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THE POLYPHASIC RISE OF THE CHLOROPHYLL *A* FLUORESCENCE (O-K-J-I-P) IN HEAT-STRESSED LEAVES

BY

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ABSTRACT

The chlorophyll *a* (Chl *a*) fluorescence induction kinetics from the minimum yield Fo to the maximum yield Fm, is an indicator of the accumulation of net reduced primary bound plastoquinone (Q_A^-). In this paper we have examined the effect of high temperature (above 44°C on the chl *a* fluorescence kinetics in potato and pea leaves using a shutter-less system which allows to measure the fluorescence signals from 10 µs to 120 s. After the heat treatment the variable Chl *a* fluorescence was dramatically quenched. Furthermore, by increasing the temperature or the duration of the heat treatment, the typical O-J-I-P transient is transformed to an O-K-J-I-P transient with a new rapid step called "K-step" detected at about 200 µs. A big dip appears after the "K-peak", followed by an increase of the Chl *a* fluorescence intensity. In order to investigate the origin and to proceed to a photochemical interpretation of these changes, we have studied the effect of NH_2OH which acts as an electron donor to PSII, as well as the effect of DCMU which is known to block the electron transport chain between Q_A^- and Q_B. Based on all these data, we propose that the appearance of this new "K-step" is due as well as to an severe inhibition of the water-splitting system and a partial inhibition of the electron transport flow between $PPhe^-Q_A^-$ and $PPheQ_A^-$.

Key-words: Chlorophyll, Fluorescence, DCMU, Hydroxylamine, Photosystems Reaction Center; Heat-stress; *Solanum tuberosum* L.

INTRODUCTION

Photosynthetic adaptation to temperature is an important factor determining the survival of plants in hot climates as the photosynthetic processus is the most sensitive to high temperature (Björkman, 1975). In higher plants, the electron flow goes from H_2O to $NADP^+$ through two principal pigment-protein-complexes of the photosynthetic apparatus: photosystem II (PSII) and photosystem I (PSI) (see Govindjee and Govindjee, 1975). It has been documented earlier that the primary site of environmental stress damage is associated with components of the photosynthetic system located in the thylakoids membranes, most probably with PSII (see Havaux, 1993).

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Already in 1931, the variable chlorophyll *a* (Chl *a*) fluorescence of plants has been related to the time course of photosynthesis by Kautsky (Kautsky and Hirsch, 1931). The wide use of Chl *a* fluorescence measurements in stress research can be attributed to the following advantages that the method provides: it is easy, rapid, efficient, non-destructive and can be used on small samples of all green plant material (Lichtenthaler *et al.*, 1988; Luuk *et al.*, 1992). Plants live in a heavy-changing environment and their growth is affected by a multitude of environmental factors, such as temperature, light, water and CO_2. Lack or excess of any of these or other factors causes stress on plants (Lichtenthaler *et al.*, 1988).

At room temperature, the Chl *a* fluorescence transient induction follows an O-J-I-P pattern from the initial low fluorescence level (Fo), to the maximum fluorescence level (Fp) or (Fm) (as described in detail by Strasser *et al.*, 1995). We have used a Plant Efficiency Analyser (PEA) to investigate the effect of high temperature stress on the Chl *a* fluorescence transient kinetics in leaf discs of potato and pea plants. We have observed that by increasing either the temperature or the duration of the heat treatment, the Chl *a* fluorescence transient exhibits many changes as following: an increase in the initial low fluorescence (Fo), a decrease in the maximum fluorescence (Fp) and therefore a large quenching in the whole variable Chl *a* fluorescence transient. These effects have been mentioned earlier in the literature as damages in the PSII activity (see Weis, 1981; Havaux *et al.*, 1992; Klinkovsky *et al.*, 1994). The leaves and isolated chloroplasts show a marked reduction in their photosynthetic activity after exposure to temperatures above 40–45°C (Naus, 1992).

In this study we demonstrate, for the first time, that high temperature above 43°C transforms the O-J-I-P Chl *a* fluorescence transient of all green leaves to an O-K-J-I-P fluorescence transient with a new rapid step detected at 200 μs which we call "K-step".

In respect to historical reasons (see Strasser *et al.*, 1995), the intermediate steps between Fp and Fo of the fluorescence transient are labelled in alphabetical order as I-J-K from longer to shorter times. In this paper we report the phenomenological appearance of this "K-step", and propose some possible interpretations of this new phenomenon.

MATERIALS AND METHODS

a) Plant materials

All the experiments were done with 6 mm leaf discs from dark adapted fully matured intact leaves of potato (*Solanum tuberosum* L.) and pea (*Pisum sativum* L.) plants. During the first three weeks, the plants were grown in 50 ml culture tube containing 15 ml of MS medium (Murashige and Skoog, 1962), and then tranferred in pots in a soil mixture Optima (Optima Werke, Munchenstein, Switzerland) in the green house under 22°C/18°C and 16h/8h (day/night). Plants were watered daily.

