Destabilization by high pH of the S_1-state of the oxygen-evolving complex in photosystem II particles

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Received 11 November 1985

The S-state distribution in a dark-adapted photosystem II preparation, isolated from spinach chloroplasts, can be changed by pH. The period 4 oscillation of the oxygen-releasing reaction, as measured by UV absorbance changes, is shifted by one flash at pH 8.3. This is not due to the generation of the EPR signal II on the first flash, or to the loss of a positive charge to some other electron donor after the first flashes. It is concluded that at pH 8.3 the S_1-state is reduced to S_0. The half-time of the reduction of S_1 to S_0 is 45 s, and the reduction is reversed in the dark if the pH is readjusted to pH 6.0. The oxygen release at pH 8.3 is irreversibly slowed down to about 3.2 ms.

Oxygen-evolving complex *S-state* *Photosystem II* *Manganese* *pH effect* *Destabilization*

1. INTRODUCTION

The oxygen-evolving complex of photosynthesis carries out the oxidation of 2 water molecules to one dioxygen molecule by 4 successive photoreactions of PS II (review [1]). So the complex can be in 5 different redox states, the so-called S-states [2,3]. S_0 and S_1 are believed to be stable in the dark, S_2 and S_3 revert to S_1 in minutes and S_4 is reduced rapidly to S_0 with release of oxygen. As a result the S_1-state predominates after a period of darkness and subsequent illumination by a series of short saturating flashes yields an oscillating amount of oxygen with maxima on flash number 3, 7, 11, etc.

In chloroplasts it is reported that the S_1-state is stable and cannot be reduced to S_0 [4,5]. Bouges [6,7] reported the oxidation of S_0 to S_1 in the dark in hydroxylamine-treated chloroplasts, thus restoring the original $S_0:S_1$ ratio of 1:3. The remarkable invariance of this ratio was also reported by Velthuys and Kok [8]. Vermaas et al. [9] reported the oxidation of S_0 to S_1 in pea thylakoids during a long dark incubation, resulting in a dark S-state distribution of 100% S_1.

A powerful tool to study the S-state turnover is the UV absorbance change which has been found to oscillate with a periodicity of 4 [10]. Making use of the pure PS II preparation as described by Berthold et al. [11], denoted as the BBY PS II preparation here, Dekker et al. [12,13] recently showed that the $S_0 \rightarrow S_1, S_1 \rightarrow S_2$ and $S_2 \rightarrow S_3$ transitions each cause the same absorbance increase in the UV [13], which was attributed to the oxidation of Mn(III) to Mn(IV) [12]. During the $S_3 \rightarrow (S_4) \rightarrow S_0$ transition, which took 1–1.5 ms in these preparations, all 3 Mn(IV) ions are reduced simultaneously with the oxidized secondary donor Z^+ [13]. In addition to Z a similar molecule D is present, the function of which is unknown. D^+ and Z^+ are characterized by a similar EPR signal, the so-called Signal II. The appearance of the EPR signal due to Z^+ is dependent on the sample preparation procedure, while a contribution of D^+ is nearly always present.

Abbreviations: Chl, chlorophyll; DCBQ, 2,5-dichloro-*p*-benzoquinone; Mes, 4-morpholineethane sulfonic acid; PS II, photosystem II; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

De Groot et al. [14] showed that in the BBY PS II preparation, at pH 6.0, the spin-lattice relaxation time of Signal II, measured with electron spin echo spectroscopy, decreases with increasing oxidation state of the oxygen-evolving complex. The relaxation behaviour at pH 8.3 suggested that at this pH the stability of the S_1-state was decreased such that in dark-adapted samples nearly 100% of the reaction centers were in the S_0-state.

In this study we analyzed the period 4 oscillation of the UV absorbance changes of the oxygen-evolving complex in a dark-adapted BBY PS II preparation at pH 8.3. It will be shown that this high pH indeed causes a reduction of the S_1 to the S_0-state, which can be reversed by lowering the pH in absolute darkness.

