# Electron Spin Resonance Study of Chloroplast Photosynthetic Activity in the Presence of Amphiphilic Amines

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**Abstract**. Electron spin resonance spectroscopy (ESR) was used to study the effects of amphiphilic amines of the carbamate, amide, and ester type and amine oxide on the photosynthetic system of spinach chloroplasts. The ESR signal II connected to the photosynthetic center PS II donor side was observed to diminish in the presence of amines, whereas that of PS I remained unchanged. The inhibition of PS II increased with the increasing of amine concentration. In the presence of amines, the light: dark chloroplast ESR signals ratio as well as the intensity of the ESR signal of unbound $Mn^{2+}$ increased. It is suggested that the amphiphilic amines affect the structure of PS II and the electron transfer to PS I. The effects of the amines tested on the photosynthetic system correlate with their potency to perturb the lipid membrane structure.

**Key words**: Photosynthesis — Spinach chloroplasts — Amphiphilic amines — ESR spectroscopy — Membrane perturbation

## Introduction

The study of compounds able to affect photosynthesis is of great importance for understanding of the physico-chemical mechanisms of this process itself. It is commonly accepted that a pair of water molecules are oxidized in the reaction centre PSII accompanied by the release of oxygen. In green plants, PSII is linked to a cluster of 4 $Mn^{2+}$ atoms, and to 18, 24, and 33 kDa extrinsic polypeptides. The 33 kDa polypeptide stabilizes the $Mn^{2+}$ cluster, and its absence in the reaction centre results in a decrease of oxygen production. The 24 and 18 kDa proteins are assumed to act to concentratre $Ca^{2+}$ and $Cl^-$ ions. Cofactors shield the $Mn^{2+}$ clusters from exogenous influences that induce the release of $Mn^{2+}$ from the membrane. The role of $Ca^{2+}$ in PSII is connected with oxygen evolution (Rutherford 1985; Bossac and Rutherford 1980).

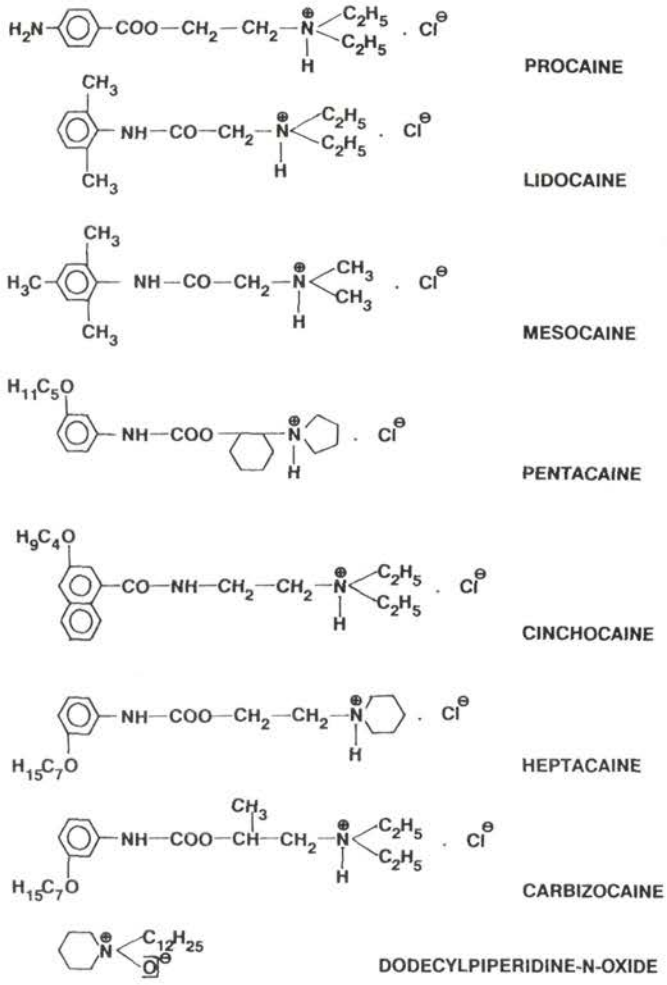


**Fig. 1.** The chemical structures of the amphiphilic amines used.

It is well known that the biological activities of amphiphilic amines (AA) are connected with release of $Ca^{2+}$ ions and with changes of the membrane structure (Račanský et al. 1984; Nosál et al. 1985). Since photosynthetic centers form an integral part of thylakoid membranes, and $Ca^{2+}$ ions are expected to play an important role in the photolytic process (Govindjee 1986) an effect of AA can also be assumed on plant photosynthesis resulting from a decrease of oxygen production. Semin et al. (1987, 1988, 1989) have found that AA of

