THE EFFECT OF ALKALINE pH ON CHLOROPLAST PHOTOSYSTEM I REACTIONS AT CRYOGENIC TEMPERATURE

Richard MALKIN* and Alan J. BEARDEN**

**Department of Cell Physiology and **Donner Laboratory, University of California, Berkeley, California 94720 USA*

Received 9 August 1976

1. Introduction

Although the identity of the reaction center chlorophyll of Photosystem I (*P*-700) has been well documented [1], the chemical nature of the primary electron acceptor remains controversial (see [2] for recent review). After the demonstration in 1971 of the photoreduction at cryogenic temperature of a membrane-bound iron-sulfur center [3], it was proposed that the center functions as the Photosystem I primary electron acceptor [4–7]. Recently, two groups [8,9] have proposed that another component, of unknown chemical identity, is located in the electron transport chain before the iron-sulfur center and functions as the primary electron acceptor of Photosystem I. In many of these recent studies, because of the extremely electronegative oxidation-reduction potential of the Photosystem I primary electron acceptor, it has been necessary to study the reaction at an alkaline pH (pH 9.0 to 11.0) to achieve complete chemical reduction of the bound iron-sulfur centers. During the course of studies similar to those reported by other workers we observed that alkaline pH has a marked effect on the reactions of *P*-700 at liquid-helium temperatures. These findings, which will be reported in this communication, are relevant to recent identification of components of the primary charge separation of chloroplast Photosystem I.

Correspondence to: Dr. Richard Malkin, 251 Hilgard Hall, University of California, Berkeley CA 94720 USA

2. Methods

Chloroplast fragments enriched in the Photosystem I reaction center (D-144) were prepared by treatment of spinach chloroplast fragments with digitonin by the procedure of Anderson and Boardman [10] and a more highly enriched Photosystem I fragment was prepared by treatment of chloroplasts with lauryl dimethylamine oxide (LDAO) by the procedure of Malkin [11]. The fragments were resuspended in a solution containing 10 mM Tricine buffer (pH 8.0), 10 mM NaCl, 1 mM EDTA (pH 8.0), and 10 mM sodium ascorbate. The fragments were stored in the dark at 4°C prior to dilution in an appropriate buffer at the desired pH.

Electron paramagnetic resonance (EPR) spectra were recorded at 15 K with an X-band JEOL EPR spectrometer equipped with a liquid-helium cooling system, as previously described [12,13]. Samples were illuminated in the EPR cavity with wide-band red light (Corning 2-64 filter) for 30 s at 15 K and recordings of the spectra were stored in an on-line PDP-8L computer for later data treatment. No differences in the extent of light-induced signals were observed when spectra were recorded during or after illumination.

3. Results

The photoreduction at 15 K of a bound iron-sulfur center can be demonstrated in D-144 Photosystem I