2. MATERIALS AND METHODS

PS II particles were prepared from spinach

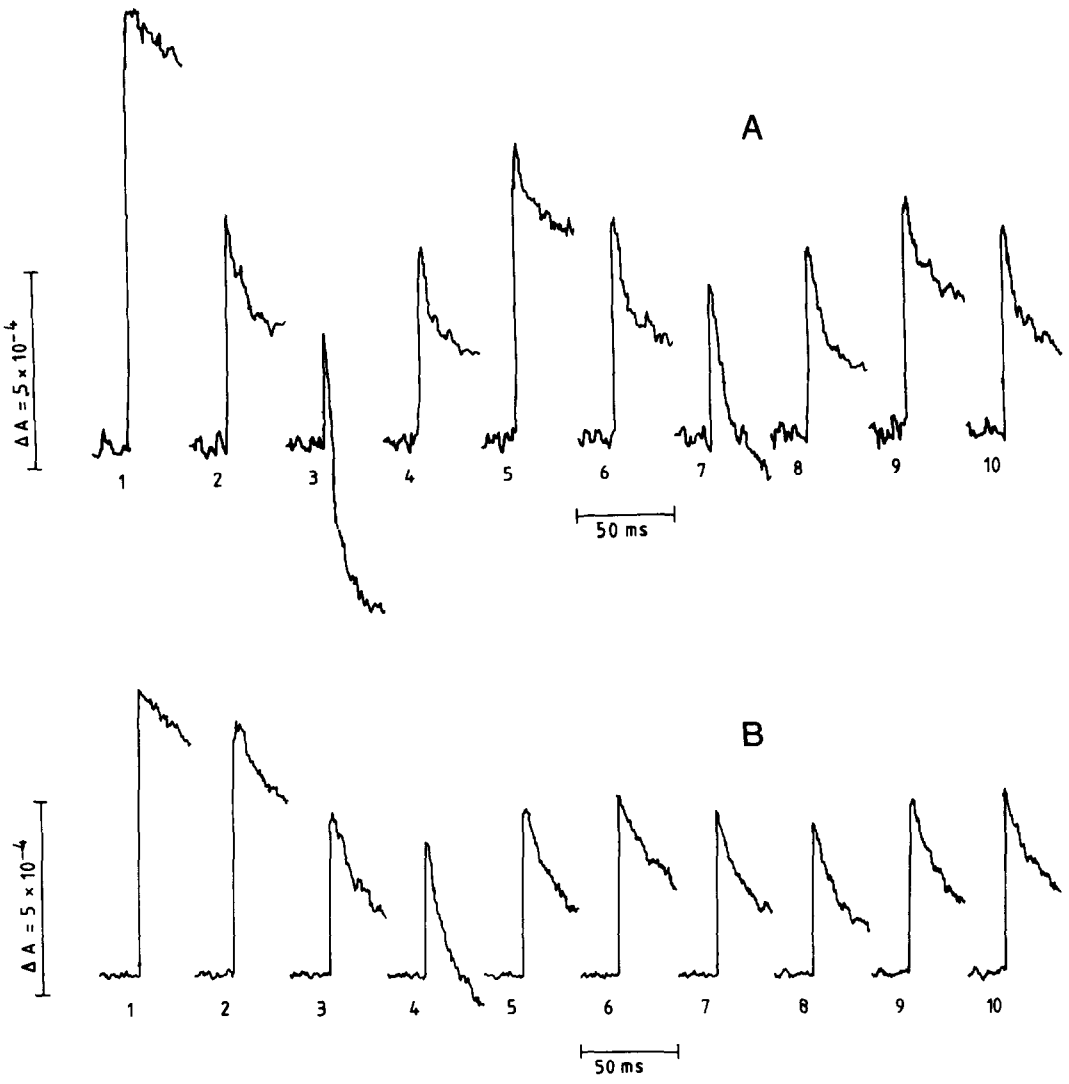

Fig.1. Absorbance changes at 345 nm in a dark-adapted BBY PS II preparation suspended at 200 μg/ml Chl in 50 mM Mes-NaOH (pH 6.0) (A) or 50 mM Tricine-NaOH (pH 8.3) (B), both with 5 mM $MgCl_2$, 15 mM NaCl and 100 μM DCBQ. The flashes were spaced at 300 ms. The traces are the average of 25 measurements.

chloroplasts according to Berthold et al. [11] with the exception that the second Triton X-100 incubation was omitted. The final preparations were stored in 400 mM sucrose/50 mM Mes/15 mM NaCl/5 mM $MgCl_2$ (pH 6.0) in liquid nitrogen at a Chl concentration of 3 mg/ml.