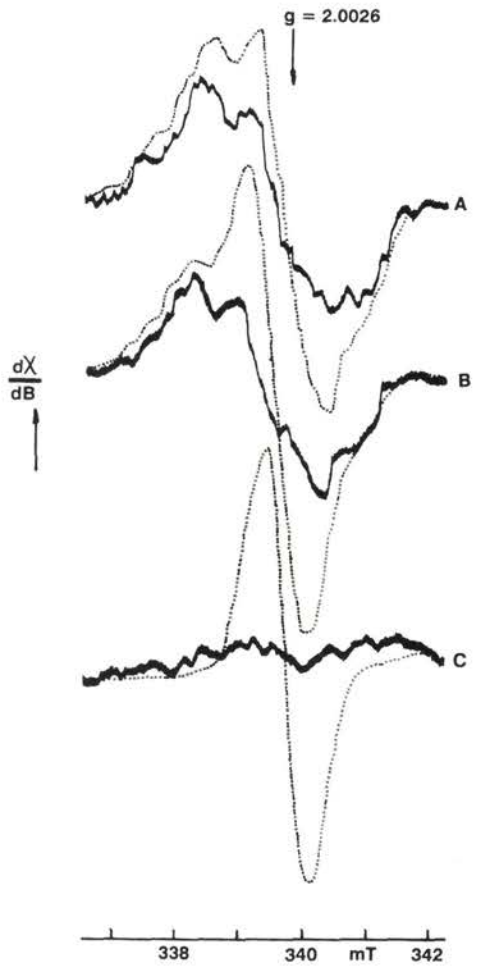


**Fig. 2.** The ESR spectra of chloroplasts at 23 °C in the dark (solid line) and in the light (dotted line). *A* — in the absence of AA; *B* — in the presence of 0.01 mol/l cinchocaine; *C* — in the presence of 0.05 mol/l cinchocaine. The dotted line in spectrum C was recorded at 0.5 amplification.

procaine type act on the photolytic complex. In the present work, the influences of amphiphilic amines on the photosynthetic activity of chloroplasts were studied by the method of electron spin resonance spectroscopy (ESR).

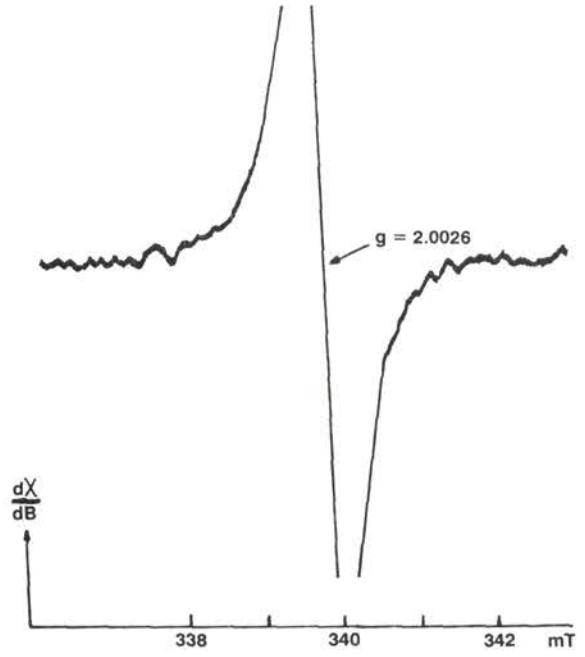


**Fig. 3.** The ESR spectrum of chloroplasts in the presence of 0.05 mol/l heptacaine at −170 °C in the light. The spectrum was recorded at the same amplification as spectra *A* and *B* in Fig. 2.

## Materials and Methods

The chloroplasts were isolated from fresh spinach leaves by destruction in an ice cooled homogenizer (2 × 30 s) with a buffer containing 0.4 mol/l sucrose, 20 mmol/l Tris, 0.2 mmol/l $MgCl_2$. The green juice was filtrated through 8 layers of nylon cloth and a 10 mm layer of cotton wool. Chloroplasts were precipitated by centrifugation at 5000 × *g* for 5 min, and the pellet was resuspended in the original buffer at the concentration of about 5 mg Chla/ml. To keep the samples active over prolonged periods, they were immersed in liquid nitrogen and frozen in original buffer supplemented with 10% glycerol. No decrease of activity could be detected. The concentration of chlorophyll a was determined according to Arnon (1949). To detect ESR activity, the particular amount of AA was added to the sample immediately before the measurement.

