THERMOLUMINESCENCE B AND Q BANDS ARE AT THE SAME TEMPERATURE IN AN AUTOTROPHIC AND A HETEROTROPHIC D1 PROTEIN MUTANT OF *SYNECHOCYSTIS* SP. PCC 6803.

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Key words: cyanobacteria, D1 protein, mutants, photosystem 2, thermoluminescence

1. Introduction

The photosynthetic electron transfer chain of D1 protein mutants of the cyanobacterium *Synechocystis* sp. PCC 6803 was studied with thermoluminescence (TL). The mutants have different deletions in the stromal loop between the fourth and the fifth membrane spanning helix of the D1 protein: the so called PEST-like sequence was deleted from PD (Δ(R225-F239)) and both the PEST-like sequence and the putative cleavage region of the D1 protein were deleted from PCD (Δ(R225-V249)). The control strain AR has antibiotic resistance cassetes interrupting *psbA*-1 and *psbA*-3 genes, so that *psbA*-2 is the only active D1 gene in all strains [1].

The control strain AR and the PD mutant strain grow autotrophically whereas the PCD mutant is heterotrophic. All strains are able to evolve oxygen at considerable rates if supplied with a suitable artificial electron acceptor. Reoxidation of Q_A^- is much slower in the mutants than in the control strain [1].

2. Materials and methods

2.1 Research material

The cyanobacteria were grown in liquid culture in BG-11 medium supplied with appropriate antibiotics [1] and 5 mM glucose under 40 μmol m^{-2} s^{-1} PPFD, at 30°C. Oxygen evolving thylakoids were isolated as described [2]. TL was measured from thylakoid samples using a commercial instrument (DSTMI1, A & A Overseas Co.). The sample (0.3 ml, 100μgChl/ml) was dark adapted for 5 min. before cooling to -80°C. One single turnover flash was given during the cooling at -10°C. TL emission was recorded during heating from -80°C to 80°C. The heating rate was 25 °C/min.

2.2 Thermoluminescence measurements

The TL glow curve contains information of thermodynamic properties of different components acting during photosynthetic electron transfer. At least three different models for explaining the form of the TL glow peak are available [3, 4, 5]. The first order model [4] is based on the generalized Randall-Wilkins theory. DeVault *et al.*

G. Garab (ed.), Photosynthesis: Mechanisms and Effects, Vol. II, 1145–1148.

[3] introduced a model in which reactions occuring before the final recombination step are taken into account in order to explain the unphysically large values of the frequency factor s and the activation energy ΔE obtained from the first-order analyses.

The general-order model [5] takes into account the possibility that the recombining charge pairs may undergo other "retrapping" reactions (e.g. triplet formation) in addition to the recombination. These retrapping reactions are governed by the order parameter b [5]. In the first-order model the value b is 1 (no retrapping). A b value of 2 would mean that retrapping and trapping occur with the same probability. The general-order model yields usually lower ΔE values for photosynthetic glow peaks than the other two models, and the values calculated for the frequency factor s are physically sound [5].

We used both the first order model and the general order model to analyze TL curves recorded from AR control strain and the two mutants. The thermodynamic parameters were determined with a stochastic curve fitting method using Matlab. The glow curves were fitted to a sum of the minimum number of component curves described by either the first-order or the general-order model.

3. Results

TL measurements from isolated thylakoids of the control strain (AR) in the absence of 20 μM DCMU showed a B band (Fig. 1A, solid line) arising from $S_2Q_B^-$ recombination. The peak position was 35°C, in agreement with earlier measurements from the same strain [6]. In the presence of 20 μM DCMU the AR control strain showed a TL glow curve peaking at 14°C corresponding to $S_2Q_A^-$ recombination (Fig 1B, solid line).

In contrast to the control strain AR the mutants PD and PCD did not have a band at 30 - 40 °C (Fig. 1). Glow curves recorded from the two mutants both in the presence and absence of 20 μM DCMU resembled curves of the control strain recorded in the presence of DCMU but had slightly higher peak temperatures (Fig. 1B). Addition of DCMU shifted the peak position to a slightly higher temperature in the autotrophic mutant PD (Fig. 1B, dashed line).

Multiple peaks were required for a good fit to both first-order and general-order equations, but all general order fits could be accomplished with 2-3 major peaks whereas the first order fits required 5 - 6 bands (not shown). The first-order model suggested that the frequency factor s varies between $6 \cdot 10^9$ and $3 \cdot 10^{16}$ s^{-1} and ΔE between 0.78 and 1.27 eV for different bands in the three strains. Because the first-order model would suggest an unreasonably high number of reactions, we used the general order model in further analyses.

