0 0 0 4 0 0 3 3 3 2

Submitted to Federation of European
Biochemical Societies Letters

LBL-3624
Preprint c.1

OBSERVATION OF A NEW EPR TRANSIENT IN CHLOROPLASTS THAT MAY REFLECT THE ELECTRON DONOR TO PHOTOSYSTEM II AT ROOM TEMPERATURE

Robert E. Blankenship, Gerald T. Babcock,
Joseph T. Warden and Kenneth Sauer

December 18, 1974

RECEIVED
LAWRENCE
RADIATION LABORATORY

JAN 30 1975

LIBRARY AND
DOCUMENTS SECTION

Prepared for the U. S. Atomic Energy Commission
under Contract W-7405-ENG-48

For Reference

Not to be taken from this room

LBL-3624
c.1

DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.

0 0 4 3 0 0 6 3 3

OBSERVATION OF A NEW EPR TRANSIENT IN CHLOROPLASTS THAT MAY REFLECT THE ELECTRON DONOR TO PHOTOSYSTEM II AT ROOM TEMPERATURE

Robert E. BLANKENSHIP, Gerald T. BABCOCK,* Joseph T. WARDEN,** and Kenneth SAUER

Department of Chemistry, and Laboratory of Chemical Biodynamics, Lawrence Berkelev Laboratory, University of California, Berkeley, California 94720, USA

Received

1. Introduction

Knowledge of the reactions leading to production of oxygen by green plants and algae has expanded in the past several years through measurements of O_2 flash yields [1-3], fluorescence changes [4-6], H^+ production [7], and absorbance changes [8,9]. Most of these observations provide only indirect measures of the O_2 producing reactions, and interpretation of the results can be difficult. Direct observation of the components involved in the reactions leading to the oxidation of water would greatly facilitate progress in this area of research.

Recently experiments performed by Chen and Wang [10] and Babcock and Sauer [11-13] have focused attention on an EPR Signal II component that is

*Present Address: Department of Biochemistry, Rice University, Houston, Texas 77001, USA.

**Present Address: Department of Chemistry, Rensselaer Polytechnic Institute, Troy, N. Y. 12181, USA.

observed upon the inhibition of O_2 evolution. This component, which has been designated Signal IIf, is stoichiometric with P700 and is formed with high quantum efficiency in less than 500 μs following a flash. On the basis of these results, coupled with potentiometric evidence indicating that the reduction potential of the radical is high (E_m > +0.5 v), it was proposed that Signal IIf arises from $Z\bullet^+$, the electron donor to Photosystem II (PS II) [12]. Upon inhibition of oxygen evolution the rapid reduction of $Z\bullet^+$ is retarded, and the radical intermediate can be observed with conventional EPR methods.

In this communication we report an EPR transient with a spectrum similar to Signal II which is observed in O_2-evolving spinach chloroplasts at room temperature. We interpret this transient as arising from the electron donor to PS II.

2. Materials and Methods

Broken spinach chloroplasts were prepared as previously described [14]. Identical results for an experiment similar to that presented in Fig. 3 of this paper were obtained with three different isolation buffers: 0.4 M sucrose, 0.05 M HEPES, pH 7.6, 0.01 M NaCl; 0.4 M sucrose, 0.02 M Tricine, pH 7.2, 0.01 M KCl; or 0.4 M NaCl, 0.05 M phosphate, pH 7.6. Tris-washed and Tris-washed, reactivated chloroplasts were prepared as previously described [15]. All samples contained 10^{-4} M NADP, 20 μg/ml ferredoxin, and 10^{-3} M ascorbate. Chlorophyll content for all samples was 3-4 mg/ml.

ABBREVIATIONS: PS II, Photosystem II; DCMU, 3-(3,4-dichlorophenyl)-1, 1-dimethylurea.

EPR measurements were made using a Varian E-3 (X band, 9.3 GH) spectrometer. To improve the time response of the system, the signal from the E-3 detector preamplifier was processed with a Princeton Applied Research Model 210 selective amplifier and Model 220 lock-in amplifier. The reference signal for the lock-in amplifier was the 100 KHz magnetic field modulation signal of the E-3. The response time of the system, measured by the rise of Signal I, was 100 μs.

Xenon flashes (10 μs at half height) were obtained as described by Babcock and Sauer [16]. The light was transmitted through a lucite light pipe 8 feet long to minimize flash artifacts. The flash rate was 2 sec^{-1} for all data shown.

The signal averaging system described by Babcock and Sauer [11] was used for all experiments except those presented in Fig. 1, where a Nicolet NIC-80 computer was employed. All experiments were carried out at room temperature.

3. Results

Fig. 1a shows a kinetic trace of a transient EPR signal observed at 3380 Gauss in untreated spinach chloroplasts. This field position corresponds to the low-field maximum of Signal II [17,18]. The signal rises with the 100 μs time constant of the instrument, and decays by an apparent first-order process with a $t_{1/2}$ of 700 μs. Fig. 1b shows that no light-induced signal is observed in the presence of DCMU. The DCMU sensitivity, plus the observation that off-resonance controls also show no light-induced changes, demonstrate that the transient is not a flash artifact.

