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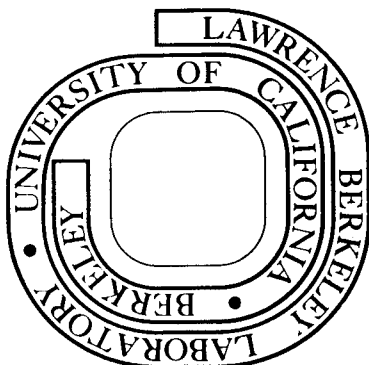
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Absorption Changes of P700 Reversible in Milliseconds
at Low Temperature in Triton-Solubilized
Photosystem I Particles*

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SUMMARY

Triton-solubilized Photosystem 1 particles from spinach chloroplasts exhibit largely reversible P700 absorption changes over the temperature range from 4.2 K to room temperature. For anaerobic samples treated with dithionite and neutral red at pH 10 and illuminated during cooling, a brief (1 μ s) saturating laser flash produces absorption changes in the long wavelength region that decay in 0.95 ± 0.2 ms from 4.2 to 50 K. Above 80 K a faster (100 ± 30 μ s) component dominates in the decay process, but this disappears again above about 180 K. The major decay at temperatures above 200 K occurs in about 1 ms. The difference spectrum of these absorption changes between 500 and 900 nm closely resembles that of P700. Using ascorbate and 2,6-dichlorophenolindophenol as the reducing system with a sample of Photosystem 1 particles cooled in darkness to 4.2 K, a fully reversible signal is seen upon both the first and subsequent flashes. The decay time in this case is 0.9 ± 0.3 ms.

The reversibility of the absorption change or EPR signal associated with P700⁺ induced at low temperature appears to depend on the redox potential of the medium and the nature or presence of exogeneous electron donors [1-5] and whether the sample was illuminated during cooling [6-8]. The temperature of the measurement is an important factor in determining the decay kinetics [1,8-11]. In addition, kinetic differences have been observed that may depend on the duration and intensity of the excitation pulse and the time response of the detection system [8,10,12-14]. There are several reports in the literature that the P700 signals are formed irreversibly upon illumination at low temperature [15-18].

We have studied the decay kinetics of laser flash-induced absorption changes of P700 in Triton-solubilized Photosystem 1 particles from 4.2 K

to room temperature. We found reversible signals with decay times of milliseconds or shorter throughout this temperature range in the presence of dithionite/neutral red or ascorbate/2,6-dichlorophenolindophenol. This confirms two earlier reports of low temperature reversible signals in chloroplasts or Photosystem 1 fragments in this time range [8,12]. We have found decay kinetics and a temperature dependence that differ from those reported previously, however.

Triton-solubilized Photosystem 1 particles from spinach were prepared following the method of Vernon and Shaw [19] and stored at -20 C, as described previously [20]. For low temperature studies a freshly thawed portion (0.1 ml) of the particle suspension in the appropriate reaction mixture (0.4 ml) was mixed with glycerol (1.0 ml). For low potential studies the reaction mixture contained sodium dithionite (2 mg), dimethyltriquat (50 μ M), neutral red (75 μ M) and glycine buffer (0.15 M, pH 10). The solution was degassed under vacuum prior to addition of the solid dithionite in a nitrogen-filled glove box. In other studies a reaction mixture containing sodium ascorbate (5 mM), 2,6-dichlorophenolindophenol (20 μ M), sucrose (0.4 M) and tricine buffer (50 mM, pH 7.6) was used, and the degassing was omitted. The sample suspensions were placed in a special lucite cuvette (1.0 or 1.5 mm path), covered tightly and transferred to a cryostat (Meric, Arpajon, France) that was cooled by flowing helium gas at a controlled temperature (4.2 to 294 K). Some samples were illuminated during cooling using attenuated light from the measuring beam source.