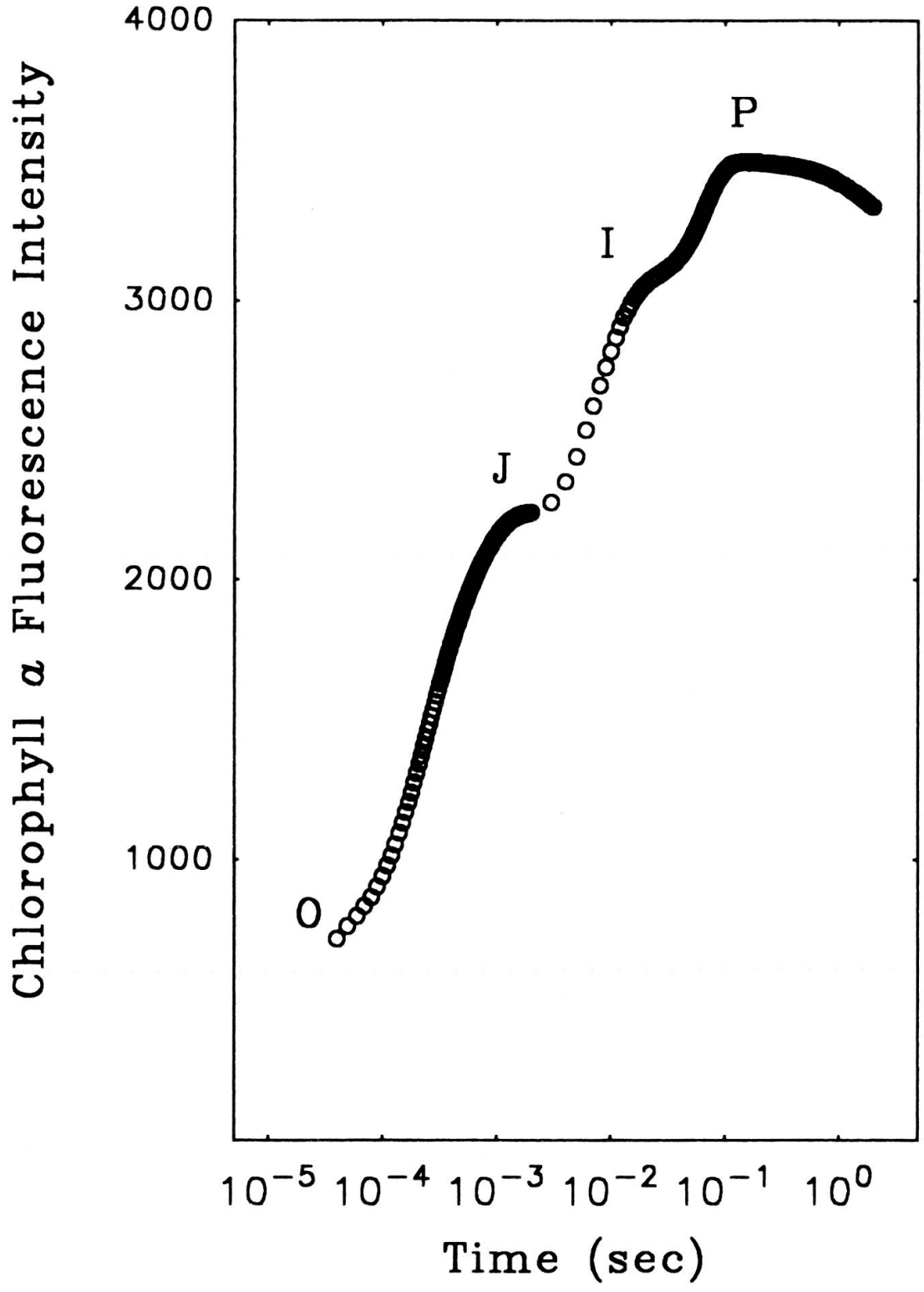

FIG. 1.

Chl *a* fluorescence transients of a dark adapted potato leaf, excited with a red (650 nm) LEDs of 600 Wm^{-2}. The graph plotted on a logarithmic time scale shows the typical polyphasic fluorescence rise, showing the steps O, J, I and P.

b) Heat-stress treatment

Heat stress was induced by immersing the leaf discs directly in the circulating waterbath at different temperatures ranging from 20°C to 50°C for different durations, in the dark. A varying time for re-adaptation at room temperature follows the heat treatment before the Chl *a* fluorescence signals were recorded.

For the NH_2OH treatment, the leaf discs were first heat treated at 47°C for 5 min and then immediately vacuum infiltrated for 30 sec in darkness at room temperature in an infiltration medium (100 mM sorbitol, 50 mM HEPES, 0.5 mM $CaCl_2$ and 0.5 mM $MnSO_2$, pH 7.5) with or without 40 mM NH_2OH. For the DCMU treatment, the leaves were heat treated at 47°C for 5 min and then immersed in H_2O containing 5 mM DCMU for 30 min at room temperature in dark. The control measurements were done on leaf discs heat treated at 47°C and immersed immediately in distilled water at 20°C for 30 min in the dark.

c) Chl *a* fluorescence measurements

The Chl *a* fluorescence induction was measured at room temperature using a Plant Efficiency Analyser (PEA) built by Hansatech Ltd. King's Lynn, Norfolk, England. This instrument gives an excitation light with a maximum intensity of 600 Wm^{-2} by an array of six light emitting diodes (peak 650 nm), focused on the sample surface to provide a homogeneous illumination light spot of about 4 mm in diameter. The fast fluorescence transient was recorded from 10 µs to 1 sec. The initial fluorescence Fo is considered to be around 40 µs. All the recorded fluorescence transients were plotted in logarithmic and/or linear time scale over 5 orders of magnitude (10 µs to 1 s).

RESULTS AND DISCUSSION

When a dark adapted leaf disc of any green plant is excited during 1 sec by a light intensity of 600 Wm^{-2}, it exhibits a fast rise fluorescence transient from an initial low Chl *a* fluorescence level, called Fo, considered as the emission emanating from the antenna pool of the Chl *a* pigment bed when all the PSII Reaction Centers (RC) are photochemically active (all Q_A are oxidized) (Armond *et al.*, 1980), to a maximum level, called Fp, where all Q_A are in the reduced state (see Strasser *et al.*, 1992). In control conditions (20°C in distilled water for 5 min), the fast Chl *a* fluorescence induction transient shows a polyphasic rise (see Fig. 1) called O-J-I-P fluorescence transient as described in detail elsewhere by Strasser *et al.*, 1991, 1992, 1995. This fast O-J-I-P fluorescence transient shows three different phases: O to J (around 2 ms), J to I (around 30 ms) and I to P (around 500 ms) with two intermediate steps J and I in between Fo and Fp. The fluorescence induction kinetics measured in continuous light, shows the filling up of the electron acceptor side of PSII. The O to J phase is due to the reduction of Q_A to Q_A^-. This O to J phase is also influenced by the S-states of the donor