The optical experiments were performed with 200 μg/ml Chl and 100 μM DCBQ in the indicated buffer. The following buffers were used: Mes for pH 6.0, Hepes for pH 7.0–7.5 and Tricine for pH 7.5–8.3. The concentration of the buffers was 50 mM, and 15 mM NaCl and 5 mM $MgCl_2$ were added. The experiments were carried out in a single-beam apparatus as described in [12]. The optical path length was 1.2 mm. The saturating 10 μs xenon flashes were spaced at 300 ms (unless stated otherwise). Above 290 nm, a tungsten halogen lamp was used for the measuring light. Below 290 nm, a deuterium lamp was used. The photomultiplier was protected from the flashes by using appropriate filter combinations. The EPR experiments were performed with a Varian E9 X-band spectrometer with 100 kHz field modulation. Low temperatures were obtained using an Oxford Instruments helium flow cryostat. The EPR samples were contained in suprasil quartz tubes with an internal diameter of 3 mm.

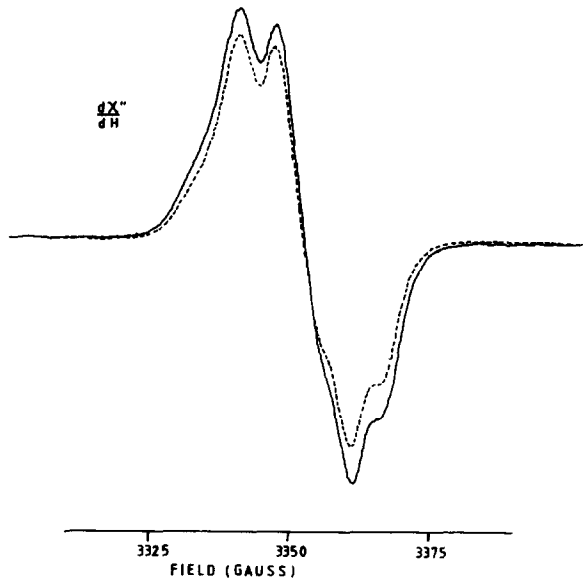

Fig.2. EPR spectra of Signal II in a dark-adapted (– – –) and an illuminated (——) BBY PS II preparation. Illumination took place before and during the freezing of the sample. Instrument settings: signal gain 8×10^2; modulation amplitude 50 G; microwave power 5 μW; temperature 4.3 K; 5 mg/ml Chl in a buffer containing 5 mM $MgCl_2$, 15 mM NaCl and 400 mM sucrose.

3. RESULTS AND DISCUSSION

Absorbance changes of the oxygen-evolving complex by a series of saturating flashes were studied in the BBY PS II preparation at pH 6.0 and 8.3. The preparations were dark-adapted for at least 15 min, after which DCBQ was added to avoid limitations of the acceptor side. A series of saturating flashes, spaced at 300 ms, was fired and the flash-induced absorbance changes were measured in successive 50 ms sweeps, the offset being adjusted before each flash. Fig.1A and B shows typical traces at pH 6.0 and 8.3, respectively, measured at 345 nm, where the manganese of the oxygen-evolving complex causes relatively large absorbance changes and no oscillation of the acceptor side occurs under these conditions [13]. It shows that the period 4 oscillation which is characteristic of an active oxygen-evolving complex is shifted one flash backwards at pH 8.3. Secondly, the fast absorbance decrease which accompanies oxygen release is slowed down to 3.2 ms at pH 8.3, compared with the 1.3 ms decay at pH 6.0 for untreated BBY PS II, but is still faster than the 5.0 ms decay for salt-washed BBY PS II [15]. It should be noted that at pH 8.3 a BBY PS II preparation does not evolve oxygen at all, as measured with a Clark electrode in continuous illumination.

Since the EPR amplitude of Signal II is the same for dark-adapted and illuminated samples at pH 8.3 (fig.2), the shift in the period 4 oscillation at pH 8.3 cannot be due to the oxidation of D or Z on the first flash.