The sample cuvette was oriented at 45° to the mutually perpendicular measuring and exciting beams. Absorbance values quoted in the figure captions were determined from absorption spectra measured at room temperature through a path perpendicular to the cuvette faces. Values given for $\Delta A/A_{\max}$ include a factor of $1/\sqrt{2}$ to correct for the increased path of the sample oriented

at 45° to the measuring beam. Flash-induced absorption changes were stimulated by dye laser (605 nm) pulses of 1 μ s duration and at 15s intervals, as described previously [20]. The measuring beam optical bandwidth was 3 nm for wavelengths below 740 nm and 10 nm for higher wavelengths. The signal from the photodetector was fed into an AC-coupled amplifier with a high pass filter so that slowly decaying components appeared to have a 12 ms ($t_{1/2}$) decay. Multiphasic decay curves were resolved using semi-logarithmic plots of measurements from photographic records of the traces.

In the presence of dithionite at pH 10 and a good electron donor, neutral red, the Photosystem 1 particles show flash-induced absorption transients at 703 nm at low temperatures that have the same magnitude as those observed at room temperature. The signals are largely or fully reversible within a few milliseconds following the flash. As illustrated in Fig. 1, the decay is nearly monophasic at temperatures between 4.2 and 50 K with a halftime of 0.9 ± 0.3 ms. A small initial fast transient was occasionally seen, but not reproducibly. With increasing temperature the decay becomes faster ($t_{1/2} \sim 180 \mu$ s at 84 K, Fig. 1). Between 100 and 180 K the decay is clearly biphasic. The larger component ($65 \pm 10\%$) occurs in $100 \pm 30 \mu$ s and the smaller (35%) with a halftime of 1.1 ± 0.3 ms (Fig. 2, 146 K). At temperatures above 200 K the fast decay is no longer predominant, and the major relaxation occurs with a halftime of 1.2 ± 0.2 ms (Fig. 2, 218 K). A summary of these rate constants versus temperature is collected in Fig. 3, where solid and open circles represent the major and minor decay components, respectively. The scatter of the results precludes any meaningful conclusions about Arrhenius activation energies; the dominant changes seem to be associated with two fairly narrow temperature ranges near 80 and 180 K, respectively. The appearance and disappearance of the rapid transient is reversible and occurs for transitions in both directions through these temperature ranges. The amplitude of the initial transient absorption change in response to the flash is not

altered during these temperature shifts, despite the dramatic changes in kinetics.

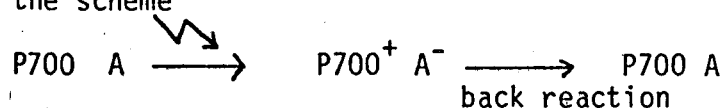
The difference spectrum of the reversible transient measured at 100-120 K was recorded at wavelengths between 500 and 560 and between 675 and 900 nm (Fig. 4). It is generally similar to the difference spectrum of P700 observed for these preparations at room temperature [20]; the appearance of the sharp positive change at 690 nm has been reported previously at low temperatures [6,8,12,18,21,]. The ratio of the magnitude of the absorbance increase at 820 nm to that of the decrease at 703 nm is 0.085 ± 0.005 at 4.2 and at 120 K, 0.130 ± 0.003 at room temperature in the absence of glycerol and 0.125 in the presence of glycerol (65%). The temperature effect may result primarily from a sharpening and deepening of the 703 nm bleaching feature. In the temperature range from 100 to 180 K, where both fast and slow decay components were detected, the relative amplitude of the slow component measured at 530 or 820 nm (40%) was greater than that at 703 nm (15%). This suggests that some species other than P700 may contribute to the slowly decaying absorption change. Analogous observations were present in the spectra at room temperature [20].