North-Holland Publishing Company – Amsterdam

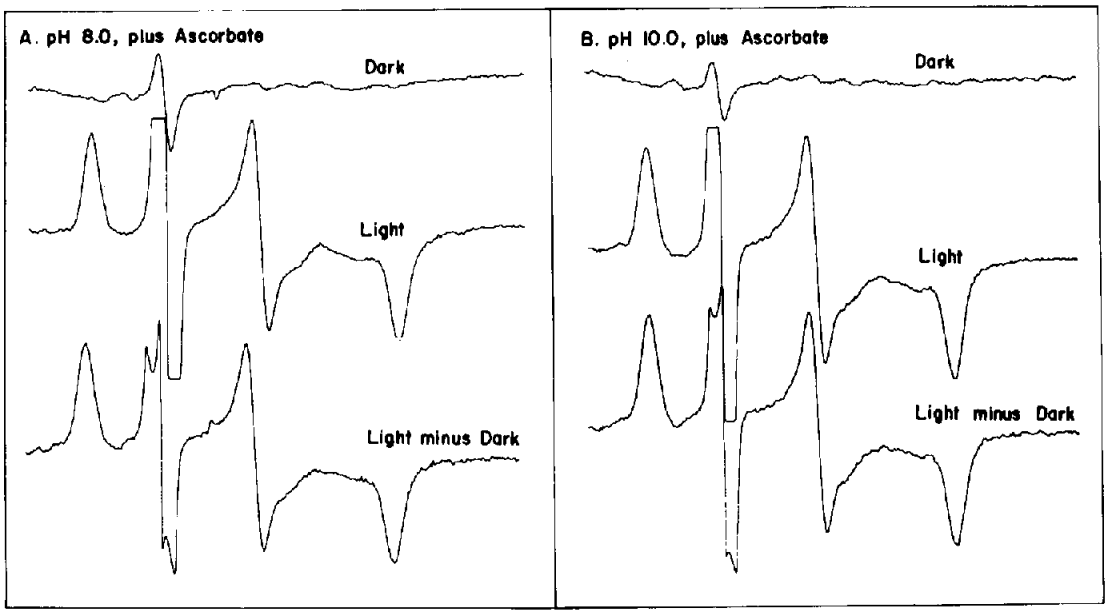

Fig.1. Effect of pH on photoreduction of the bound iron-sulfur center at 15 K in D-144 chloroplast fragments. The reaction mixture contained D-144 chloroplast fragments (0.5 mg chlorophyll per ml), either 0.1 M Tricine buffer (pH 8.0) (fig.1A) or 0.1 M glycine buffer (pH 10.0) (fig.1B), and 10 mM sodium ascorbate. Samples were incubated in the dark prior to freezing. EPR conditions: frequency, 9.2 GHz; microwave power, 10 mW; modulation amplitude, 10 G; temperature, 15 K.

fragments incubated in the presence of sodium ascorbate at either pH 8.0 (fig.1A) or pH 10.0 (fig.1B) prior to freezing. The light minus dark difference spectra at these two pH values are identical and indicate the photoreduction of an iron-sulfur center with EPR g values of 2.05, 1.94, and 1.86. As shown in fig.1A and fig.1B, alkaline pH has little effect on the magnitude of the photoreduction of the iron-sulfur center: in a series of experiments the amount of iron-sulfur center photoreduced at these two pH values differed by no more than 10%.

Concomitant with the photoreduction of an iron-sulfur center in Photosystem I, low-temperature illumination under the conditions described above photooxidizes *P*-700 the reaction center chlorophyll. This photo-oxidation is accompanied by the appearance of an EPR free-radical signal with a g value of 2.0026 and a linewidth of about 8 gauss. As shown in fig.2A, a large photo-oxidation of *P*-700 accompanies iron-sulfur center photoreduction in the sample incubated at pH 8.0 but the behavior of the sample incubated at pH 10.0 prior to freezing is markedly different. The magnitude of the *P*-700 change after illumination at 15 K of the sample at pH 10.0 (fig.2B) is only 25% of that observed in the sample incubated at pH 8.0, even though the extent of the iron-sulfur center photoreduction is unchanged at these pH values.

A more complete profile of the effect of pH on the photoreactions of the two Photosystem I components is shown in fig.3. At pH values more acidic than pH 9.0, the magnitude of the *P*-700 change is maximal and there is a marked decrease at more alkaline pH values. At the most alkaline pH tested (pH 11.0), only 15% of the amount of *P*-700* formed at pH 8.0 is observed after illumination and there is little effect on the magnitude of the photoreduction of the bound iron-sulfur center.

The effect of alkaline pH on the extent of the *P*-700 photoreaction at 15 K has also been observed in a more highly enriched Photosystem I subchloroplast fragment prepared with LDAO [11].

