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Site-directed mutagenesis of Thr A739 of photosystem I in *Chlamydomonas reinhardtii* alters significantly the excitonic and electronic coupling of the primary electron donor P700

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Introduction

Photosystem I of plants, algae, and cyanobacteria is a membrane-bound pigment-protein complex that mediates light-induced electron transfer from plastocyanin or cytochrome c_6 on the lumenal side to ferredoxin on the stromal side. In plants and algae it is composed of 13 subunits and associated with a light-harvesting complex (LHC-1). PsaA and PsaB each consisting of 11 transmembrane helices form the heterodimeric core and coordinate most of the cofactors involved in the electron transfer process and the main part of the antenna system with about 100 chlorophylls and 20 β-carotenes. Only the terminal electron acceptors F_A and F_B (two [4Fe-4S] iron-sulfur clusters) are bound by one of the extrinsic subunits on the stromal side, PsaC. After absorption of light by an antenna pigment, the excitation energy is transferred to the primary electron donor P700, an excitonically coupled dimer comprised of chlorophyll *a* (P_B) and *a´* (P_A). P700 being in the lowest excited singlet state donates an electron to the primary electron acceptor A_o, a chlorophyll *a* monomer. Charge stabilization is achieved by subsequent electron transfer to secondary acceptors, the phylloquinone A_1 and F_x, a [4Fe-4S] iron-sulfur cluster and finally to F_A and F_B.

The oxidation midpoint potential of the primary donors in photosynthetic reaction centres plays an important role in the initial redox reactions. In general, the physicochemical properties of the Chls are determined by the π-system of their macrocycles. The properties are modified by pigment-pigment- and pigment-protein-interactions. The interaction with the protein takes place via the coordinating ligands and hydrogen bonding with the peripheral substituents of the Chls. The coordinating ligands exert a specific influence on the redox cofactors´ properties but as they are identical for many cofactors, the wide range of midpoint potentials can hardly be explained by metal coordination. It is very likely that the potentials are modulated by other protein-cofactor interactions, e.g. hydrogen bonding. Studies with genetically modified bacterial reaction centres have shown that hydrogen bonding of the protein to the carbonyl group of the 2-acetyl and the $13^{(1)}$-keto group which are both part of the conjugated π-system has a significant influence on the electronic structure and the redox potential (Mattioli et al., 1995; Rautter et al., 1995).

From inspection of the crystal structure of *Synechococcus elongatus* (Jordan et al., 2001), we find T A739 to be a putative hydrogen bond donor to the $13^{(1)}$-keto group of P_A of P700. We assume that this is also the case for *C. reinhardtii* as this residue is highly conserved among different species. The coordinating ligands of P700 of PSI are provided by His from PsaA (His A676) and PsaB (His B656).

We constructed TV A739 in *C. reinhardtii* to remove the hydrogen bond to the $13^{(1)}$-keto carbonyl of P_A and analyzed the exchange of Thr against Val on the optical spectra, the oxidation midpoint potential, the spin density distribution and the parameters of the triplet state.

Materials and Methods

Site-directed mutagenesis was performed according to the altered sites mutagenesis protocol (Promega, Heidelberg, Germany). For reintroduction of *psaA-3* we used pKR154 which was a kind gift of Dr. K. Redding. Cells were grown heterotrophically on TAP or HSA. Cell harvest and thylakoid preparation was carried out as described earlier (Krabben et al., 2000). Isolation of the PSI complex was performed according to (Hippler et al., 1997) with slight modifications. Optical spectroscopy, redox titrations, ENDOR and ODMR have been performed as described earlier (Krabben et al., 2000).

Results and Discussion

Optical spectroscopy at room temperature and 77 K

Absorbance difference spectra were recorded to investigate the optical features of the primary electron donor.