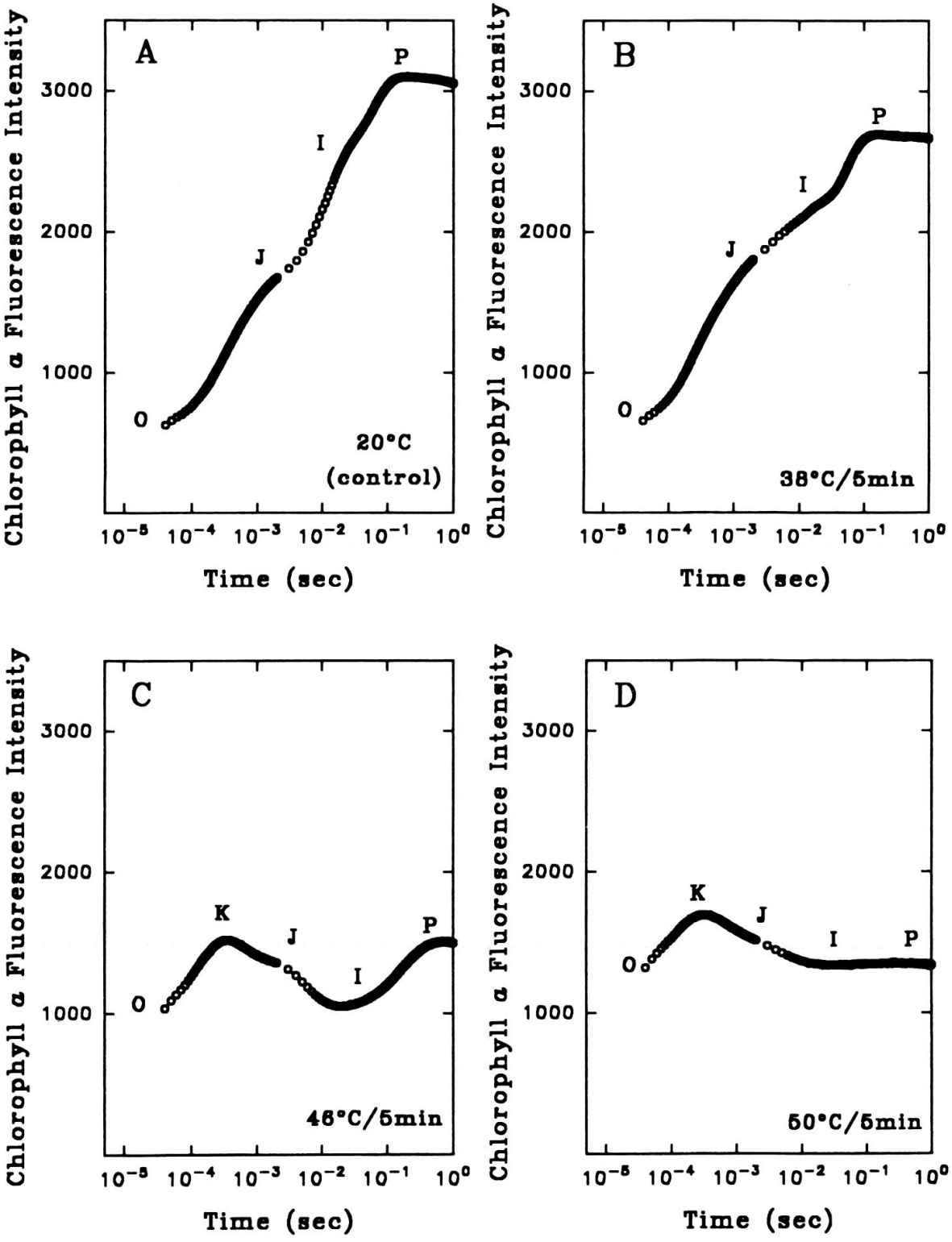

FIG. 2.

Chl *a* fluorescence transient of control (A); heated leaf discs for 5 min at 38°C (B), 46°C (C) and 50°C (D), showing the temperature dependance of the appearance of "K-step". All the samples were re-adapted at room temperature for 10 s before measurements.