The light-induced changes that have been observed at 15 K in the presence of ascorbate at either pH 8.0

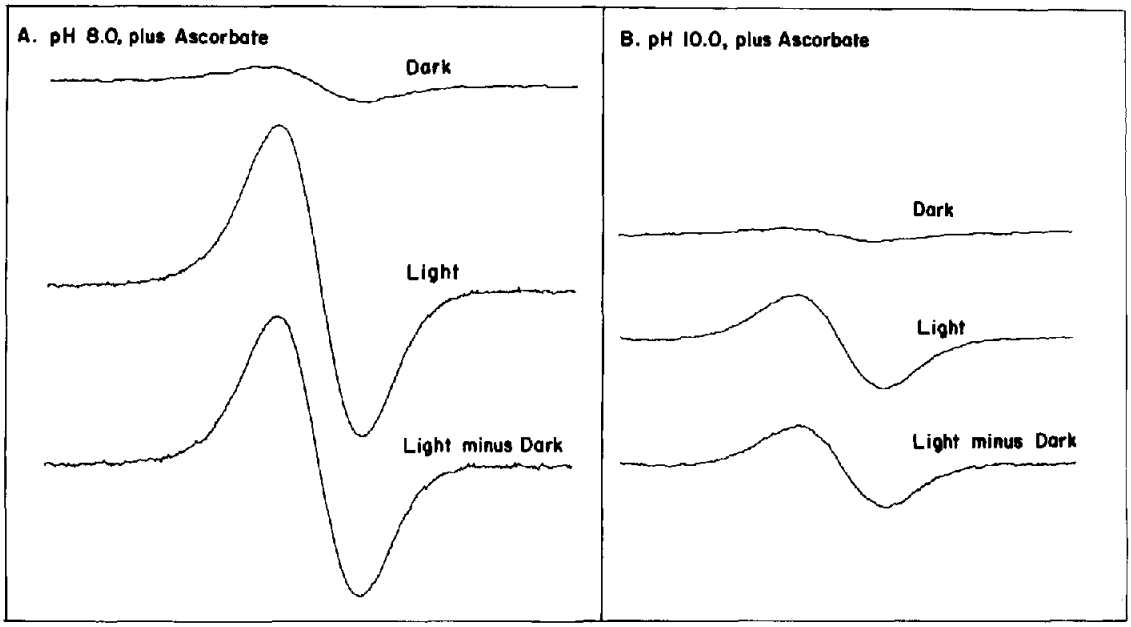

Fig.2. Effect of pH on photo-oxidation of *P*-700 at 15 K in D-144 chloroplast fragments. The reaction mixtures were as in figs.1A and 1B. EPR conditions were as in fig.1 except that a microwave power of 2 mW and a modulation amplitude of 3.2 G were used.

or more alkaline pH values are irreversible and a second illumination produces no additional light-induced changes. In addition, no decay of signals has been detected over the course of the experiments. The effect of alkaline pH was not dependent on the cryogenic temperature at which illumination occurred up to liquid-nitrogen temperatures (77 K), but the reversibility of the Photosystem I primary charge separation precluded measurements at higher temperatures (see [6,7]).

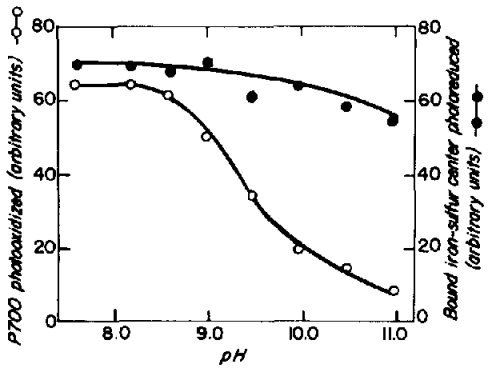

Fig.3. pH Profile for bound iron-sulfur center photoreduction and *P*-700 photo-oxidation at 15 K in D-144 chloroplast fragments. Experimental conditions as in figs.1 and 2.

In addition to having the same g value and EPR linewidth, the *P*-700 EPR signals at neutral and alkaline pH values display similar spin-relaxation properties at low temperatures, i.e., both signals display saturation at microwave powers of less than 1 mW at both 27 K and 65 K (TE_{oll} cylindrical mode cavity; $Q_{loaded} = 5 \times 10^3$).