We measured the absorbance changes caused by the first S-state transition of dark-adapted PS II particles at pH 8.3. The absorbance changes remaining at the end of the sweep, i.e. 30 ms after the first flash (see fig.1B), were measured in the presence of DCMU. The precise shape of the difference spectrum of Q^- – Q in PS II particles is known [12]. After normalization on the amplitude of the C-550 band shift the spectrum due to Q reduction was subtracted from the first flash spectrum. The resulting difference spectrum (fig.3) is

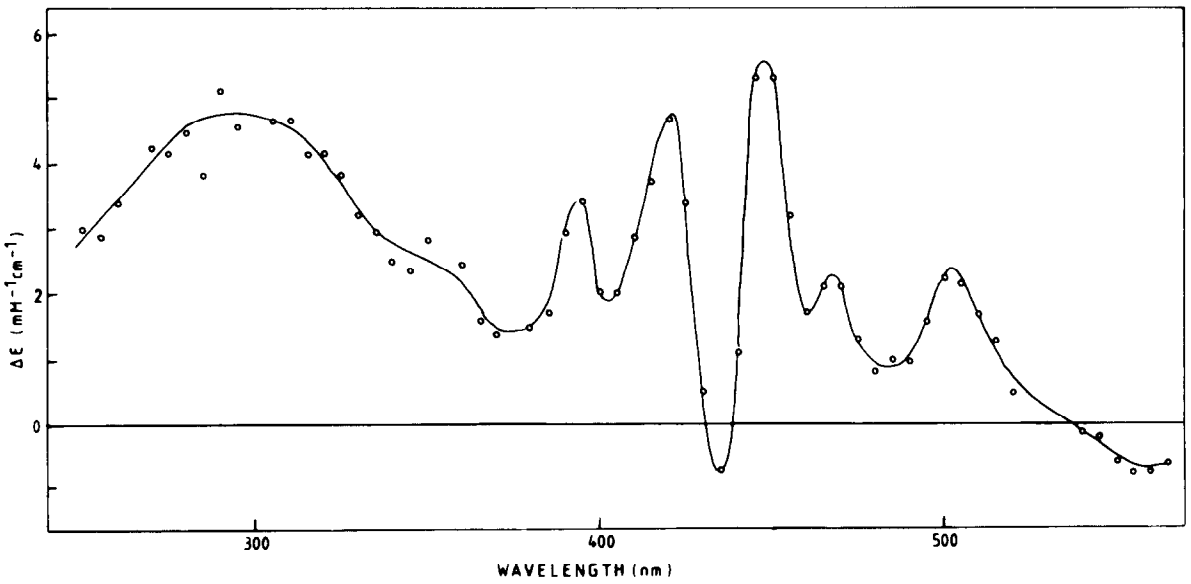

Fig.3. Spectrum of the absorbance change on the first flash in a dark-adapted BBY PS II preparation at pH 8.3 as in fig.1B, to which 10 μM DCMU was added before the measurement, and from which the changes due to Q reduction have been subtracted, as described in the text.

almost the same as that reported for the first flash at pH 6.0 in the presence of DCMU and is very similar to that of the Mn(III)-Mn(IV) transition [12]. We therefore conclude that the first transition of PS II particles at pH 8.3 is due to the oxidation of one manganese in the oxygen-evolving complex. For this transition a differential extinction coefficient of about 4.8 $mM^{-1} \cdot cm^{-1}$ at 300 nm was calculated. In a BBY PS II preparation at pH 6.0, this extinction coefficient was found to be about 6.0 $mM^{-1} \cdot cm^{-1}$ [12]. We conclude that at pH 8.3

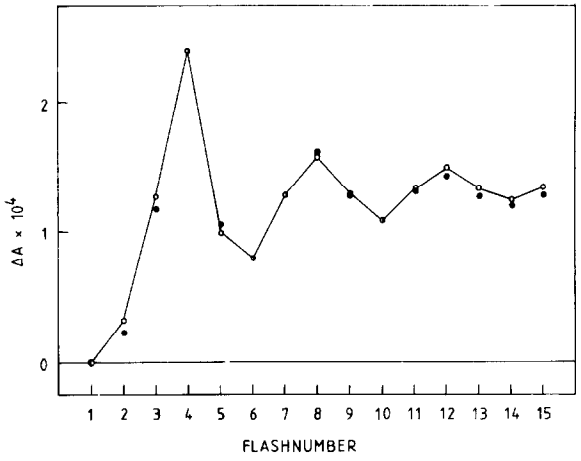

Fig.4. Amplitude of the 3.2 ms phase of the $S_3 \rightarrow (S_0) \rightarrow S_0$ transitions after the first 15 flashes in dark-adapted BBY PS II preparations at pH 8.3 as in fig.1B (○). The values are the average of 50 measurements. (●) Amplitudes of the $S_3 \rightarrow (S_4) \rightarrow S_0$ transition calculated for a dark S-state distribution of 100% S_0 and 11% misses and 11% double hits on all transitions.