A similar pattern of reversible P700 changes in the millisecond region at low temperature occurs when the reductant used is ascorbate and 2,6-dichlorophenolindophenol in sucrose/tricine buffer at pH 7.6 and with 65% glycerol present. At room temperature the amplitude of the flash-induced absorption change at 820 nm was not appreciably affected by substituting glycerol for two-thirds of the aqueous solvent. The decay with an apparent halftime of 12 ms was instrument limited; it probably corresponds to a back reaction with a real halftime of 30-50 ms, as found previously under similar conditions [20,22]. To test for any contribution from irreversible absorption changes at low temperature, we cooled a sample to 4.2 K in complete darkness and carefully protected it from even low levels of actinic light prior to

the first laser flash. Using 820 nm measuring light we recorded an absorption transient from a single flash, then the average from the next five flashes and finally the response to the seventh flash. The (average) response amplitudes agreed within 5%, and in each case the signal decayed with a halftime of 0.9 ± 0.3 ms. Although the electronic circuitry prevented the measurement of decays slower than about 12 ms, the absence of additional amplitude on the first flash after cooling in darkness indicates that no significant irreversible absorbance change occurs using ascorbate as reductant under our experimental conditions.

For the ascorbate-treated samples the amplitudes of the transients at 820 nm observed at 4.2 K were nearly twice as large as for the same sample at room temperature prior to cooling. The difference spectrum at 4.2 K shows bleaching at between 694 and 710 nm and an increase in absorption at 690 nm that are essentially indistinguishable from those measured in the presence of dithionite and neutral red (Fig. 4). The ratio of the amplitude at 820 to that at 703 nm was 0.14. Subjecting the sample to relatively strong illumination from the measuring beam (at 694 nm) for 1 min at 4.2 K did not significantly alter transient responses measured afterwards. No flash-induced absorption changes occurred at 703 or 820 nm at 4.2 K in control experiments performed with 2 mM potassium ferricyanide added to the sample in glycerol/buffer, pH 7.6.

Our observations of rapidly-reversible absorption changes in Photosystem I particles at low temperatures are most easily understood in terms of the back reaction between oxidized P700 and a reduced early electron acceptor, according to the scheme



From the amplitudes of the bleaching at 703 nm we conclude that the reversible signal at low temperature corresponds essentially to the full

magnitude of P700 observed at room temperature. In our previous study of the Triton-solubilized Photosystem 1 particles [20], we presented evidence for a set of 4 distinct electron acceptors associated with each P700. At room temperature the back reaction of $P700^+$ with A_2^- occurs with a halftime of 0.25 ms, which probably corresponds to the "millisecond" phase seen at temperatures above 180 K in this study. In both cases the reaction occurs in the presence of neutral red and dithionite (anaerobic) at pH 10 and in the absence of strong background illumination.

In studies of Photosystem 1 particles solubilized using the detergent sodium dodecylsulfate [14], we concluded that P700 is unable to transfer an electron to A_2 . Instead, a decay of $P700^+$ in 10 μ s is observed at room temperature, closer to the rate of the back reaction of $P700^+$ with A_1^- . This relaxation becomes progressively slower as the temperature is decreased to 80 K, whereupon the decay halftime of 0.6 ms remains constant to 4.2 K. The occurrence of a similar transition temperature and low temperature decay rate for the Triton particles (Fig. 3) is suggestive that we are seeing the back reaction between $P700^+$ and A_1^- in this low temperature range (4.2 to 80 K), regardless of the reducing agent, electron donor or illumination of the sample during cooling. The temperature independence indicates a tunneling process for this back reaction.

In the intermediate temperature range from 100 to 180 K the Triton particles show a decay dominated by a 100 μ s component in the presence of neutral red and dithionite at high pH. By contrast, a progressive increase in the decay rate was seen for the sodium dodecylsulfate-solubilized particles above 100 K [14]. Floyd, et al. [12] reported a 30 μ s decay component of P700 from spinach chloroplasts excited by a laser flash at 77 K; however, other reports of low temperature measurements have not had sufficient time resolution to detect sub-millisecond transients. One of us (PM) has examined spinach chloroplasts with ascorbate and 2,6-dichlorophenolindophenol at

77 K and found that a relatively large fraction (70%) of the flash-induced absorption change of P700 is reversible with a halftime of about 120 μ s. It is possible that the complex decay pattern in the intermediate temperature region results from the superposition of electron-hole recombination involving both A_1 and A_2 . Further studies are needed to clarify this situation.