Fig. 2 shows the spectrum of Signal II taken in the dark (curve 1); Signal I plus Signal II taken in continuous light (curve 2); and the transient signal observed in flashing light (dots). The spectrum of the transient signal resembles Signal II, and clearly differs from Signal I.

Fig. 3 shows a comparison of Signal I and Signal II decay kinetics. The biphasic nature of P700 (Signal I) decay in this time regime has been observed optically by Haehnel, et al.[19]. The longer decaying component of Signal I, with $t_{1/2}$ about 20 ms, is similar to that observed by other workers [8,20,21].

We conclude that the transient of Fig. 3b is a previously unobserved kinetic component of Signal II in chloroplasts. Extending the kinetic notational scheme for PS II radicals of Babcock and Sauer [12], we designate this transient Signal IIvf (very fast).

Quantitative comparison of Signal II vf with Signal I using the data of Fig. 3 indicates that Signal IIvf/Signal I = 1.1 $\pm$ 0.2. The method used is a modification of that of Warden and Bolton [22]. Since Signal II does not have a Gaussian lineshape, it was necessary to determine empirically a proportionality constant between the Signal II amplitude at the derivative maximum and the area of Signal II as measured by double integration. The method of Warden and Bolton was used to determine the area of Signal I from the flash induced response. Assumptions implicit in this technique are that Signal I is Gaussian in shape and that Signal IIvf has the same spectrum as the Signal II species seen in the dark.

From the data presented so far it is difficult to know whether Signal IIvf arises from the donor or acceptor side of Photosystem II. As considered in the Discussion, the rate of subsequent reduction of the oxidized electron

donor to PS II may be very similar to the rate of oxidation of the reduced primary electron acceptor. The initial reduction of the acceptor Q is much faster (< 1 μs) than the oxidation of the donor Z (35 μs) [23]. Unfortunately, the response time of our EPR instrument is not fast enough to distinguish between these two possibilities, so an indirect method was used to assign Signal IIvf.

Babcock and Sauer [11-13] showed that Signal II exhibits a rapid (< 500 μs) rise and relatively rapid (0.5-1 s) decay in Tris-washed chloroplasts. The addition of Mn^{+2} decreased the magnitude of this kinetic component, called Signal IIf (fast), presumably by direct donation to the oxidized reaction center, $P680^{+}$ [13].

Fig. 4 shows the result of an experiment using Tris-washed chloroplasts. Curve a shows the light-induced response of untreated chloroplasts, where only the 700 μs EPR component is observed. Curve b shows the light-induced response of Tris-washed chloroplasts, where only an EPR signal decaying in 0.5-1 sec is observed. The rise occurs in <100 μs; previous measurements were instrument-limited at 500 μs. Curve c shows the response of Tris-washed chloroplasts to which 10^{-3}M Mn^{+2} has been added. No light-induced change is observed. This result supports assignment of Signal IIvf to the donor side of PS II, as considered in greater detail in the Discussion.

Fig. 5 shows a comparison of the Signal II response in untreated, Tris-washed, and Tris-washed, reactivated chloroplasts. The untreated sample shows a typical Signal IIvf response, and the Tris-washed sample a typical Signal IIf response. The Tris-washed, reactivated sample shows a response similar to that of the untreated sample, only the decay kinetics of Signal IIvf are somewhat slower (1 ms vs. 500 μs in the untreated sample).

4. Discussion

The primary photochemistry of Photosystem II and secondary electron transfer reactions leading to the evolution of O_2 by green plants and algae comprise one of the most interesting and yet one of the least understood aspects of photosynthesis. For reviews, see Refs. 8, 9, 24. Our current concept of the reactions of Photosystem II centers around the reaction center chlorophyll, P680. After a photon is absorbed and transferred to the reaction center, P680 loses an electron and becomes $P680^+$. The absorption change resulting from P680 oxidation occurs within 1 μs following a flash [23].

$$P680\ Q \xrightarrow{h\nu} P680^*Q \xrightarrow{\leq 1\ \mu s} P680^+Q^- \qquad (1)$$

After the initial charge separation, further reactions occur to stabilize the oxidant and reductant. The first of these reactions is the reduction of $P680^+$ by an unknown component usually called Z, the electron donor of PS II. Gläser *et al.* [23] have reported τ = 35 μs for $P680^+$ reduction at room temperature.

$$Z\ P680^+Q^- \xrightarrow{35\ \mu s} Z^+P680\ Q^- \qquad (2)$$

The redox state of the acceptor Q can be monitored by fluorescence measurements. If Q is oxidized, chloroplast fluorescence is low; but when Q is reduced, chloroplast fluorescence is high [25]. This dependence of fluorescence on the redox state of Q appears to be valid only at times greater than 10-35 μs after a flash [4,5,26]. At shorter times the fluorescence behavior is very complex and does not seem to correlate with the redox state of Q. Zankel [4] and Mauzerall [5] have found the kinetics of

oxidation of Q^- to be biphasic, with components of 200 μs and 2 ms. Earlier work by Forbush and Kok [27] gave a single decay time of 0.6 ms.