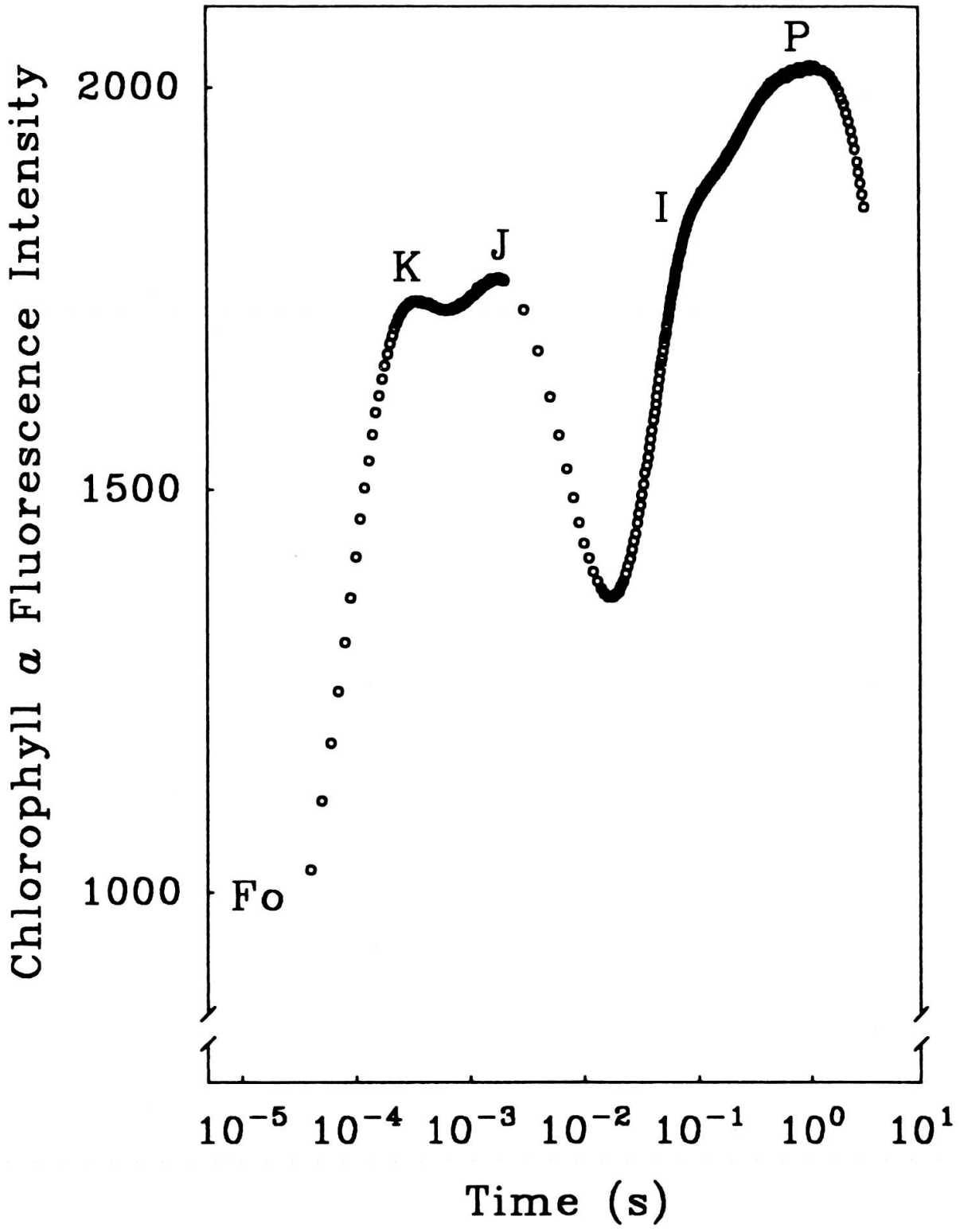

FIG. 3.

Chl *a* fluorescence transients of potato leaf disc after heat treatment for 13 min at 44°C and dark adaptation at room temperature for 10 s. The graph plotted on logarithmic time scale shows distinctly 4 steps: O (Fo), K, J, I and P.

side of PSII (Delosme, 1967; Hsu, 1993). The intermediate step I and the final step P has been proposed to be due to the existence of fast and slow reducing plastoquinol (PQ) pool-centers as well as to different redox states of the RC, which reduces the PQ pool (Strasser *et al.*, 1995).

The temperature stress provokes many changes in the O-J-I-P fluorescence transient. For 5 min of heat treatment, even at 30°C, Fp exhibits a decrease and Fo an increase. The extent of both changes increases continuously with temperature. The same type of observation was indicated earlier (Weis, 1981; Havaux *et al.*, 1992; Klinkovsky *et al.*, 1994). However, along with these changes, at high temperature, the O-J-I-P transient exhibits a gradual transformation into an O-K-J-I-P Chl *a* fluorescence transient, with a new intermediate step "K-step" appearing at about 200 μs. This new "K-step", between O and J-step, becomes dominant step in the O-K-J-I-P transient at 46°C (Fig. 2 C).

The effect of heat treatment duration was also studied. Fig. 4 shows that, by increasing the duration of the heat treatment, Fp decreases but Fo shows no significant changes. Concerning the transformation of the O-J-I-P transient to O-K-J-I-P transient, we have found that, prolongation of the heat treatment causes a more pronounced emerging of the "K-step' (see Fig. 4).

Since it has been shown earlier (Strasser *et al.*, 1995) that the O-J-I-P steps can be shifted depending on light intensity used, it was important to show that this O-K-J-I-P transient is not resulting by such a shift. Thus, our effort was to find an optimal combination of temperature and duration of treatment which would lead to a Chl *a* fluorescence transient exhibiting undoubtfully that "K" is a new step. Fig. 3 shows clearly, for the first time, the Chl *a* fluorescence transient of potato leaf disc heated at 44°C during 13 min, with three distinct intermediate steps K, J and I between Fo and Fp. We have observed that, whenever the "K-step" appears, the original shape of O-J-I-P can not be restored even after re-adaptation of heat treated leaf discs at room temperature for more than 1 h (data not shown). We suggest that, the appearance of the "K-step" could be correlated to an irreversible heat-damage in PSII.

Fig. 5 shows the fraction of each step K, J, I, P where Fsum = Fk + Fj + Fi + Fp. The fraction Fk shows a big increase around 40°C which we consider as the threshold temperature between mild temperatures and high temperatures for potato leaves. The J-level fraction was totally unaffected after heat treatment but the fraction of both I-level and P-level decreases at high temperatures.

We suggest that the increase observed in the initial low fluorescence level (Fo) could be due to the increase of the slope of the initial rise resulting from the appearance of the new "K-step". This observation is in agreement with Buhkov *et al.* (1990), where they said that: the increase in the Chl *a* fluorescence observed after preheating a sample is not solely linked to an increase in the Fo-level.

Concerning the dip observed after the "K-peak" (Fig. 6 curve a), it can be related to the major injury caused by high temperature, which is known to be on the water oxidizing side (Schreiber *et al.*, 1997; Havaux, 1993). In order to verify this hypothesis,