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REFERENCES

1. Vernon, L.P., Ke, B. and Shaw, E.R. (1967) *Biochem.* 6, 2210-2220
2. Evans, M.C.W. and Cammack, R. (1975) *Biochem. Biophys. Res. Commun.* 63, 187-193
3. Bearden, A.J. and Malkin, R. (1976) *Biochim. Biophys. Acta* 430, 538-547
4. Ke, B., Dolan, E., Sugahara, K., Hawkrigde, F.M., Demeter, S. and Shaw, E.R. (1977) in *Photosynthetic Organelles* (Miyachi, S., Katoh, S., Fujita, Y. and Shibata, K.) Special Issue of *Plant and Cell Physiology*, No. 3, 187-199
5. Demeter, S. and Ke, B. (1977) *Biochim. Biophys. Acta* 462, 770-774
6. Lozier, R.H. and Butler, W.L. (1974) *Biochim. Biophys. Acta* 333, 465-480
7. Evans, M.C.W., Cammack, R. and Reeves, S.G. (1975) *Proc. 3rd Internat. Congr. Photosyn.* (Avron, M., ed) pp 383-388 Elsevier, Amsterdam
8. Ke, B., Sugahara, K. and Sahu, S. (1976) *Biochim. Biophys. Acta* 449, 84-94
9. Visser, J.W.M., Rijgersberg, K.P. and Amesz, J. (1974) *Biochim. Biophys. Acta* 368, 235-246
10. Warden, J.T., Jr., Mohanty, P. and Bolton, J.R. (1974) *Biochem. Biophys. Res. Commun.* 59, 872-878
11. Ke, B., Sugahara, K., Shaw, E.R., Hansen, R.E., Hamilton, W.D. and Beinert, H. (1974) *Biochim. Biophys. Acta* 368, 401-408
12. Floyd, R.A., Chance, B. and De Vault, D. (1971) *Biochim. Biophys. Acta* 226, 103-112
13. Amesz, J. and De Grooth, B.G. (1975) *Biochim. Biophys. Acta* 376, 298-307
14. Mathis, P., Sauer, K. and Remy, R. (1978) *FEBS Lett.* in press
15. Rumberg, B. and Witt, H.T. (1964) *Z. Naturforsch.* 19b, 693-707
16. Cost, K., Bolton, J.R. and Frenkel, A.W. (1969) *Photochem. Photobiol.* 10, 251-258
17. Malkin, R. and Bearden, A.J. (1975) *Biochim. Biophys. Acta* 396, 250-259

18. Witt, K. (1973) FEBS Lett. 38, 112-115
19. Vernon, L.P. and Shaw, E.R. (1971) Methods in Enzymol. 23, 693-707
20. Sauer, K., Mathis, P., Acker, S. and Van Best, J.A. (1978) Biochim. Biophys. Acta, in press
21. Vermeglio, A., Breton, J. and Mathis, P. (1976) J. Supramolec. Struct. 5, 109-117
22. Ke, B. (1973) Biochim. Biophys. Acta, 301, 1-33