X320, a component having a light-induced absorbance change at 320 nm, has been observed by Stiehl and Witt [28] and Witt [29] and assigned as the primary electron acceptor to PS II. X320 has rise kinetics of <30 μs and decay kinetics of 600 μs. The reaction on the acceptor side is thus represented by Eq. 3.

$$P680\ Q^-A \xrightarrow{0.2 - 2\ ms} P680\ Q\ A^- \qquad (3)$$

The kinetics of the rate-limiting steps of O_2 evolution have been studied using polarographic detection of O_2 produced in flashing light [1-3,8]. The results indicate that PS II photochemistry is limited by dark reactions which occur with a halftime of 0.2 - 1 ms in dark-adapted chloroplasts [2,3] or 0.6 ms in systems with a large number of previous flashes [8]. It is difficult to determine whether this rate-limiting reaction occurs on the donor or acceptor side of the photoact, since both the donor and acceptor must be restored to their original redox states before another flasn can be effective. The time constant for the reduction of Z^+ must therefore be less than about 1 ms.

$$S\ Z^+\ P680 \xrightarrow{<1\ ms} S^+Z\ P680 \qquad (4)$$

The transient species reported in this communication has kinetic properties consistent with a location on either the donor or acceptor side of PS II. The results of Fig. 4 show that upon Tris-washing dramatic changes occur in the light-induced EPR signals observed in the magnetic field region of Signal II. The rapid ($t_{1/2}$ = 700 μs) decay seen in untreated chloroplasts is about 1000 fold slower ($t_{1/2}$ = 0.5 - 1 s) in Tris-washed chloroplasts. Addition of Mn^{+2} eliminates most of the light-induced change.

If Signal IIvf arose from a species on the acceptor side of PS II, we would expect it to be still present in Tris-washed chloroplasts and not to be affected by the addition of exogenous Mn^{+2}. Fig. 4 shows that there is no very fast component of Signal II in Tris-washed chloroplasts and, furthermore, that the Signal IIf observed is abolished by added Mn^{+2}. This is the expected result if Signal IIvf arises from the same species as does Signal IIf and Mn^{+2} competes with it as a donor to $P680^{+}$ [13]. We tentatively conclude that Signal IIvf arises from Z, the electron donor to PS II. A definite assignment can best be made upon measurement of the rise time of the transient in untreated chloroplasts, avoiding the ambiguities associated with treated chloroplast systems.

The experiments shown in Fig. 5 are also consistent with the hypothesis of Babcock and Sauer [12] that Signal IIf in Tris-washed chloroplasts is the electron donor to PS II with altered kinetics. Upon reactivation of O_2 evolution Signal IIvf returns and Signal IIf disappears. The inverse correlation between Signal IIf and O_2 evolution has been described in earlier publications [11,15]. Signal IIvf appears to have a direct correlation with O_2 evolution capacity.

Acknowledgment

This work was supported by the U. S. Atomic Energy Commission.

References

[1] Joliot, P., Barbieri, G. and Chabaud, R. (1969) Photochem. Photobiol. 10, 309-329.

[2] Kok, B., Forbush, B. and McGloin, M. (1970) Photochem. Photobiol. 11, 457-475.

[3] Bouges-Bocquet, B. (1973) Biochim. Biophys. Acta 292, 772-785.

[4] Zankel, K. L. (1973) Biochim. Biophys. Acta 325, 138-148.

[5] Mauzerall, D. (1972) Proc. Nat. Acad. Sci. U.S. 69, 1358-1362.

[6] Delosme, R. (1972) in: Proc. 2nd Int. Congr., Photosynthesis Research, Stresa (Forti, G., Avron, M. and Melandri, A., eds.), Vol. 1, pp. 187-195, Dr. W. Junk N.V. Publishers, The Hague.

[7] Fowler, C. F. and Kok, B. (1974) Biochim. Biophys. Acta 357, 299-307.

[8] Witt, H. T. (1971) Quart. Rev. Biophys. 4, 365-477.

[9] Butler, W. L. (1973) Accounts Chem. Res. 6, 177-184.

[10] Chen, K.-Y. and Wang, J. H. (1974) Bioinorg. Chem. 3, 339-352.

[11] Babcock, G. T. and Sauer K., (1974) Biochim. Biophys. Acta, 368, 282-294.

[12] Babcock, G. T. and Sauer, K., (1975) Biochim. Biophys. Acta, in press.

[13] Babcock, G. T. and Sauer, K., Biochim. Biophys. Acta, submitted.