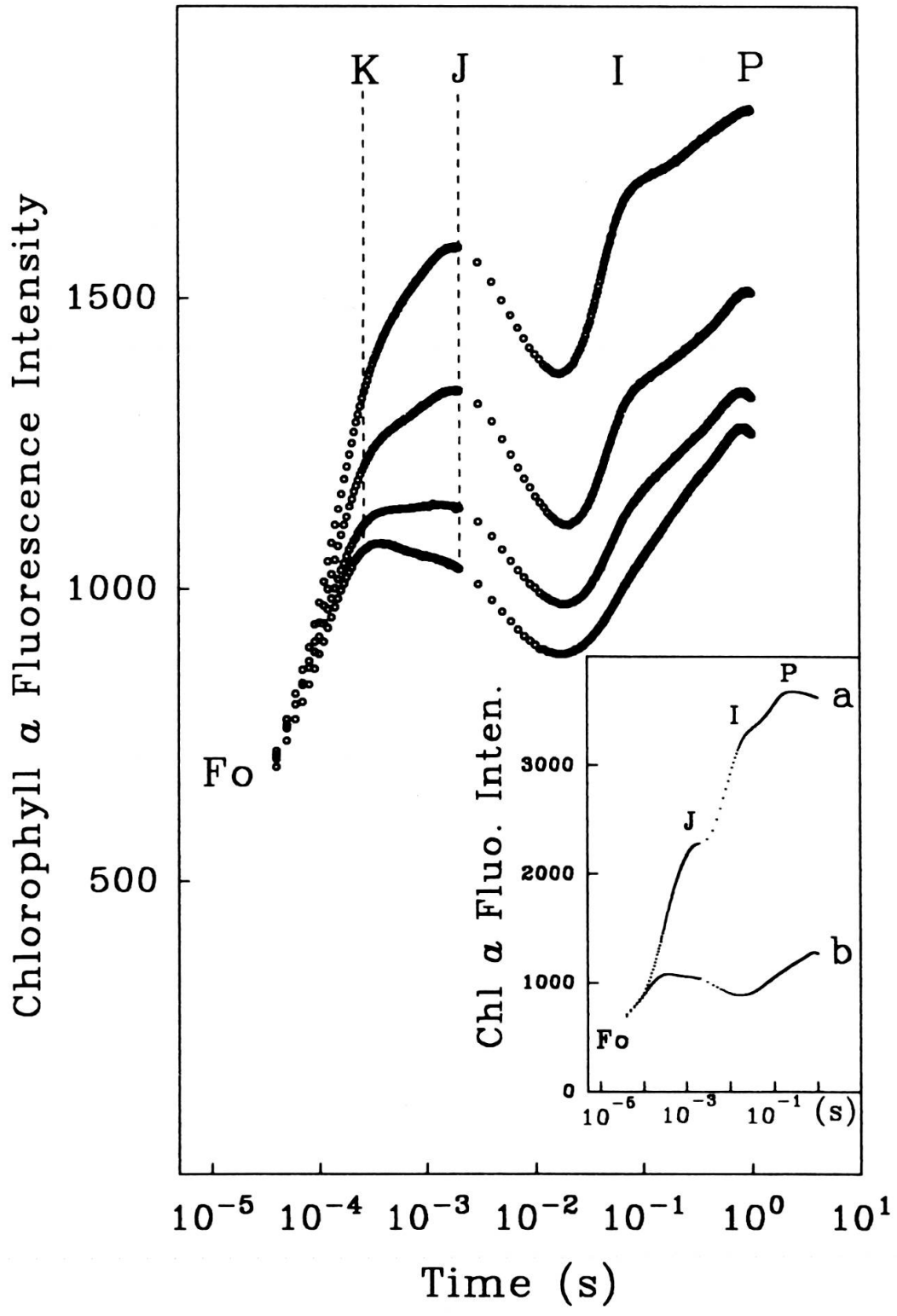

Fig. 4.

Time course change in the Chl *a* fluorescence transient of potato leaf discs after heat treatment at 44°C in the dark. From top to bottom, leaf discs were heat treated for 5, 8, 13 and 15 min respectively. After heat treatment, leaf discs were re-adapted at room temperature for 10 s before fluorescence measurements. Note the appearance of the "K" peak after longer heat treatment. Insert, Chl *a* fluorescence transient of potato leaf disc heated either at 20°C (a) or 44°C (b) for 15 min. All the graphs are plotted on a logarithmic time scale.

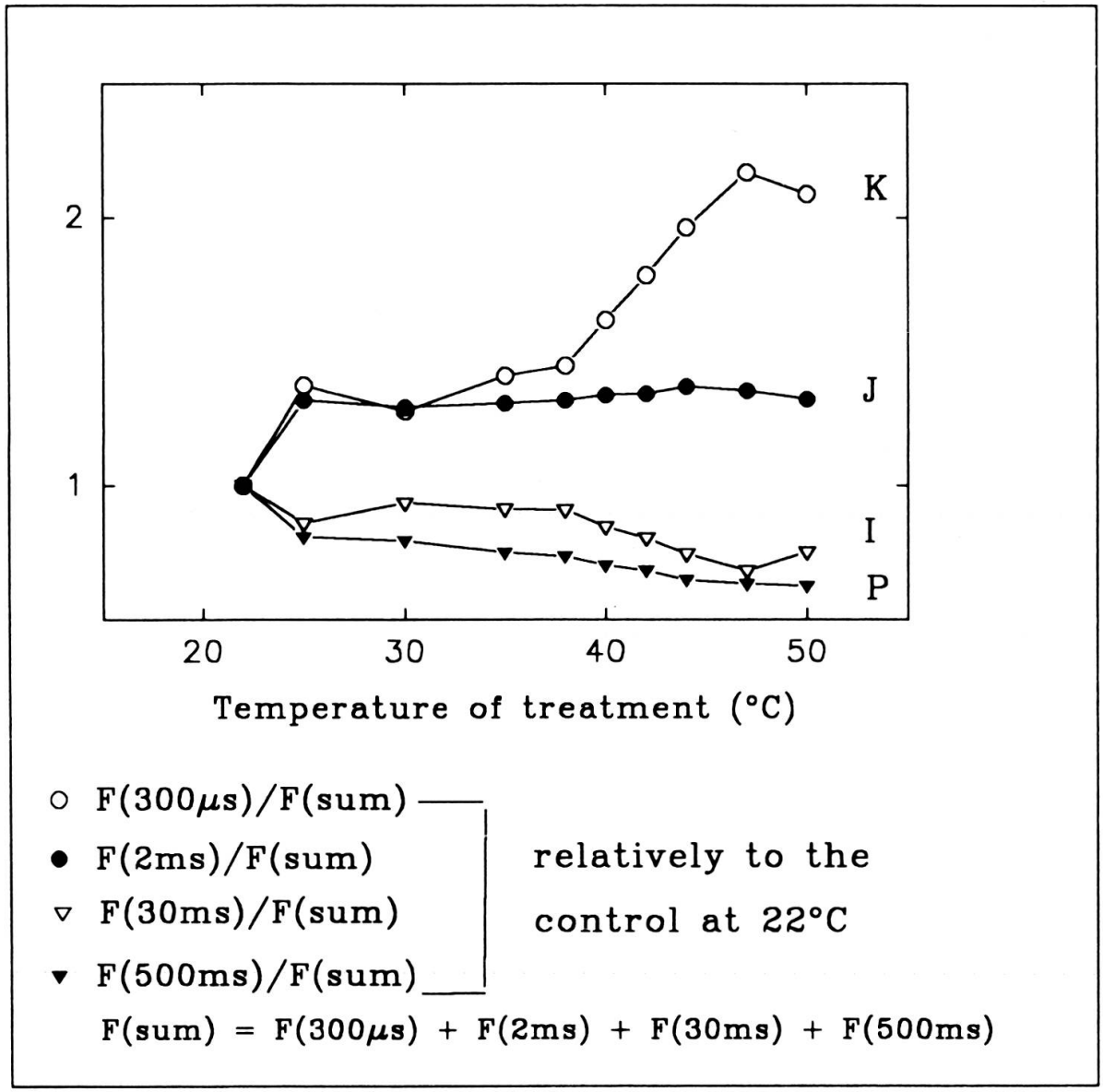

FIG. 5.