The decrease in the magnitude of the *P*-700 EPR signal at alkaline pH values could also be observed when other reductants replaced ascorbate in the incubation medium prior to freezing and in the presence of buffers other than glycine. The extent of the effect was, however, dependent on the particular reductant or buffer at alkaline pH values. In the presence of hydroquinone (5 mM) or ascorbate (10 mM) plus phenazine methosulfate (10 μM), the magnitude of the *P*-700 signal at pH 10.0 was only 10% of that observed at pH 8.0. Ferrocyanide (10 mM) was less effective than the other reductants, although

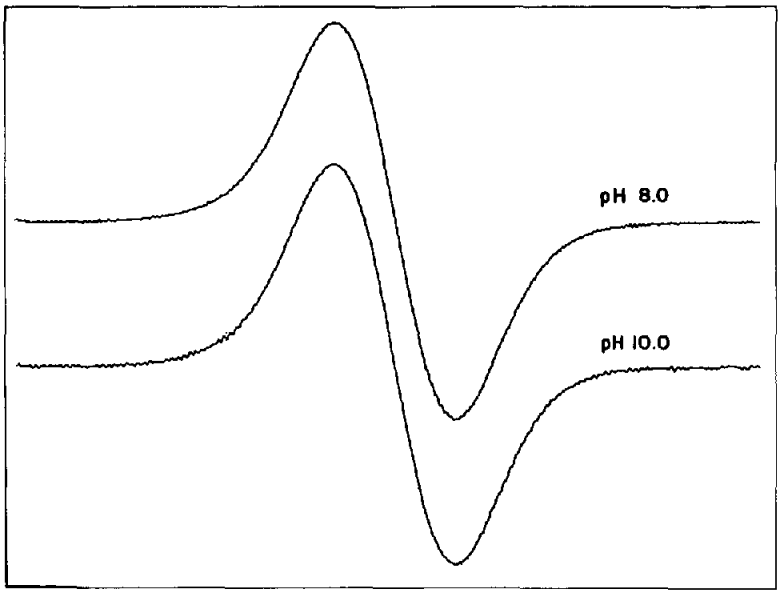

Fig.4. Effect of pH on *P*-700 photo-oxidation at 300 K in D-144 chloroplast fragments. The reaction mixture was as in fig.1 except that ascorbate was replaced by 10 μM methyl viologen. Samples were illuminated with red light (Corning 2-64 filter) at 300 K and frozen to 77 K in the light. Samples were then run at 15 K in the dark with the conditions as described in fig.2.

a significant effect could still be detected in the presence of this weak reductant.

As shown in fig.4, when D-144 fragments are illuminated at 300 K in the presence of methyl viologen (10 μM) and the samples are frozen in the light, *P*-700 can be trapped in the oxidized form (see also [14]). No effect of pH is observed, an indication that alkaline pH is not altering the EPR properties of *P*-700 per se and that alkaline pH has no effect on the photoreaction of *P*-700 when a sample is illuminated at 300 K and examined in the EPR instrument at 15 K.

4. Discussion

Our previous findings on the charge separation in Photosystem I [12] indicated that a stoichiometric relationship exists between photo-oxidized *P*-700 and the photoreduced bound iron-sulfur center after illumination at cryogenic temperatures. These results were obtained in both chloroplasts and Photosystem I fragments at 20 K in the presence of the reductant, ascorbate, at pH 7.8. The results of the present work indicate that at alkaline pH values (pH > 9.0) this stoichiometry no longer exists and that there is a decrease in *P*-700 but no decrease in the amount of the photoreduced iron-sulfur center. If we assume that the extent of the photoreduction of the bound iron-sulfur center at 15 K is a valid indicator of the primary charge separation reaction of Photosystem I, our results indicate that no inactivation of the reaction center of Photosystem I has occurred at alkaline pH. Thus, alkaline pH appears to affect uniquely the donor of Photosystem I.