[14] Blankenship, R. E. and Sauer, K. (1974) Biochim. Biophys. Acta 357, 252-266.

[15] Blankenship, R. E., Babcock, G. T. and Sauer, K., Biochim. Biophys. Acta., submitted.

[16] Babcock, G. T. and Sauer, K. (1973) Biochim. Biophys. Acta 325, 483-503.

[17] Weaver, E. C. (1968) Ann. Rev. Plant Physiol. 19, 283-294.

[18] Warden, J. T., and Bolton, J. R. (1974) Accounts Chem. Res. 7, 189-195.

[19] Haehnel, W., Döring, G. and Witt, H. T. (1971) Z. Naturforsch. 26b, 1171-1174.

[20] Warden, J. T., Jr., and Bolton, J. R. (1974) Photochem. Photobiol. 20, 263-269.

[21] Ke, B. (1973) Biochim. Biophys. Acta 301, 1-33.

[22] Warden, J. T., Jr., and Bolton, J. R. (1973) J. Am. Chem. Soc. 95, 6435-6436.

[23] Gläser, M., Wolff, Ch., Buchwald, H.-E. and Witt, H. T. (1974) FEBS Letters 42, 81-85.

[24] Cheniae, G. M. (1970) Ann. Rev. Plant Physiol. 21, 467-498.

[25] Duysens, L.N.M. and Sweers, H. E. (1963) in: Studies on Microalgae and Photosynthetic Bacteria (Miyachi, S., ed.), pp. 353-372, Univ. of Tokyo Press, Tokyo.

[26] Duysens, L.N.M., van der Schatte Olivier, T. E. and den Haan, G. A. (1972) Abst. VI. Int. Congr. Photobiol. Bochum 1972, No. 277.

[27] Forbush, B. and Kok, B. (1968) Biochim. Biophys. Acta 162, 243-253.

[28] Stiehl, H. H. and Witt, H. T. (1969) Z. Naturforsch. 24b, 1588-1598.

[29] Witt, K. (1973) FEBS Letters 38, 116-118.

Figure Captions

Fig. 1. Flash-induced change in EPR absorption at 3380 G in spinach chloroplasts at room temperature. a) untreated chloroplasts, b) chloroplasts plus 10^{-4} M DCMU. Each trace is the average of 4,000 events, with the sample changed after 1,000 flashes. Microwave power 20mw, modulation amplitude 3.15 G.

Fig. 2. Spectra of light-induced changes in EPR signals in chloroplasts. Spectrum 1 was recorded in the dark followed by spectrum 2 in continuous light. The spectrum of the transient signal, represented by ●, was done point by point using flashing light and signal averaging. The transient spectrum was normalized to spectrum 1 at 3380 G. In the region where Signals I and II overlap, the different kinetic behavior of the two signals permitted determination of the contribution of each to the total signal. A fresh sample was used for each point. Each point is the average of 1,024 events. Microwave power 20mw, modulation amplitude 6.3 G. For spectra 1 and 2, time constant 0.3 sec, scan rate 25 G/min.

Fig. 3. Flash-induced change in EPR Signal I (curve a) and Signal II (curve b). The magnetic field was 3391 G for Signal I and 3379 G for the Signal II transient. Curve b was recorded on a four times more sensitive scale than curve a. Each curve is the average of 1,024 events. Microwave power 20mw, modulation amplitude 3.15 G.

Fig. 4. Flash-induced changes in EPR signals at 3382 G. a) Untreated chloroplasts; b) Tris-washed chloroplasts plus 10^{-4} M EDTA; c) Tris-washed

chloroplasts plus 10^{-3} M Mn^{+2}. Each curve is the average of 1,024 events. Microwave power 100mw, modulation amplitude 5.0 G.

Fig. 5. Flash-induced changes in EPR signals at 3375 G. a) Untreated chloroplasts; b) Tris-washed chloroplasts; c) Tris-washed, reactivated chloroplasts. Each curve is the average of 1024 events. Microwave power 100mw, modulation amplitude 5.0 G.