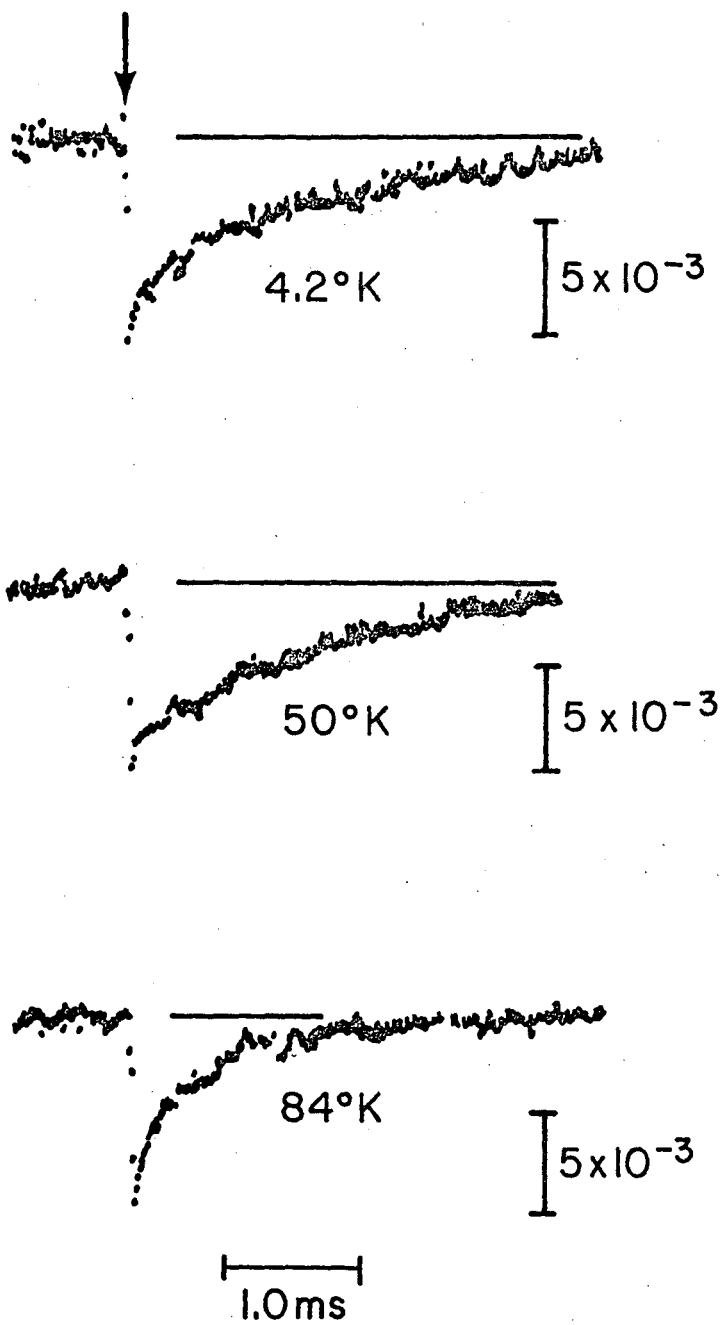
FIGURE CAPTIONS

Fig. 1. Absorption transients at 703 nm induced by a dye laser flash (1 μ s duration) incident on a sample of Triton-solubilized photosystem 1 particles at 4.2, 50 and 84 K, respectively, from top to bottom. Anaerobic reaction mixture obtained by adding two parts glycerol to one part of a solution containing dithionite (2 mg/ml), neutral red (75 μ M), dimethyltriquat (50 μ M), glycine buffer (0.15 M, pH 10) and sufficient Photosystem 1 particles to give $A_{670 \text{ nm}} = 0.65$ in a 1 mm path after mixing with glycerol. The vertical bars give ΔA with light path at 45° to surface.