The current results do not permit us to present a mechanism to explain these observations. A mechanism we currently are investigating requires the assumption of a rapid, pH-dependent re-reduction of oxidized *P*-700 after illumination at low temperature. Such a reduction could be caused by the added reductant or by an internal reductant generated only at alkaline pH values.

Our finding that the *P*-700 EPR signal is not fully developed at 15 K in samples incubated at alkaline pH values is crucial to recent interpretations of studies in which a correlation was attempted between the low-temperature reactions of *P*-700 and those of iron-sulfur centers. In many such studies, the extent of the

P-700 reaction in the presence of ascorbate [9,15–17] (*P*-700 reduced and iron-sulfur centers oxidized prior to illumination) is compared with the extent of the reaction in the presence of dithionite (*P*-700 reduced and iron-sulfur centers reduced prior to illumination). In general, these measurements have been performed at pH values greater than 10.0 in order to obtain reproducible chemical reduction of the iron-sulfur centers. It is clear that the *P*-700 formed after illumination at 15 K in the presence of ascorbate at pH greater than 9.0 represents only a small fraction of the total *P*-700 present and that the amount of photo-oxidized *P*-700 can vary from one preparation to another. Thus, comparisons of the extent of the *P*-700 photoreaction under various conditions at alkaline pH values may produce results that lead to widely divergent opinions about the nature of the primary reaction of Photosystem I. These observations may explain some of the discrepancies in the current literature about the identity of the Photosystem I primary electron acceptor.

Acknowledgements

This work was supported in part by grants from the National Science Foundation (PCM 76-02968 to A.J.B. and BMS-75-18879 to R.M.) and research funds to Donner Laboratory (to A.J.B.) from the Energy Research and Development Administration.

References

[1] Kok, B. (1956) Biochim. Biophys. Acta 22, 399–401.
[2] Bearden, A. J. and Malkin, R. (1975) Quart. Rev. Biophys. 7, 131–177.
[3] Malkin, R. and Bearden, A. J. (1971) Proc. Natl. Acad. Sci. USA 68, 16–19.
[4] Bearden, A. J. and Malkin, R. (1972) Biochem. Biophys. Res. Commun. 46, 1299–1305.
[5] Evans, M. C. W., Telfer, A. and Lord, A. V. (1972) Biochim. Biophys. Acta 267, 530–537.
[6] Visser, J. W. M., Rijgersberg, K. P. and Amesz, J. (1974) Biochim. Biophys. Acta 368, 235–246.
[7] Ke, B. (1974) in: Proc. Third Int. Congress on Photosynthesis (Avron, M. ed), pp. 373–382, Elsevier, Amsterdam.
[8] McIntosh, A. R., Chu, M. and Bolton, J. R. (1975) Biochim. Biophys. Acta 376, 308–314.
[9] Evans, M. C. W., Sihra, C. K., Bolton, J. R. and Cammack, R. (1975) Nature 256, 668–670.
[10] Anderson, J. M. and Boardman, N. K. (1966) Biochim. Biophys. Acta 112, 403–421.
[11] Malkin, R. (1975) Arch. Biochem. Biophys. 169, 77–83.
[12] Bearden, A. J. and Malkin, R. (1972) Biochim. Biophys. Acta 283, 456–468.
[13] Malkin, R. and Bearden, A. J. (1973) Biochim. Biophys. Acta 292, 169–185.
[14] Ke, B. (1972) Biochim. Biophys. Acta 267, 595–599.
[15] Bearden, A. J. and Malkin, R. (1976) Biochim. Biophys. Acta 430, 538–547.
[16] Evans, E. H., Cammack, R. and Evans, M. C. W. (1976) Biochem. Biophys. Res. Commun. 68, 1212–1218.
[17] Evans, M. C. W., Sihra, C. K. and Cammack, R. (1976) Biochem. J., in press.