0 0 4 3 0 0 6 3 9

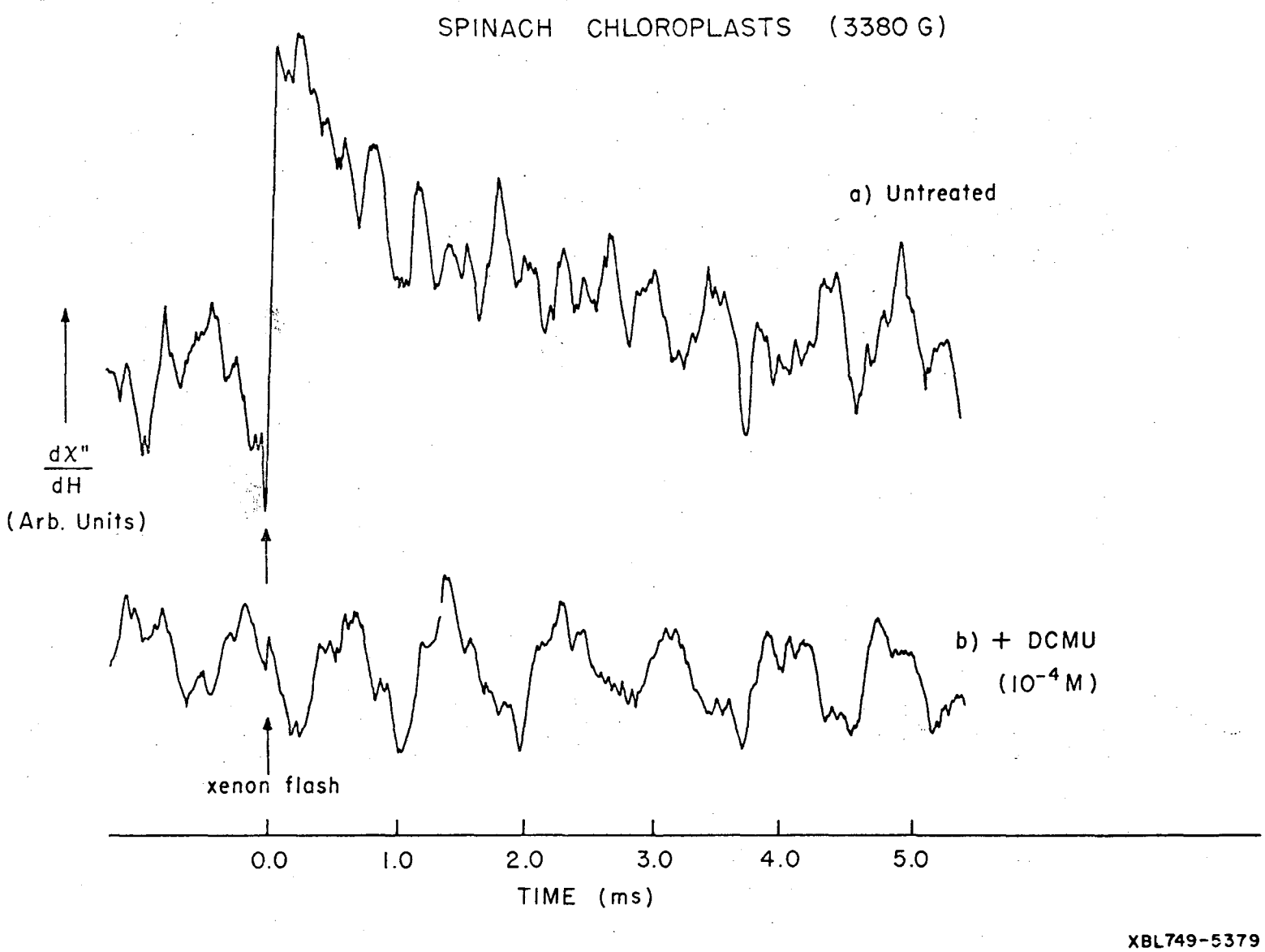
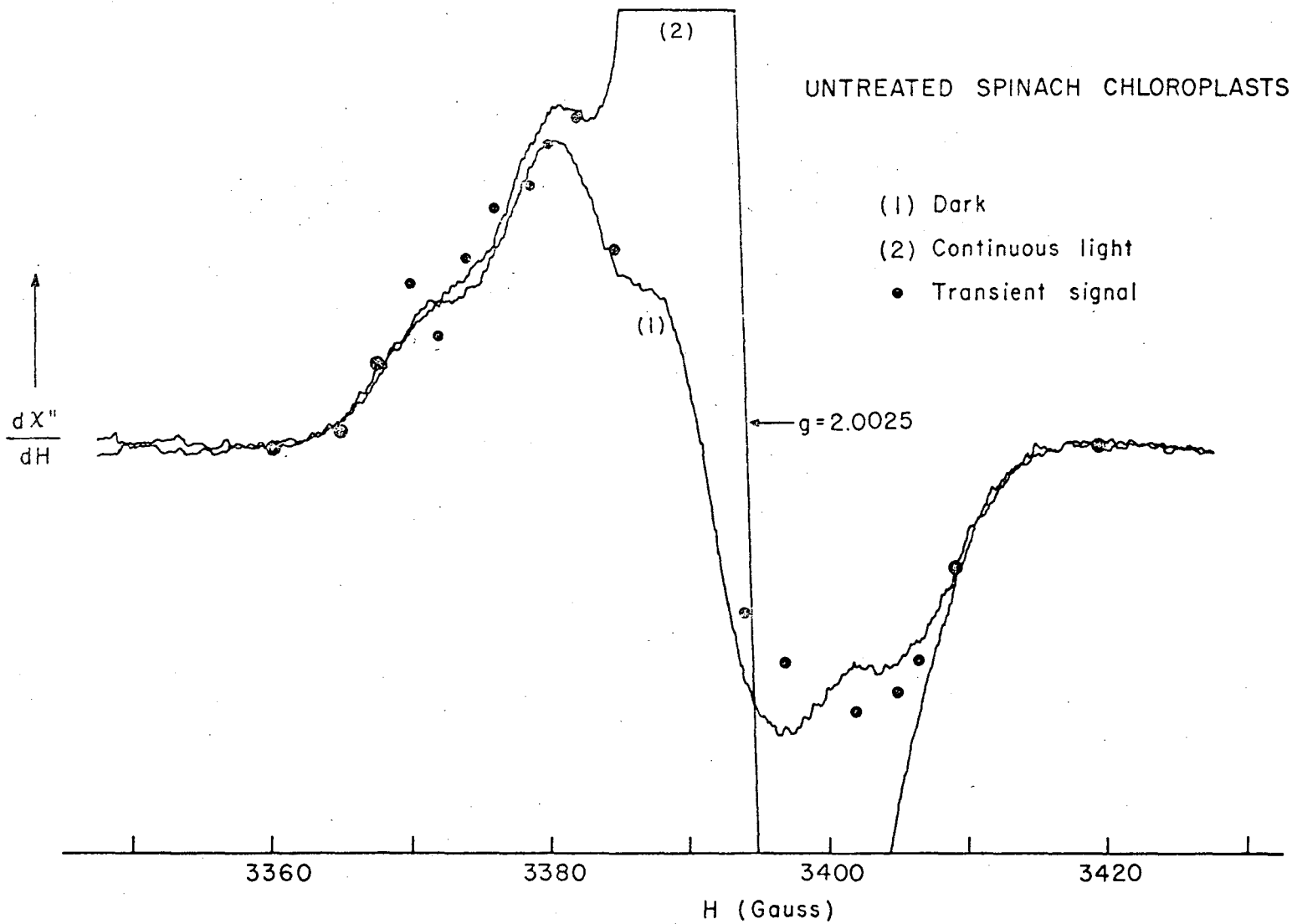