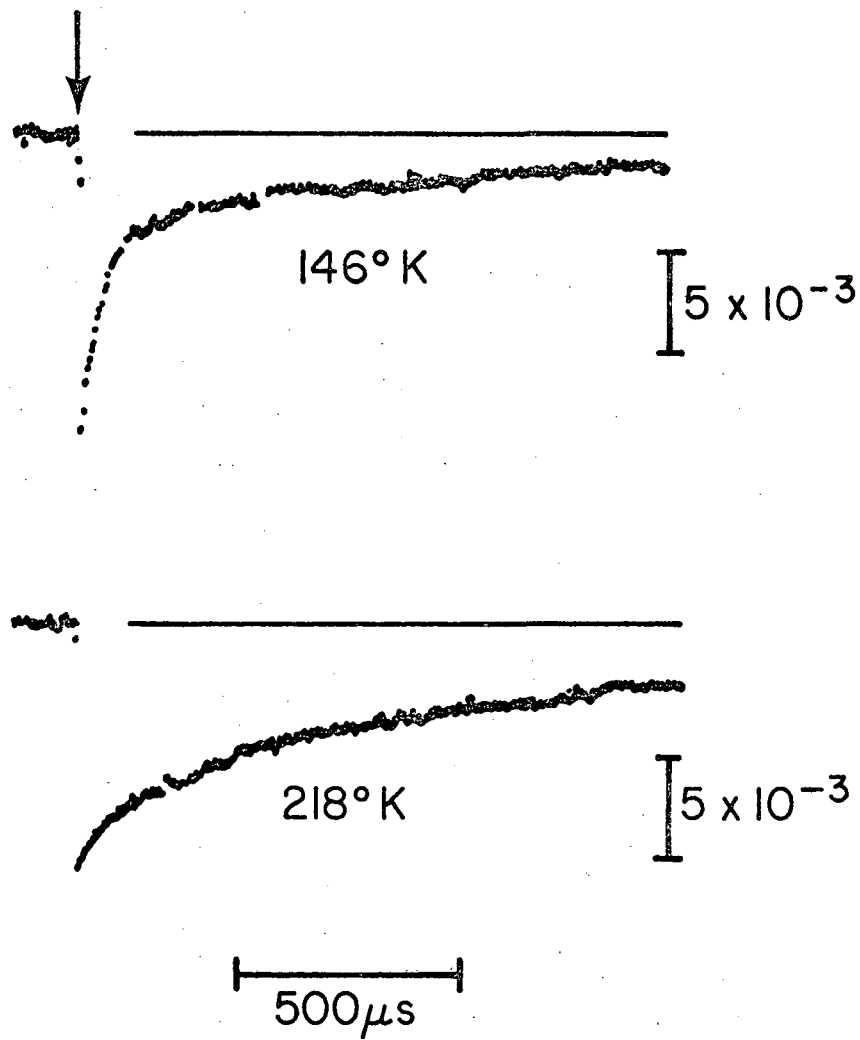
Fig. 2. Absorption transients at 703 nm induced by a dye laser flash incident on a sample of Triton-solubilized Photosystem 1 particles at 146 and 218 K, respectively, from top to bottom. Anaerobic reaction mixture prepared as described in Fig. 1. $A_{670 \text{ nm}} = 0.81$ in 1.5 mm path normal to cuvette surfaces. The sample was illuminated by weak red light during cooling prior to the experiments. Analysis of decays gives: at 146 K, $t_{1/2} = 68 \mu\text{s}$ (75%), 0.85 ms (25%); at 218 K, $t_{1/2} = 115 \mu\text{s}$ (25%), 0.95 ms (70%).

Fig. 3. Plot of logarithm of first-order rate constants versus temperature for decay of absorbance changes at various wavelengths between 695 and 820 nm. Triton-solubilized Photosystem 1 particles; conditions as in Fig. 1. Decay constants were extracted from semi-logarithmic plots of decay curves like those shown in Figs. 1 and 2. The solid circles ● indicate the major decay component, the open circles ○ indicate a secondary component. Half-filled circles ◐ represent components responsible for about half of the decay.