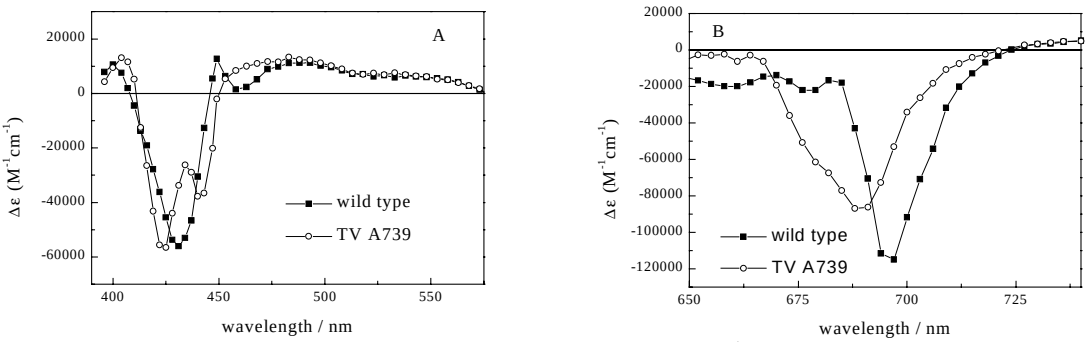
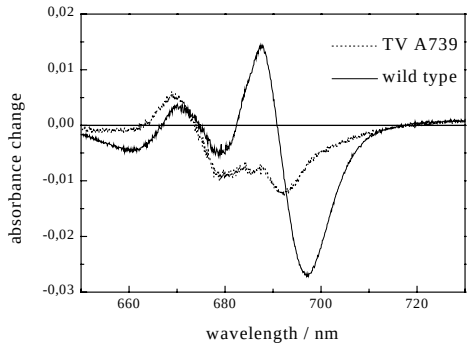


Fig. 1A and B: Flash-induced absorbance difference spectra of $P700^+$/P700 of PSI complexes measured at room temperature.

The absorbance changes due to the oxidation of P700 were monitored as a function of wavelength. Samples were excited by saturating Xe laser flashes of about 15 µs duration. The molar extinction difference coefficient was determined for WT ($\Delta\varepsilon_{697nm}$ = 115000 $M^{-1}cm^{-1}$) and TV A739 ($\Delta\varepsilon_{688nm}$ = 87000 $M^{-1}cm^{-1}$) from the flash-induced absorption change of N,N,N,N-tetramethyl-p-phenylen-diamin hydrochlorid (TMPD).

In the Soret region, two exponentials are required to fit the flash-induced absorbance changes. The first phase can be attributed to the reoxidation of FeS^- while the second phase is due to the reduction of $P700^+$. To obtain the pure $P700^+$/P700 spectrum for wild type and TV A739 mutant only the amplitudes of the second phase are depicted as a function of wavelength (fig 1A). Compared to wild type with the main bleaching located at 431 nm, the main bleaching band of TV A739 is displaced to 425 nm and a second bleaching appears at

440 nm. The difference spectra in the Q_y region are shown in fig 1B. The main bleaching band of the wild type is located at 697nm (while another very small band is observed at about 679 nm). In TV A739 the main bleaching is shifted to the blue by 9 nm and a shoulder appears at 679 nm. Comparison with mutant bacterial reaction centres shows that the introduction of a hydrogen bond at the $13^{(1)}$-keto groups leads to a red shift of the Q_y-absorption band (Williams et al, 1992; Mattioli et al, 1995). The blue shift of the main bleaching band could be the first hint that the hydrogen bond to the $13^{(1)}$- keto group of P_A is broken. The breakage of this hydrogen bond presumably leads to a blue shift of the Q_y absorption band of P_A and as a consequence to a blue shift of the low-energy exciton band of P700. Additionally, changes in the geometry of the dimer may contribute to the observed spectral changes.



The light-minus-dark absorbance difference spectra at 77 K (fig. 2) were obtained by subtracting the spectra in the dark adapted state of those after illumination by 20 saturating Xe flashes. The spectra are normalized to the same bleached area between 600 and 720 nm.

Fig. 2: Light-minus-dark-difference spectra of PSI complexes at 77 K. The curves were obtained by subtracting the absorbance spectra in the dark adapted state of those after illumination by 20 saturating Xe flashes. The spectra are normalized to the same bleached area between 600 and 720 nm.

The spectra are normalized to the same bleached area between 600 and 720 nm. For TV A739, the main bleaching band is displaced 6 nm to the blue and, probably as a consequence, the increase at 687 nm is no longer visible. This is another evidence for a change in the excitonic coupling of the dimer.

Triplet-minus-singlet (T-S) difference spectra

The T-S absorbance difference spectra show again the change in the excitonic interaction between the two Chls constituting P700. The minimum of the main bleaching band which has been assigned to the disappearance of the low-energy excitonic band upon triplet formation is blue-shifted by about 6 nm for TV A739 in comparison to WT. The zero-field-splitting parameters $|D|$ and $|E|$ of the mutant are almost unchanged. This indicates that the distribution of the triplet state is significantly altered in the mutant.