Effect of temperature on the fraction of each step K, J, I and P. The major changes were observed after treatment above 40°C. All the measurements were done after heat treatment for 5 min and re-adaptation at room temperature for 10 s.

we studied the effect of NH_2OH on the Chl *a* fluorescence transient of heat stressed leaf discs. NH_2OH is known to be an electron donor of PSII (Trebst, 1974, 1980).

A heat stressed leaf disc after NH_2OH treatment shows a Chl *a* fluorescence transient without the dip after the "K-peak" (Fig. 6 curve b). Thus, the disappearance of the dip after the "K-peak" in presence of NH_2OH appears to be associated with the restoration, by NH_2OH, of the lack of electrons caused by a partial inhibition of the water splitting-system. Although the dip after the K-peak was abolished after NH_2OH treatment, the quenching in the variable Chl *a* fluorescence (Fp - Fo) still exits. The Chl *a* fluorescence intensity after NH_2OH treatment remains lower at the Fp-level, suggesting that an electron transport towards PSI is still going on. From this result we deduce that the quenching of variable Chl *a* fluorescence and the decrease of the Fp level after the heat treatment can not only be due to the inhibition of the water splitting-system. This deduction is in agreement with the observation of Bukhov *et al.* (1990).

We therefore proceeded to investigate whether heat treatment causes an effect on the acceptor side of PSII as well using DCMU, which blocks the electron transport beyong Q_A^- (see Trebst, 1974). The DCMU treated leaf discs of the control (without heat treatment) shows the maximum fluorescence intensity at about 2 ms (Fig. 7 curve c), i.e. around the time at which the J-step appears in the untreated control leaf as reported earlier (Strasser *et al.*, 1995). However, the DCMU treatment of heat treated leaves leads to the appearance of the maximum Chl *a* fluorescence transient 10 times earlier, at about 200 µs (Fig. 7 curve d), which corresponds to the "K-step". It is clear that the appearance of "K" is not affected by DCMU. Neither does DCMU abolish the quenching on the Fp-level. It thus appears that the site of the heat treatment injury must be before the site of DCMU inhibition. According to Klimov and Krasnovskii (1981), the accumulation of reduced pheophytin (Phe^-) can provoque quenching of the variable Chl *a* fluorescence. We believe that, the accumulation of "closed RCs of the type $P680^+Phe^-$ can create, because of an enhanced recombination, a fast fluorescence rise in the antennae (our "K-step"). Therefore, we propose that, beside inhibing of the water-splitting system, heat treatment partially inhibits the electron transport between $PPhe^-Q_AQ_B$ and $PPheQ_A^-Q_B$. Such an inhibition leads to the accumulation of Phe^-P680^+ which can explain both the quenching of Fp and the appearance of "K-step".

We believe that the constellation in a leaf of higher plants which is able to exhibit the rapid step "K" in the fluorescence rise is a normal phenomenon. It is always present but for dynamic reasons it does not appear clearly in an unstressed system (Eggenberg *et al.*, 1992). However the K-step becomes dominant after heat treatment as reported here. All plants we have investigated so far showed this rapid step "K" in the polyphasic fluorescence transient after heat treatment, although the sensibility of each plant to the temperature is different. We therefore conclude that the sequence O-K-J-I-P exhibits the phenomenological sequence of redox states in the heterogeneous reaction center of PSII in leaves of higher plants.