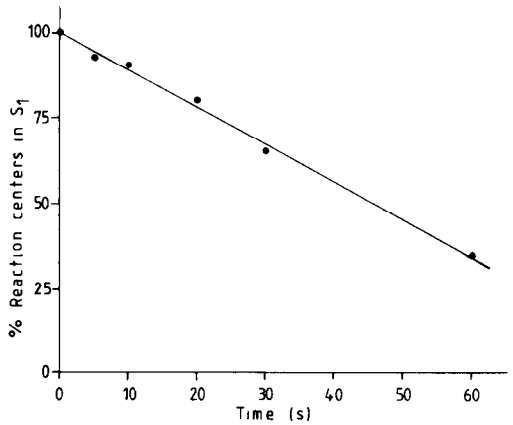

Fig.5. Percentage of reaction centers in the S_1-state vs the time after one saturating flash in a BBY PS II preparation at pH 8.3. The percentage was determined as described in the text.

20% of the reaction centers did not produce a stable charge separation. An inactivation at high pH of a part of the oxygen-evolving complex was also described for chloroplasts by Briantais et al. [16,17].

In fig.4 the open circles indicate the extent of the 3.2 ms phase at 345 nm. This phase accompanies the oxygen release and could be fitted with a dark S-state distribution of 100% S_0, 11% misses and 11% double hits on all transitions (solid circles).

Since each step in the sequence from S_0 to S_3 is accompanied by the same UV absorbance difference spectrum [13] the dark-adapted state could still be S_1 mainly and one positive charge could be lost after the first or second flash to some electron donor other than D. At 30 ms after the flash (end of the sweep) the period 4 oscillation in absorption at 345 nm can still be described with the same parameters as the oxygen release. So there is no loss of a positive charge from manganese in the first 30 ms after the flash. Probably such a loss did not take place at all within the 300 ms time between successive flashes because with a flash spacing of 100 ms the oscillation of the 3.2 ms phase (oxygen release) could also be fitted with the same parameters (not shown). We therefore conclude that there is no loss of a positive charge after the first few flashes and that the dark-adapted state at pH 8.3 is not S_1 but S_0.

Fig.5 shows the percentage of the S_1-state which is not reduced to S_0 at pH 8.3 in the time between a saturating pre-flash and the measurement of flash-induced absorbance changes. This is calculated by fitting the measurement with a variable S-state distribution and the fixed transition parameters of fig.4. The half-time of the reduction of the S_1-state to S_0 at pH 8.3 is 45 s.

To verify whether the reduction of S_1 to S_0 at pH 8.3 is reversible or irreversible, the following experiment was done. PS II particles were suspended in a buffer containing 50 mM Tricine-NaOH (pH 8.3), 5 mM $MgCl_2$ and 15 mM NaCl, incubated in the dark for 15 min during which a complete reduction of S_1 to S_0 takes place. Then the pH was brought back to pH 6.0 by a change of buffer. This was done in total darkness so that no light-induced transition could take place. Immediately after the readjustment of the pH to 6.0 the flash-induced absorbance changes at 345 nm were measured. Although part of the reaction centers were inactivated during the experiment, and the oxygen release was still as slow as at pH 8.3, the period 4 oscillation was clearly shifted to the original oscillation pattern at pH 6.0 (fig.6). One has to conclude that the reduction of S_1 to S_0 at pH 8.3 is reversed when the pH is readjusted to pH 6.0. The oxidant/reductant involved in the dark equilibration of the S_0 to S_1 ratio remains unknown.