In the AR control strain, the B band measurements showed a component at 24 - 28°C ($\Delta E = 0.87$ eV) with variable intensity, in addition to the main peak at 35°C ($\Delta E = 0.90$ eV). The 24 - 28°C peak was more intense in measurements done with intact cells (data not shown). The glow curves of both mutants, measured both in the absence and the presence of DCMU, consisted of two component bands: one peaking at 24 - 28°C ($\Delta E = 0.87$ eV) and another one peaking at 14-18°C ($\Delta E =$

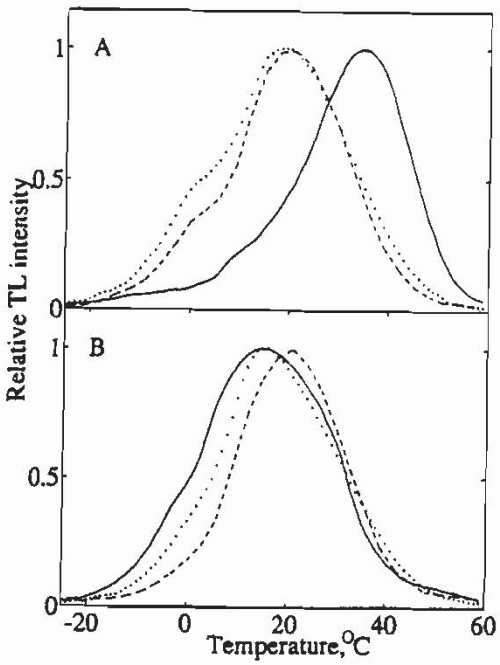

Figure 1. The TL glow curves measured from the isolated thylakoids of *Synechocystis* sp. PCC 6803 strains AR (solid line), PD (dashed line), and PCD (dotted line). The measurements were done in the absence (A) and in the presence (B) of 20 μM DCMU.

0.85 - 0.86 eV in the mutants). Two component bands with similar characteristics were found in the Q band measurements of AR control strain; the lower component was at 11°C with ΔE value of 0.82 eV.

Values of the frequency factor s calculated for all component bands were in the range of $1.5 - 5.4 \cdot 10^{13}$ s^{-1}. The s value of the component band peaking at 11 - 18°C was smaller in the two autotrophic strains AR ($s = 1.9{\cdot}10^{13}$ s^{-1}) and PD ($s = 2.3{\cdot}10^{13}$ s^{-1}) than in the heterotrophic PCD strain ($s = 5.4{\cdot}10^{13}$ s^{-1}). The order of the kinetics was in the range of 1.2 - 1.6.

4. Discussion

The TL curve of the AR control strain has earlier been measured from intact cells in conditions comparable to the present one, and the peak temperature of the B band was the same [1].

The mutants show little effect of DCMU on TL: The TL B band found in the control strain was not present in the two mutants. We conclude that electron transfer from Q_A to Q_B is so inefficient in both mutants that the concentration of Q_B^- becomes too small to be detected with TL. The results could also be explained by assuming that the B band has moved to the same position as the Q band in the mutants implying a change in the redox potential of Q_B/Q_B^- to the more negative in the mutants than in AR. Photosynthesis in all three strains, even the traces observed in PCD, is sensitive to DCMU [7], indicating that the lack of a DCMU effect is not due to incomplete displacement of Q_B by DCMU.

Order of kinetics: TL peaks of photosynthetic material are wider than bands predicted by the Randall-Wilkins theory. Analysis of our data with a general order model suggests a high order of the reaction kinetics ($b = 1.2{-}1.6$). This high reaction order means that retrapping of the recombining charge pair by other processes (that could include triplet formation) is $0.2 - 0.6$ times as probable as recombination

resulting in the luminecent excited state of $P680$. The order parameter b was larger in our analysis than the values obtained earlier from spinach thylakoids [5].

Band at 24 - 28°C: The origin of the 24−28°C band is not clear but this component was usually seen both in the presence and the absence of DCMU and it seems to be a characteristic feature of *Synechocystis* 6803, especially when intact cells are studied. It may arise either from the recombination of the $S_2Q_A^-$ state in active PSII centers or from the recombination of $S_2Q_B^-$ state of inactive PSII centers [8].

Thermodynamic parameters: Values of the activation energy and frequency factor of the component peaks produced by the earlier first-order analyses are usually much higher [4, 6] than what we have obtained now. Especially values of the frequency factor have traditionally been too high exceeding the expected value $< 10^{14}$ s^{-1} (higher values are outside of the kinetics of the molecular reactions). Activation energies and frequency factors determined using the model of general order kinetics are in the same range as obtained with the same model from spinach tylakoids [5]. The activation entropy dS of the recombination reaction is a function of the frequency factor ($dS = k\ ln(s\ h/k)$) and therefore the differences in the frequency factor s suggest that the mutations have restructured the PSII electron transfer chain so that the back reaction $S_2Q_A^- \rightarrow S_1Q_A$ requires less reorganization in the heterotrophic mutants than in the autotrophic strains.

6. Acknowledgements

E.T, P.M. and E.M.A. were supported by Academy of Finland, and M.K. by Kone Foundation. G. Was supported by the University of Geneva during the preparation of this manuscript.

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