An ESR spectrometer type ERS 230 (ZWG AdW, Berlin, GDR) working in the X-band was used in the study. The samples having a concentration of approx. 5 mg Chla/ml were placed into flat cuvettes. The samples were irradiated directly in the resonator using a 250 W halogen lamp. Forty mm wide water filter was used to remove the infrared component of the light. ESR spectra were recorded using the modulation amplitude of 0.5 mT and a microwave power of 5 mW.

The chemical structures of the AA compounds used are shown in Fig. 1.

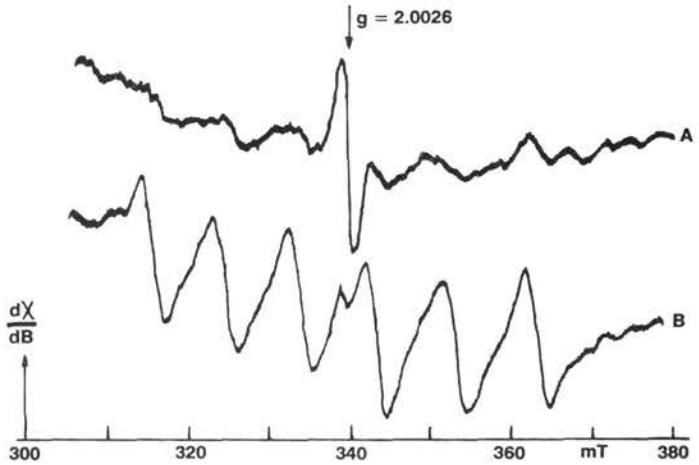


**Fig. 4.** The ESR spectra of $Mn^{2+}$ ions in intact chloroplasts (spectrum A) and in the presence of 0.05 mol/l cinchocaine (spectrum B).

## Results and Discussion

Fig. 2. presents ESR spectra of chloroplasts in the region of $g \approx 2$ measured in dark as well as after irradiation. In both cases the spectra, represent superposition of signals I and II. Signal I characterized by $g = 2.0026$ and $\Delta B = 0.72$ mT pertains to the photosynthetic centrum PS I, particularly to the dimer of Chla (Hoff 1979; Hoff 1987). Signal II with $g = 2.0045$ and $\Delta B = 2$ mT probably corresponds to the semiquinone cation radical of photosynthetic centrum PS II (Govindjee 1986; Hoff 1987). From Fig. 2 it is evident that the light: dark signal intensity ratio is approx. 1.7 for pure chloroplasts, while being considerably higher for chloroplasts upon interaction with AA. Moreover, Fig. 2 shows that upon increasing the concentration of AA, signal II intensity decreases and that of signal I (corresponding to PS I) increases. For some AA (heptacaine, pentacaine, cinchocaine, carbizocaine) signal II completely dissapears upon illumination as well as in the dark at concentrations $\geq 0.05$ mol/l, whereas the signal corresponding to PS I is very intensive upon illumination.

As a rule, upon the decreasing the temperature intensities of signals I and II increase. Fig. 3 illustrates the spectra recorded at $-170$ °C. It is evident from this Figure that even at very low temperatures, no signal II was detected for chloroplasts treated by AA whereas the intensity of signal II was increased for pure chloroplasts (not shown).

For chloroplasts affected by inhibitors of the 3-(3′,4′-dichlorophenyl)-1,1-

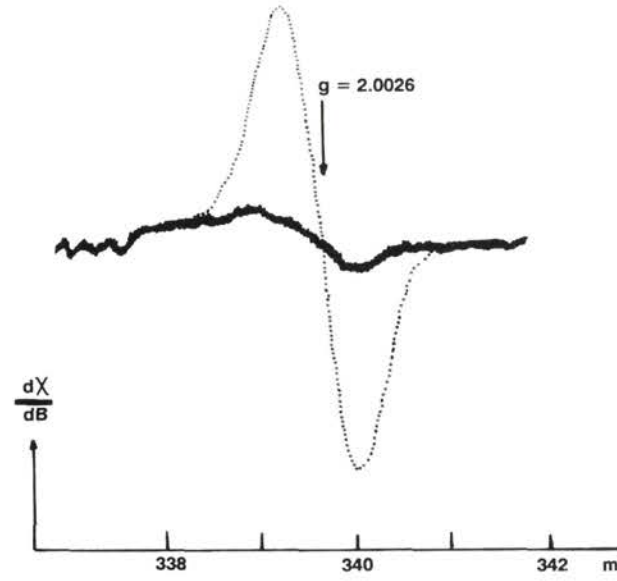


**Fig. 5.** The ESR spectra of chloroplasts in the presence of 0.05 mol/l dodecylpiperidine-N-oxide at 23 °C in the dark (solid line) and in the light (dotted line, amplification 0.5 ×).

dimethylurea type the increase of the PS I signal intensity has been interpreted as caused by the interruption of electron transfer chain between PS II and PS I (Hoff 1979). In the studied case, i.e. in chloroplasts treated by AA, the situation is similar but now AA probably block completly the activity of PS II (absence of signal II). Consequently, the electron transfer from PS II to PS I is unabled and this is manifested by an enormous intensity increase of the PS I signal.