Fig. 1.



XBL749-5378

Fig. 2.

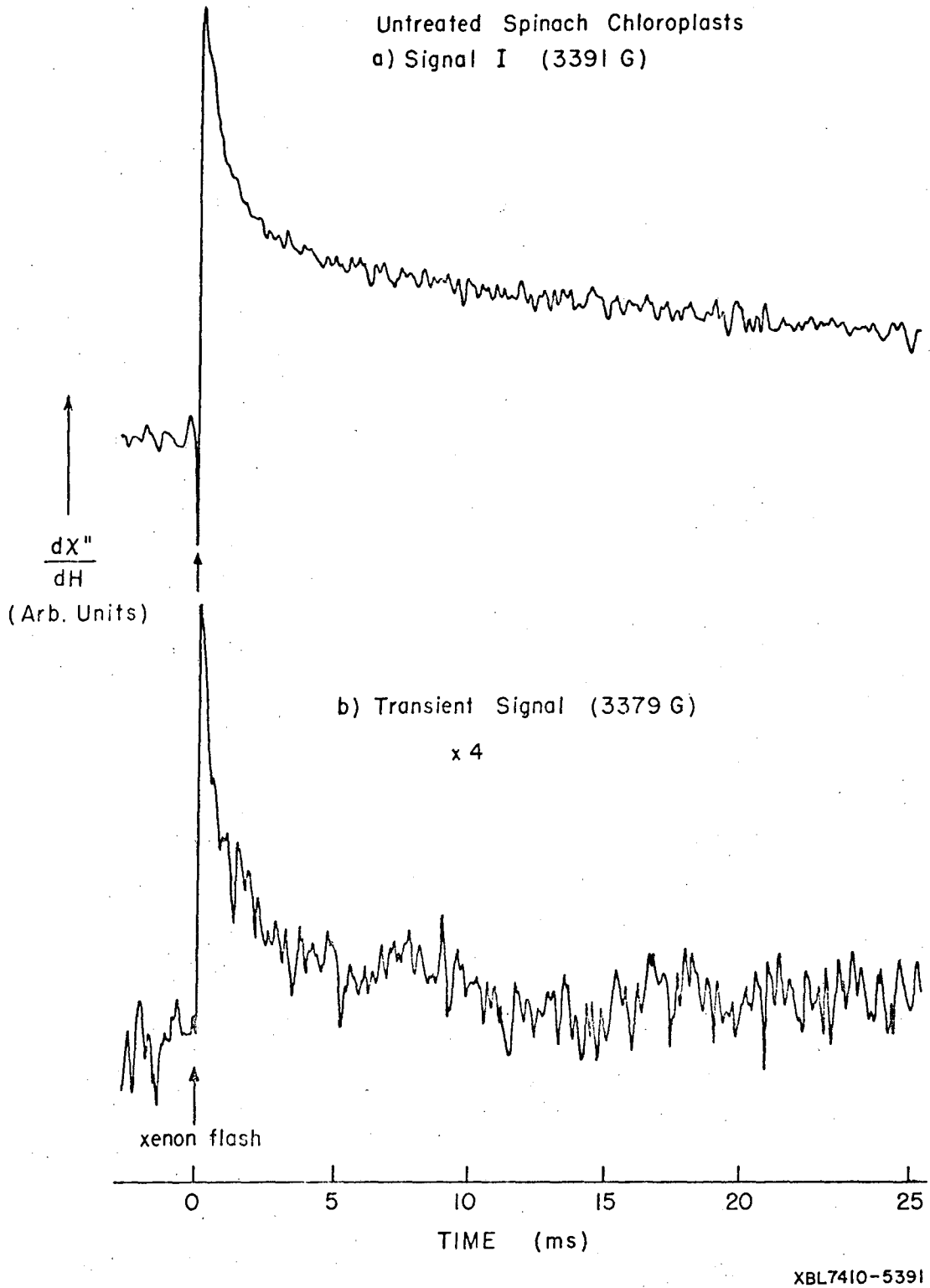

Untreated Spinach Chloroplasts
a) Signal I (3391 G)
dχ"/dH
(Arb. Units)
b) Transient Signal (3379 G)
x 4
xenon flash
0
5
10
15
20
25
TIME (ms)
XBL7410-5391

Fig. 3.

