown in Fig. 1. Each point represents the difference of absorbance at 255 nm before and after reduction with $NaBH_4$ of the ethanol solution prepared from the petroleum ether-extract of spinach chloroplasts poised at the potential indicated. The experimental data fitted well with the theoretical curve based on the Nernst equation for a two-electron redox reaction with the midpoint potential of +82 mV.

The difference spectra (without $NaBH_4$ minus with $NaBH_4$) of ethanol solutions prepared from chloroplasts stabilized at +205 mV and −53 mV at pH 7.0 were shown in Fig. 2 as curves 1 and 2, respectively. A positive peak at 262 nm with a shoulder at 255 nm, a negative peak at 294 nm, and isosbestic points at 232, 283 and

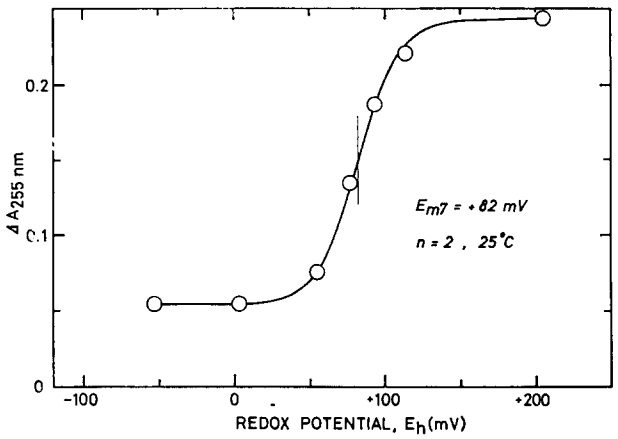


Fig. 1. $NaBH_4$-induced absorbance changes of ethanol solution of petroleum ether-extracts from chloroplasts as a function of the redox potential, at which chloroplasts were poised at pH 7.0 and 25 °C. Circles, experimental data; solid curve, a theoretical curve based on the Nernst equation for two-electron transition with $E_m = +82$ mV.

309 nm found in curve 2 seem to correspond to the spectra of an impure preparation of plastoquinone C [6] possibly contaminated with α-tocopherolquinone, suggesting these quinones are still oxidized at −53 mV, or autooxidized during the extraction. The difference spectrum (curve 3) between curves 1 and 2 showed a positive peak at 255 nm with a shoulder at 262 nm, a negative peak at 290 nm and isosbestic points at

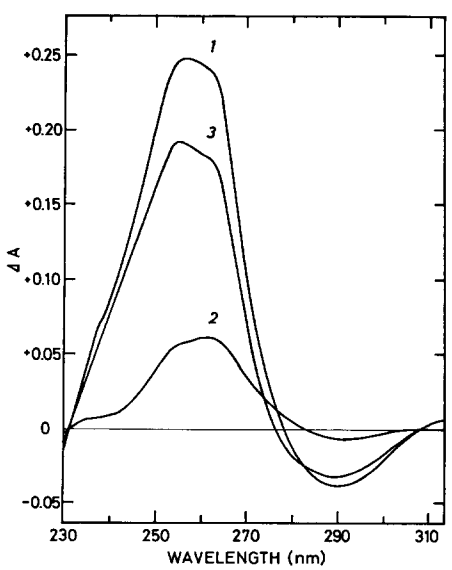


Fig. 2. Difference spectra without $NaBH_4$ minus with $NaBH_4$ of ethanol solutions prepared from chloroplasts stabilized at +205 mV (Curve 1), and at −53 mV (curve 2) at pH 7.0. Curve 3, difference spectrum between curves 1 and 2.

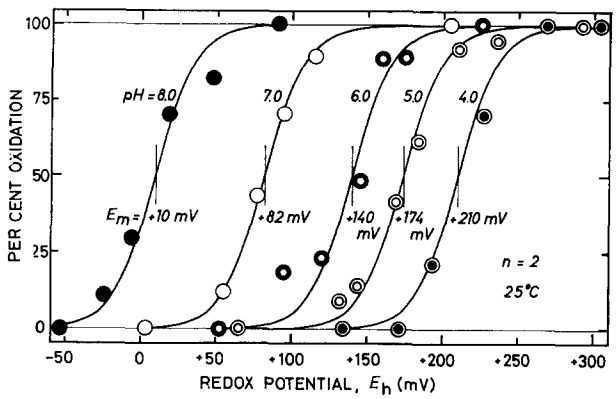


Fig. 3. Redox titration curves for plastoquinone A in chloroplasts at pH 4.0, 5.0, 6.0, 7.0 and 8.0. Circles, experimental data; solid curves, theoretical curves based on the Nernst equation for two electron transition with $E_m$ = +10 mV (pH 8.0), +82 mV (pH 7.0), +140 mV (pH 6.0), +174 mV (pH 5.0) and +210 mV (pH 4.0).