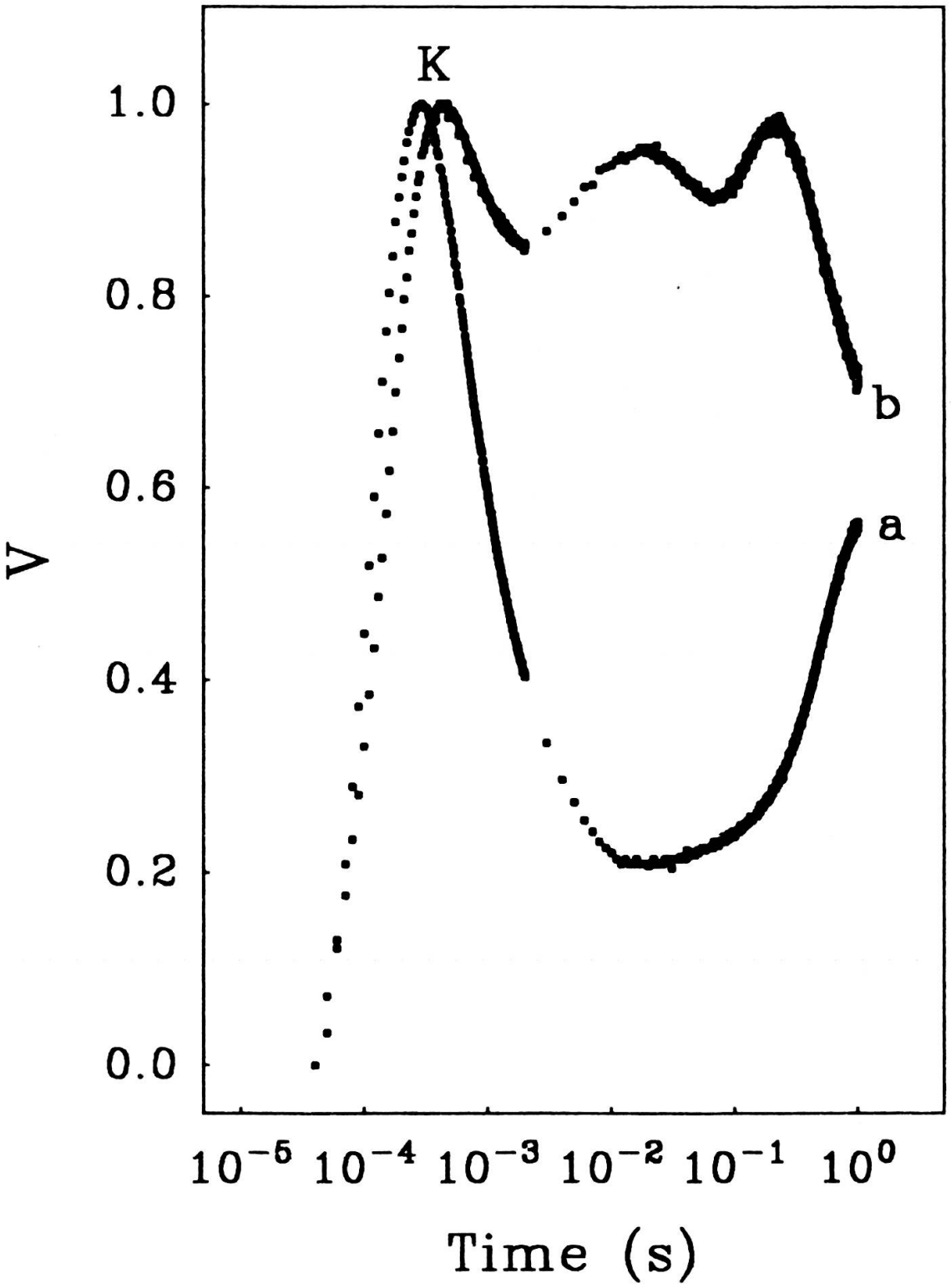

FIG. 6.

Relative variable Chl *a* fluorescence (V) = (Ft – Fo) / (Fm – Fo) of pea leaf disc heated at 50°C for 5 min and vacuum infiltrated in medium as described in the materials and methods either in absence (a) or presence (b) of 40 mM NH_2OH for 30 s. Waves a and b are normalized on K-step.

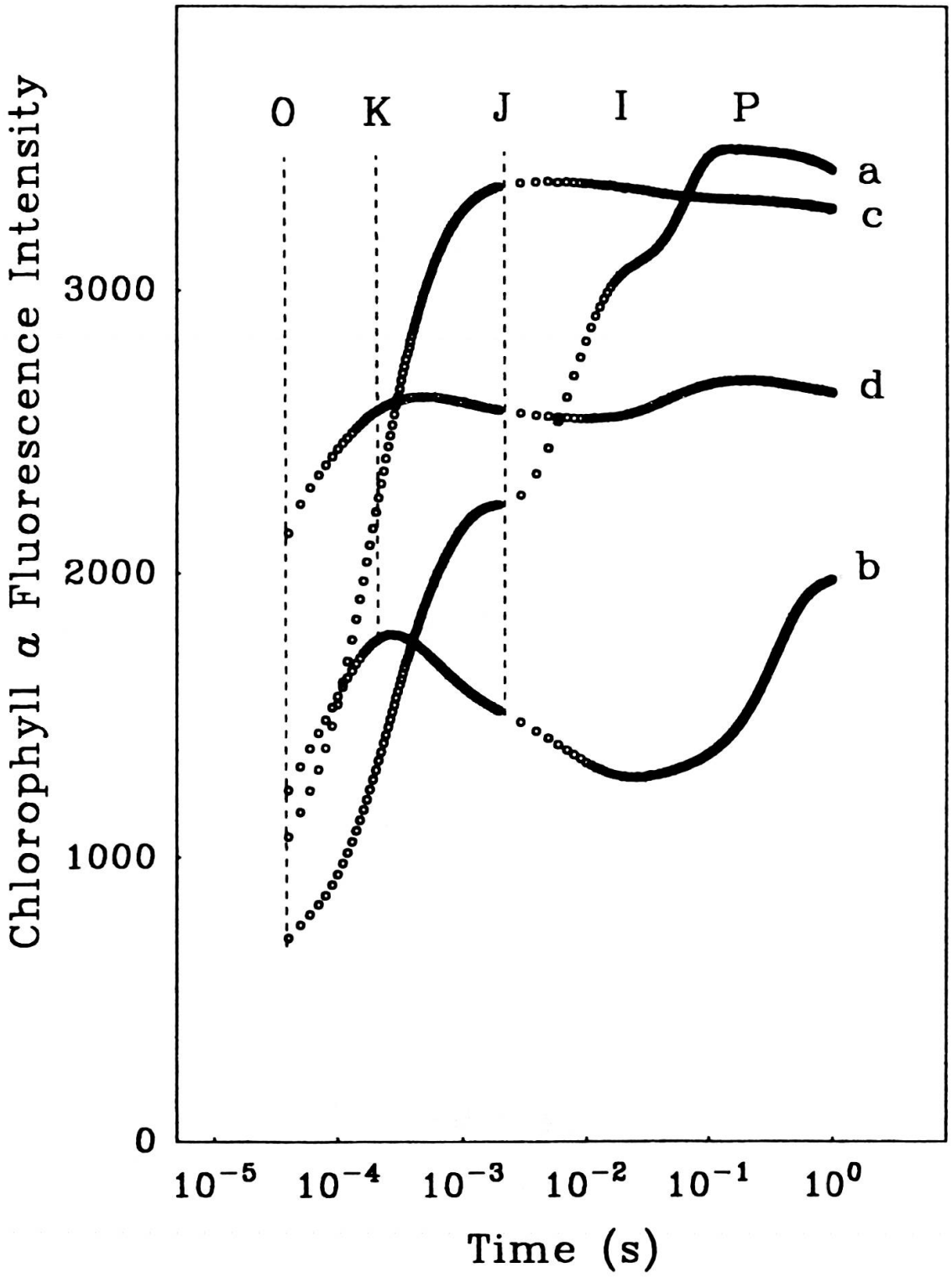

FIG. 7.

Effect of DCMU and heat treatment on Chl *a* fluorescence transient of potato leaf disc. Whenever used, DCMU was 5 mM, heating was at 47°C for 5 min and incubation was at room temperature. Trace (a): leaf disc was incubated at room temperature in distilled water for 30 min. Trace (b): leaf disc was first heated and then incubated in distilled water for 30 min. Trace (c): leaf disc was incubated in 5 mM DCMU for 30 min. Trace (d): same as trace (b) but incubated in 5 mM DCMU for 30 min. All the graphs were plotted in logarithmic time scale.