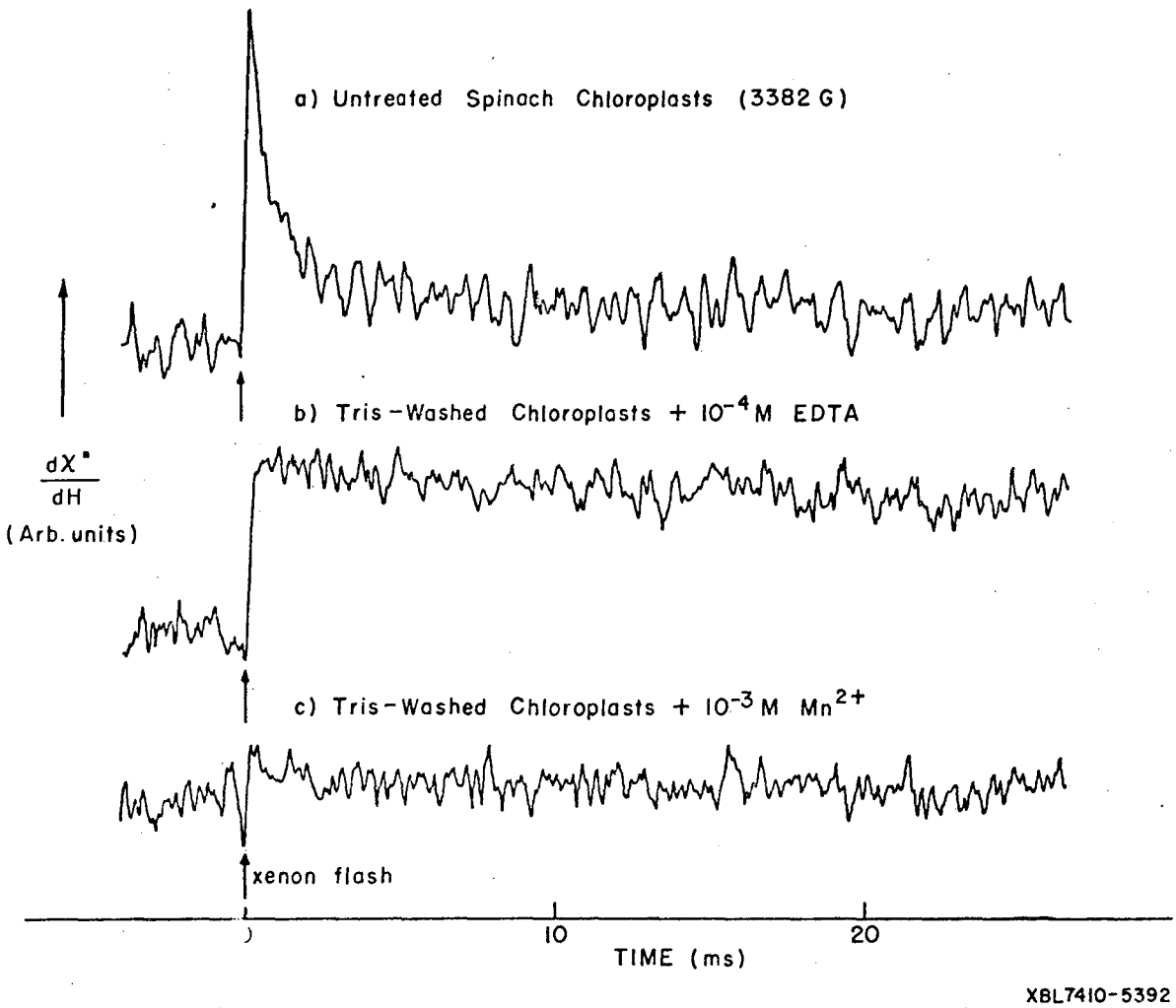

a) Untreated Spinach Chloroplasts (3382 G)
b) Tris-Washed Chloroplasts + 10^{-4} M EDTA
c) Tris-Washed Chloroplasts + 10^{-3} M Mn^{2+}
$\frac{d\chi''}{dH}$
(Arb. units)
xenon flash
10
20
TIME (ms)
XBL7410-5392

Fig. 4.