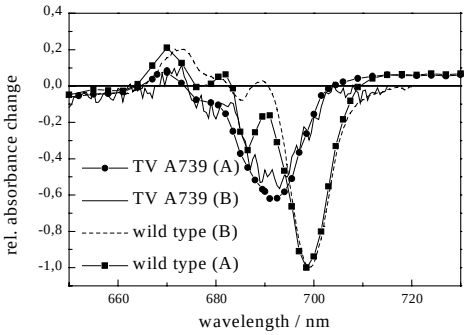
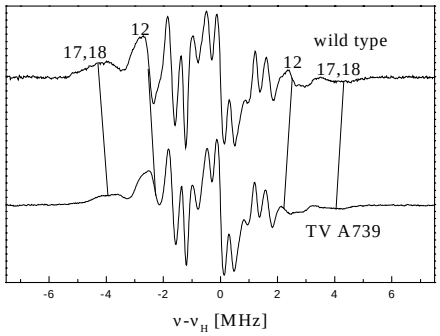


Fig. 3: T-S spectra of PSI. (A) Flash-induced T-S spectra, (B) ADMR monitored T-S spectra (for experimental set-up, see Carbonera et al., 1997; experimental conditions: T=1.8K, 735 MHz microwave frequency, 500 mW microwave power, 33 Hz modulation frequency).

Oxidation midpoint potential of $P700^+$/P700

P700 is the first redox component in the electron transfer chain of PSI. Therefore, its midpoint potential is an important property and assumed to be sensitive to changes in the environment of P700. To determine the oxidation midpoint potential of P700, the flash-induced absorbance change at 826 nm, associated with oxidation of P700, was measured as a function of the redox potential. The data were fitted using the one-electron Nernst equation. The midpoint potential of the mutant TV A739 is decreased by 32 mV compared to wild type (469 ± 6 mV).

Electron nuclear double resonance spectroscopy of $P700^{+\bullet}$



ENDOR spectroscopy was used to characterize the electronic structure of $P700^{+\bullet}$ by resolving the individual proton hyperfine couplings (hfcs). In comparison to WT, the hfcs of the methyl protons at position 12 of the spin carrying Chl P_B are decreased for TV A739 by about 0.5 MHz (A_{iso} (WT) = 5.32; A_{iso} (TVA739) = 4.85). A similar shift is observed for the β-protons (pos. 17, 18) but for the methyl groups at position 7 and 2 the decrease of the hfcs is close to the error limit. The decrease of the hfcs might indicate a spin density shift from P_B to P_A.

Fig. 4: ENDOR spectra of $P700^{+\bullet}$. Measurements were performed as described before (Krabben et al., 2000).

From our results we conclude that the hydrogen bond between T A739 and the $13^{(1)}$-keto group of P_A is removed in the mutant TV A739. Introduction of a hydrogen bond at the analogous position of T A739 in the bRC mutant LH M160 leads to an increase of the redox potential and a more asymmetric spin density distribution compared to wild type. Krabben et al have demonstrated that the positive charge and the unpaired electron of $P700^+$ are mainly localized on P_B. If we assume a dimer model for P700 similar to the bRC with energetically different orbitals for P_A and P_B, breakage of the hydrogen bond between T A739 and the $13^{(1)}$-keto group of P_A would increase the energy of the highest occupied π-orbital (HOMO) of P_A leading to a more symmetric charge distribution and a shift of the spin density towards P_A. At the moment, the decrease of the hfcs of the 12-methyl group and the 17/18 β-protons could be an indication for such a spin density shift, nevertheless, a redistribution of spin density within the macrocycle of P_B cannot be excluded. Another consequence of this model would be the destabilization of the neutral state of P700 compared to the oxidized state and a decrease of the $P700/P700^+$ midpoint potential which is confirmed by our results.

Acknowledgements

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References

Carbonera, D., Giacometti, G., and Agostini, G. (1997) FEBS Lett. 343, 200-204

Hippler, M., Drepper, F., Farah, J., and Rochaix, J.-D. (1997) Biochemistry 36, 6343-6349

Jordan P., Fromme P., Witt H.T., Klukas O., Saenger W., Krauss N. (2001) Nature 411, 909-917

Krabben, L., Schlodder, E., Jordan, R., Carbonera, D., Giacometti, G., Lee, L., Webber, A.N., and Lubitz W. (2000) Biochemistry 39, 13012-13025

Mattioli, T.A., Lin, X., Allen, J.P., and Williams, J.C. (1995) Biochemistry 34, 6142-6152

Rautter, J., Lendzian, F., Schulz, C., Fetsch, A., Kuhn, M., Lin, X., Williams, J.C., Allen, J.P., and Lubitz, W. (1995) Biochemistry 34, 8130-8143

Williams, J.C., Alden, R.G., Murchison, H.A., Peloquin, J.M., Woodbury, N.W., and Allen, J.P. (1992) Biochemistry 31, 11029-1103