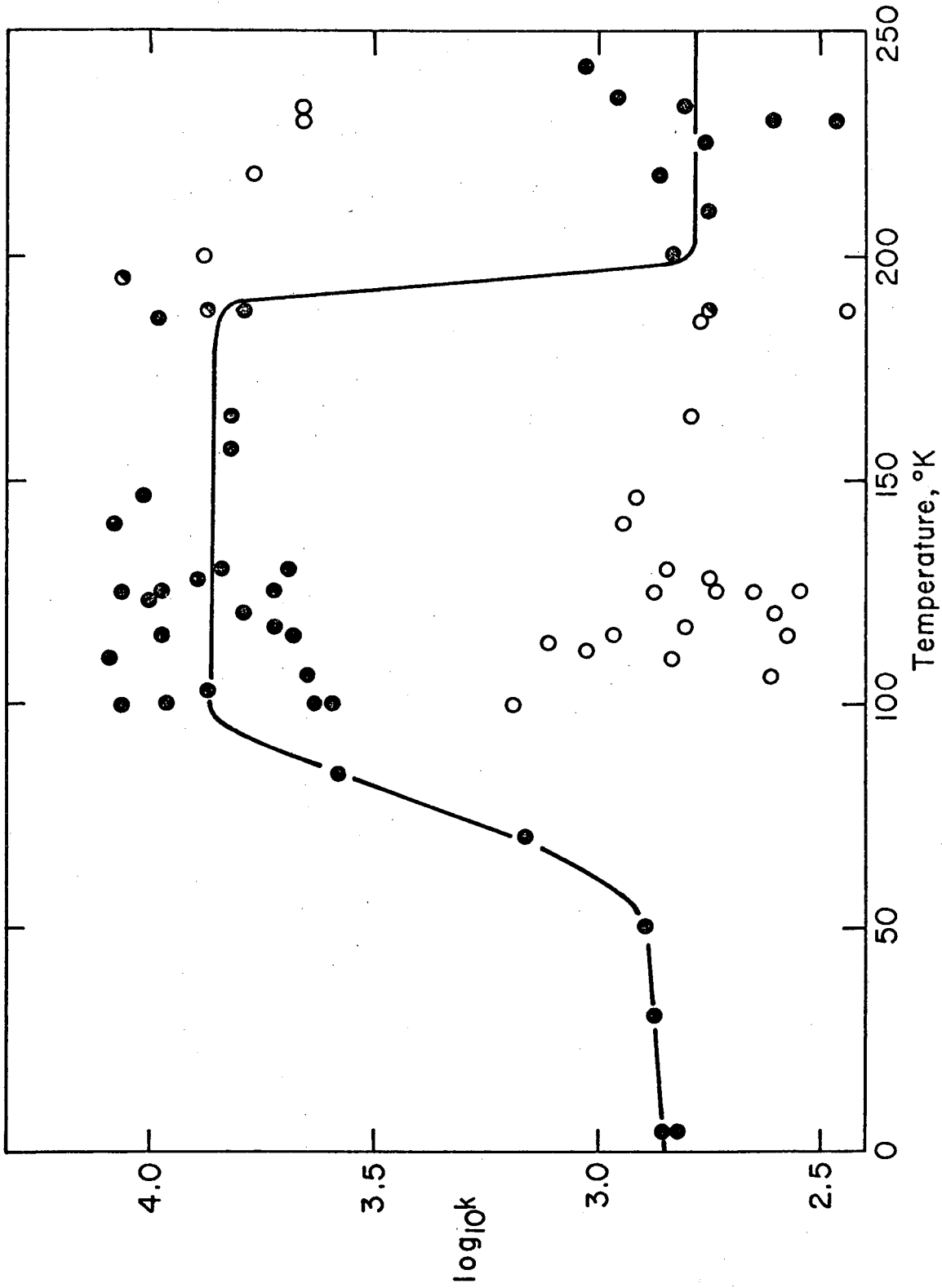
Fig. 4. Difference spectrum for absorbance changes of Photosystem 1 particles in the presence of dithionite and neutral red (anaerobic) in glycerol: buffer (2:1) at 100-120 K (open circles) and at 4.2 K (open triangles). Ordinate shows $10^3 \Delta A/A_{\max}$, where A_{\max} is the absorbance of the sample at 670 nm in the measuring beam tilted at 45° . Path length (normal to cuvette surfaces), 1.5 mm. $A_{\max} = 1.15, 0.92, 2.4$ for different samples. Reaction conditions as described in Figs. 1 and 2.