Inactivation of PS II is connected with an increase in the intensity of ESR signal of $Mn^{2+}$ ions which are released from the 33 kDa protein on the PS II donor side into the interior of thylakoid membranes (Blankenship and Sauer 1974; Cheniae 1980). The effect of cinchocaine on $Mn^{2+}$ ions release is shown in Fig. 4. It is clearly seen that in the presence of amine the intensity of the ESR signal of released ions increases significantly. Similar intensity increases were observed for AA with strong inhibition effects on PSII (pentacaine, heptacaine, carbizocaine, dodecylpiperidine-N-oxide); AA with weak inhibition effects on PSII (procaine, lidocaine, mesocaine) induced but insignificant by increases of signal intensity.

The AA action on PSII can be interpreted in two ways:

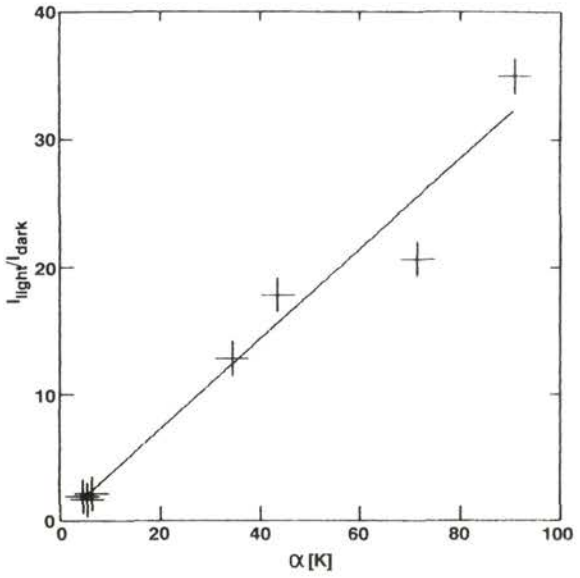


**Fig. 6.** Correlation of the inhibition effect of AA (at a final concentration of 0.05 mol/l in the sample) on PS II activity (expressed as the $I_{light}/I_{dark}$ ratio, where $I$ is the intensity of the corresponding ESR signal) with a decrease of the gel-liquid crystal phase transition temperature expressed by parameter $\alpha = \Delta T_c/\Delta$ ([AA]/[DPPC]). The values of $\alpha$ were taken from the paper of Račanský et al. (1984).

1. The decrease of PS II activity can be due to the incorporation of AA molecules into the thylakoid membrane with resulting perturbation the membrane structure and induction of conformational changes of PS II proteins leading to $Mn^{2+}$ and $Ca^{2+}$ release.

2. The decrease of PS II activity can be connected also with the binding of charged AA molecules, thus changing the electrostatic potential of the membrane surface; the result is release of $Ca^{2+}$ and $Mn^{2+}$ ions that are important components of PSII proteins.

To test the second possibility mentioned the effects of nonionic AA (dodecylpiperidine-N-oxide) on the inhibition of signal II were studied. The effects were principially the same as those of charged AA (Fig. 5). Owing to this the effects of electrostatic changes of the membrane surface could be excluded.

In our previous papers we have found the amphiphilic amines studied to perturb the structure of model and biological membranes (Národa et al. 1983; Ondriaš et al. 1983; 1984; Nosál et al. 1985; Ondriaš et al. 1987a; 1987b; Šeršeň et al. 1989). A convenient parameter characterizing the membrane perturbation is the change in the gel — liquid crystal phase transition temperature $T_c$ in

dipalmitoyl phosphatidylcholine (DPPC) model membranes expressed as $\alpha = \Delta T_c/\Delta$ ([AA]/[DPPC]), where [AA]/[DPPC] is the molar ratio of AA and DPPC (Račanský et al. 1984). As clearly seen in Fig. 6 the effect of AA on PS II correlates well with this parameter ($r^2 = 0.96$). This finding supports the explanation of the effect of AA on PSII as being due to membrane perturbation.

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