232, 276 and 308 nm. The spectrum corresponds well to the difference spectrum (oxidized-minus-reduced) of pure plastoquinone A [7]. From the difference in absorbance at 255 nm, the amount of this plastoquinone was calculated to be 0.05 mol per mol chlorophyll. Thin-layer chromatography of the hexane extracts showed that the major plastoquinone was an A-homologue. These facts indicate that the response of the redox component shown in Fig. 1 is that of plastoquinone A.

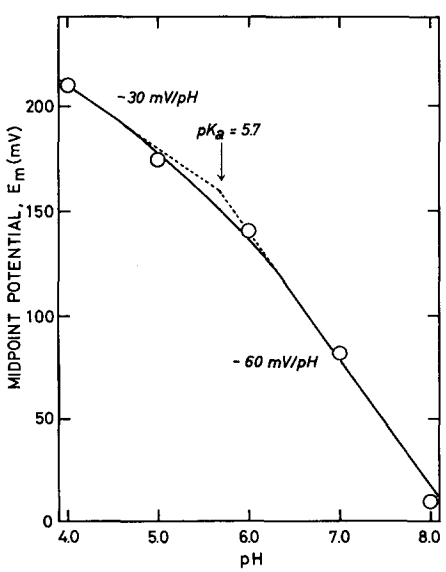


Fig. 4. Midpoint potentials of plastoquinone A as a function of pH. Circles, experimental data; solid curve, a theoretical curve based on the following equation: $E_m = E_0 + 0.03 \log [H^+]^2/([H^+] + K_a)$, where $E_0$ = +330 mV, and $K_a = 2 \cdot 10^{-6}$.

The effect of pH on the redox potential of plastoquinone A is shown in Fig. 3. The midpoint potentials at pH 4.0, 5.0, 6.0, 7.0 and 8.0 were +210, +174, +140, +82 and +10 mV, respectively. The dependence of the midpoint potentials on pH is shown in Fig. 4. The slope of the $E_m$-pH curve changed near pH 5.7 from −30 mV/pH on the lower pH side, to −60 mV/pH on the higher pH side.

## DISCUSSION

As shown in Fig. 4, the slope of the $E_m$-pH curve changed near pH 5.7 from −30 to −60 mV/pH. This change in the slope may be interpreted as indicating the protonation of the oxidized plastoquinone, the p$K_a$ of the process

$$PQH^+ \rightleftharpoons PQ + H^+$$

being 5.7, or the $K_a$ being $2 \cdot 10^{-6}$. $PQH^+$ is the assumed protonated plastoquinone in the oxidized form, PQ is plastoquinone, and $K_a$ is the dissociation constant of $PQH^+$. $K_a$ is defined by the expression:

$$K_a = \frac{[PQ][H^+]}{[PQH^+]}$$

Then the equilibrium between the oxidized and reduced forms of plastoquinone A may be formally represented by the following expression:

$$PQH^+ + H^+ + 2e \rightleftharpoons PQH_2$$

where $PQH_2$ is plastoquinol. Assuming that only $PQH^+$ dissociates in the range of pH from 4.0 to 8.0, the following equation can be derived, which is found to fit well the experimental data:

$$E_m = E_0 + 0.03 \log \frac{[H^+]^2}{[H^+] + K_a}$$

In this equation $E_0$ is a constant which is characteristic of the system and estimated to be +330 mV. The p$K_a$'s of $PQH_2^{2+}$ and $PQH_2$ may be well below 4 and above 8, respectively.

This interpretation that plastoquinone is protonated even in the oxidized form might be interesting in conjunction with the assumed buffering groups which face the inner space of the thylakoids [8]. The proton-dissociation constant of such a buffering group was estimated to be $6.3 \cdot 10^{-6}$ or p$K_a$ = 5.2 [8], a value rather close to the dissociation constant of protonated plastoquinone, $2 \cdot 10^{-6}$ or p$K_a$ = 5.7.

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