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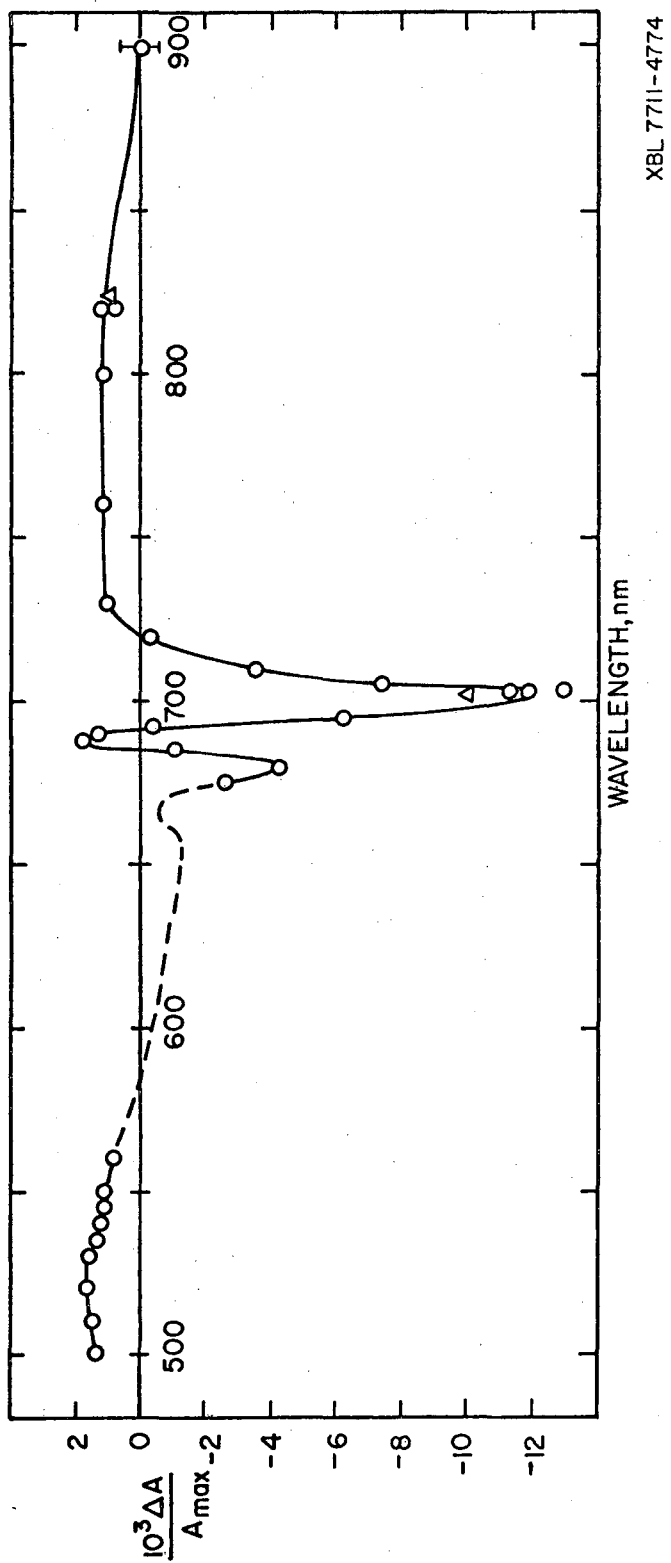


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Sauer, et al.
Fig. 3



Sauer et al.
Fig. 4

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