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RÉSUMÉ

La cinétique de l'induction de fluorescence de la chlorophyll *a* (Chl *a*) depuis sa valeur minimale Fo jusqu'à sa valeur maximale Fm, est un indicateur de la reduction nette de la plastoquinone primaire (Q_A^-). Dans ce papier nous avons examiné l'effet de la haute température (autour de 44°C) sur l'ascension de la cinétique de la fluorescence de Chl *a* des feuilles de pomme de terre et de petit pois, en utilisant un système sans obturateur qui nous permet de mesurer des signaux de fluorescence de 10 µs jusqu' à 120 s. Après un traitement à la chaleur, la fluorescence variable de la Chl *a* a été considérablement diminuée. Cependant, en augmentant la température ou la durée du traitement, la fluorescence caractéristique appelée la transition O-J-I-P est transformée en une fluorescence O-K-J-I-P avec une nouvelle étape rapide appelée "étape-K", détectée aux environs de 200 µs. Une grande dépression apparaît après l'étape "K", suivie d'une augmentation de l'intensité de la fluorescence de la Chl *a*. Nous avons essayé de déterminer l'origine et de faire une interprétation photochimique des changements observés en utilisant du NH_2OH connu comme donneur électron du PSII, et du DCMU qui est connu comme inhibiteur de la chaîne de transport d'electrons entre Q_A^- et Q_B. Avec les différentes données obtenues, nous proposons que l'apparition de cette nouvelle "étape-K" est due à une inhibition du "water-splitting-system" et à une partielle inhibition de la chaîne de transport d'électrons entre $PPhe^-Q_A$ et $PPheQ_A^-$.

REFERENCES

ARMOND, A.P., BJÖRKMAN, O. & STAEHELIN, L.A. (1980). Dissociation of supramolecular complexes in chloroplast membranes: A manifestation of heat damage to the photosynthetic apparatus. *Biochim. Biophys. Acta* **601**, 433–442.

BJÖRKMAN, O. (1975).Thermal stability of the photosynthetic apparatus in intact leaves. *Carnegie Inst. Wash. Yr. Bk.* **74**. 748–751.

BUKHOV, N.G., SABAT, S.C. & MOHANTY, P. (1990). Analysis of chlorophyll a fluorescence changes in weak light in heat treated Amaranthus chloroplast. *Photosynthesis Res.* **23**, 81–87.

DELOSME, R. (1967). Etude de l'induction de fluorescence des algues vertes et des chloroplastes au début d'une illumination intense. *Biochim. Biophys. Acta* **143**, 108–128.

EGGENBERG, P., SCHWARZ, B. & STRASSER R.J. (1992). Screening a biotope by fluorescence techniques: Two wavelengths fluorescence amplitude analysis and O-J-I-P fluorescence rise analysis. *In: Research in Photosynthesis,* Vol. **IV** (edited by N. Murata), pp. 611–614, Kluwer, The Netherlands.

GOVINDJEE & GOVINDJEE, R. (1975). Introduction to photosynthesis, *in*: *Bioenergetics of Photosynthesis (ed. Govindjee), Academic Press, NY*, pp. 1–50.

HAVAUX, M. (1993). Rapid photosynthetic adaptation to heat stress triggered in potato leaves by moderately elevated temperature. *Plant, Cell and Environment* **16**, 461–467.

HAVAUX, M. & STRASSER, R.J. (1992). Plasticity of the stress tolerance of photosystem II in vivo. In *Research in Photosynthesis, Vol. **IV***, N. Murata (ed.), pp. 149–152.

HSU, B.D. (1993). Evidence for the contribution of the S-state transitions of oxygen evolution to the initial phase of fluorescence induction. *Photosynthesis Res.* ***6***, 81–88.

KAUTSKY, H. & HIRSCH, A. (1931). Neue Versuche zur Hohlensäureassimilation. *Natuwissenschaften* ***48***, 964.

KLIMOV, V.V. & KRASNOVSKII, A.A. (1981). Pheophytin as a primary electron acceptor in photosystem II reaction center. *Photosynthetica* ***15***, 592–609.

KLINKOVSKY, T. & NAUS, J. (1994). Sensibility of the relative Fpl level of chlorophyll fluorescence induction in leaves to the heat stress. *Photosynthesis Res.* ***39***, 201–204.

LICHTENTHALER, H.K. & RINDERLE, U. (1988). The role of chlorophyll fluorescence in detection of stress conditions in plants. *CRC. Crit. Rev. Anal. Chem.* ***19*** (suppl. I) S29–S85.

LUUK, H.J.J., HILLIE, E.W. & PHILIP, R.H. (1992). Temperature dependence of chlorophyll fluorescence induction and photosynthesis in tomato as affected by temperature and light conditions during growth. *J. Plant Physiol. Vol.* ***139***, pp. 549–554.

MURASHIGE, T. & SKOOG, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarium, Vol.* ***15***, 473–497.

NAUS, J. (1992). Heat injury of barley leaves detected by the chlorophyll fluorescence temperature curve. *Biochimica et Biophysica Acta* ***1101***, 359–362.

SCHREIBER, U. & BERRY, J.A. (1977). Heat-induced changes of chlorophyll fluorescence in intact leaves correlated with damage of the photosynthetic apparatus. *Planta* ***136***, 233–238.

STRASSER, R.J. & GOVINDJEE (1991). The Fo and the O-J-I-P fluorescence rise in higher plants and algae. In *Regulation of Chloroplast Biogenesis* (edited by J.H. Argyroudi-Akoyunoglou), pp. 423–426. *Plenum Press, New York.*

STRASSER, R.J. & GOVINDJEE (1992). On the O-J-I-P fluorescence transient in leaves and D1 mutants of *Chlamydomonas reinhardtii*. In *Research in Photosynthesis*, vol. **II** (Edited by N. Murata), pp. 29–32. *Kluwer, The Netherlands.*

STRASSER R.J., SRIVASTAVA A. & GOVINDJEE (1995). Polyphasic chlorophyll *a* fluorescence transient in plants and cyanobacteria. *Photochem. & Photobiol.* **61(1)**, 32–42.

TREBST, A. (1980). Inhibitors in electron flow: tools for the functional and structural localization of carriers and energy conservation sites. *Methods in Enzymology* **69**, 675–715.

TREBST, A. (1974). Energy conservation in photosynthetic electron transport of chloroplasts. *Ann. Rev. Plant Physiol.* **25**, 423–458.

WEIS, E. (1981). Reversible heat-inactivation of Calvin cycle: A possible mechanism of temperature regulation of photosynthesis. *Planta* ***151***, 33–39.