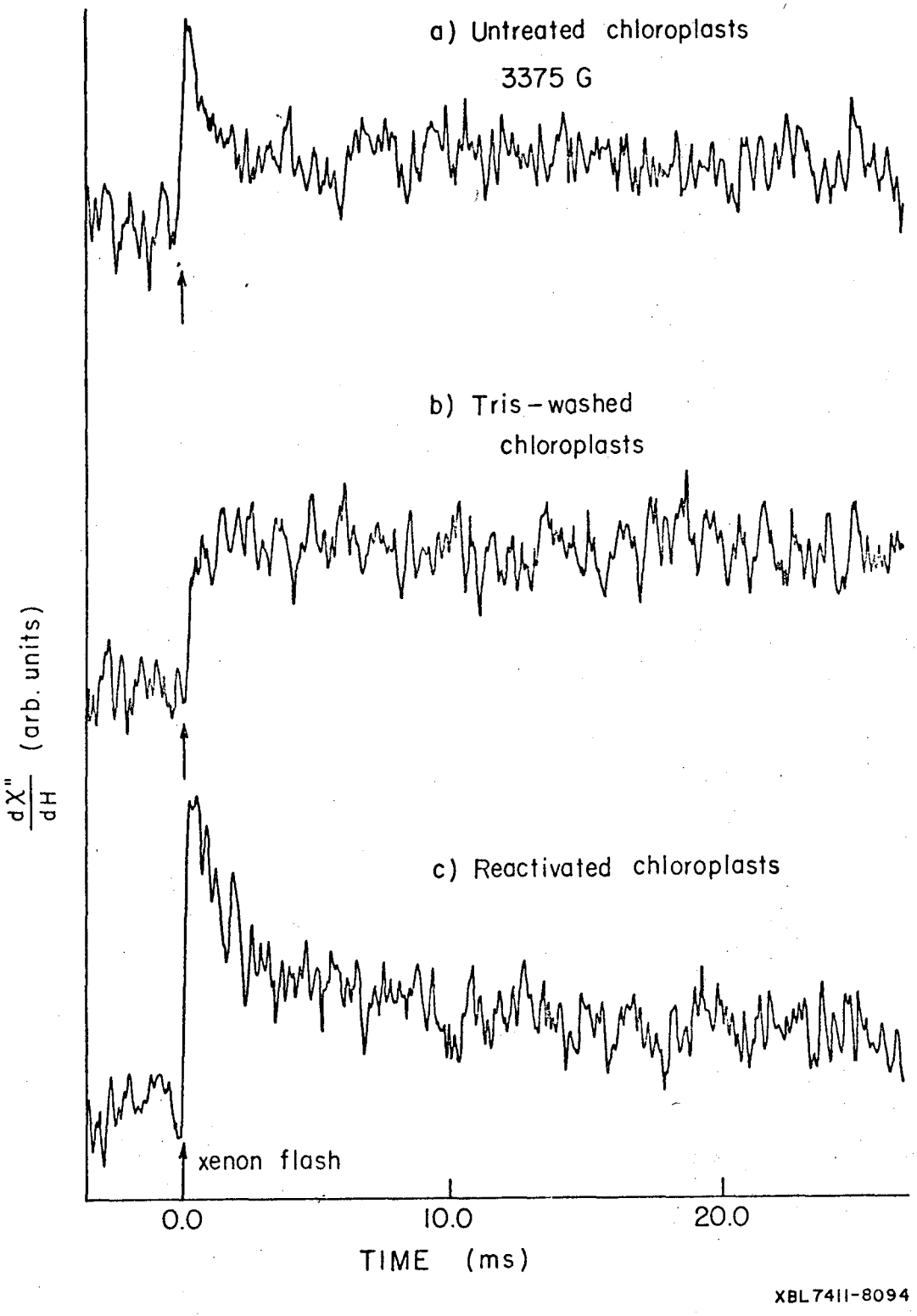

a) Untreated chloroplasts
3375 G
b) Tris-washed chloroplasts
c) Reactivated chloroplasts
xenon flash
$\frac{d\chi''}{dH}$ (arb. units)
0.0
10.0
20.0
TIME (ms)
XBL7411-8094

Fig. 5.

LEGAL NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Atomic Energy Commission, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

TECHNICAL INFORMATION DIVISION
LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720