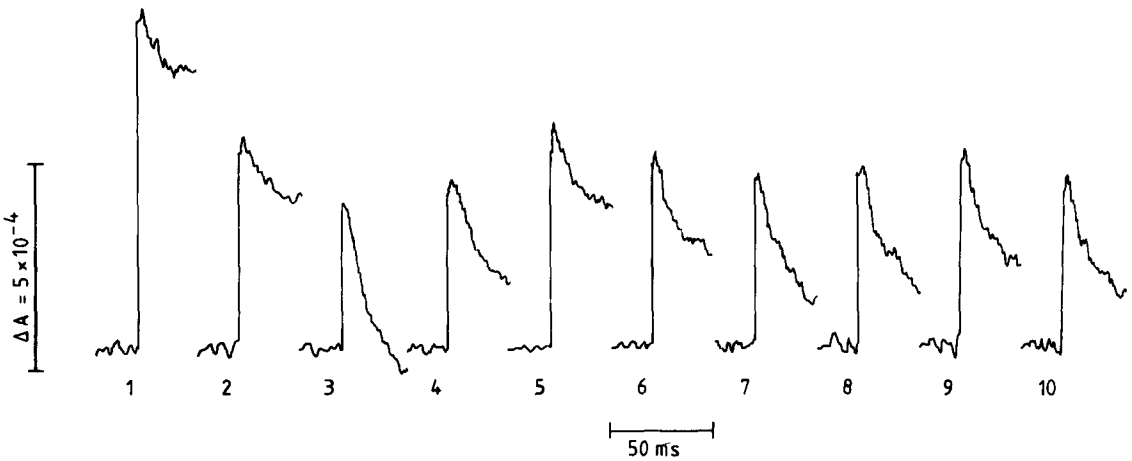

Fig.6. PS II particles were suspended in the Tricine buffer of pH 8.3 and incubated in the dark for 15 min to allow a complete reduction of S_1 to S_0. Then the pH was brought back to pH 6.0 by a buffer change in the dark so that no light-induced transition could take place. Absorbance changes were measured as in fig.1A. The recordings are the average of 50 measurements.

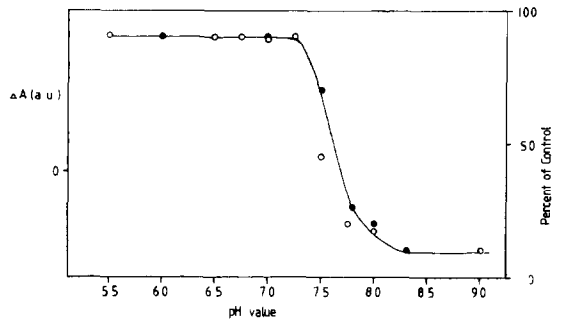

Fig.7. The effect on the reduction of S_1 to S_0 as a function of pH in a BBY PS II preparation (●). The reduction is measured as the difference between the absorbance changes at 345 nm measured 30 ms after the 4th and 3rd flash. (○) Effect of trypsin on oxygen evolution as a function of pH as shown in [19].

Völker et al. [18] have shown that in a BBY PS II preparation a structural modification of the oxygen-evolving apparatus occurs at about pH 7.6, which makes it accessible to trypsin. The reduction of S_1 to S_0 shows the same pH dependency (fig.7), suggesting deprotonation of a group with a pK_a of 7.6. We propose that this deprotonation results in a reversible structural change which causes the destabilization of the S_1-state at high pH.

4. CONCLUSIONS

In a dark-adapted PS II preparation the S-state distribution can be changed by pH. Below pH 7.6 mainly the S_1-state is present, but above this pH mainly the state S_0. At pH 8.3 the period 4 oscillation could be fitted with the same parameters as at pH 6.0 except that the dark S-state distribution is 100% S_0 instead of 75% S_1 and 25% S_0. At pH 8.3, the S_1-state is reduced to S_0 with a half-time of 45 s. This anomalous behaviour at high pH may be related to a conformational change of the oxygen-evolving apparatus in a BBY PS II preparation. The phenomenon is reversible and probably not directly related to the irreversible slowing down at high pH of the oxygen-evolving reaction and the complete inactivation of oxygen evolution in continuous illumination.

ACKNOWLEDGEMENTS

We wish to thank F.T.M. Zonneveld for the preparation of the BBY PS II. This investigation was supported by the Netherlands Foundation for Chemical Research (SON), financed by the Netherlands Organization for the Advancement of Pure Research (ZWO).

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