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Bioelectrochemistry 57 (2002) 123–128

Bioelectrochemistry

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Hypothesis

Photo-electrochemical control of photosystem II chlorophyll fluorescence in vivo

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Received 30 October 2001; received in revised form 16 January 2002; accepted 23 January 2002

Abstract

The effect of electric field on chlorophyll fluorescence is considered on the basis of the reversible radical pair model. The hypothesis is presented that the electric fields generated by photosynthetic charge separation in reaction centers and propagated laterally through the thylakoid lumen are associated with changes in chlorophyll fluorescence yield. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Chlorophyll fluorescence; Photosystem II, photochemical quenching; Photo-electrochemical quenching; Electric potential

1. Introduction

Chlorophyll fluorescence is a valuable tool for probing the energetics of photosynthetic systems. Fluorescence emission in light-collecting antenna systems is a competitive path to photochemical energy conversion in photosynthetic reaction centers resulting in fluorescence quenching. Monitoring of fluorescence kinetics provides an experimental means to study the mechanism and dynamics of the primary and secondary photosynthetic events on a time scale ranging from ns to tens of seconds. Application of fluorescence methods in research on photosynthetic performance of plants relies on the complementary relation between the chlorophyll fluorescence yield and the yield of electron transport generated in the reaction centers. This complementary relation was first demonstrated in photosynthetic bacteria [1] and later confirmed for photosystem II (PS II) in algae and higher plants [2]. Nowadays and, in particular, after the availability of several sophisticated fluorescence-monitoring and -imaging instruments, fluorescence is a standard method in fundamental and applied plant research.

Although recognized as a control parameter, the effect of electric fields on the fluorescence yield of PS II has not received much attention, mainly because the effects in vivo have been presumed to be relatively small [3]. Recently, experimental evidence was obtained that the chlorophyll fluorescence yield in plant chloroplasts with impaired PS II activity is substantially increased in association with changes in the transmembrane electric potential generated by PS I [4]. Here, we will present as a hypothesis that, in general, electric fields generated by photosynthetic charge separation in reaction centers and propagated laterally through the thylakoid lumen are associated with changes in chlorophyll fluorescence yield.

2. Background and theory

Changes in fluorescence yield that cannot easily be explained in terms of a quenching mechanism can be accommodated into a concept that electric fields in the vicinity of the reaction center have a discernible effect on chlorophyll fluorescence [4–9]. The electric field exerts its effect on the quasi-equilibrium between the excited state of reaction center chlorophyll (P680*), which is in exciton equilibrium with that of the antennas (Chl*), and the reaction center radical pair ($P680^+Pheo^-$). This equilibrium is determined by the rate constants of primary electron transfer reactions, amongst which the radical pair recombination. The PS II reaction center (RC) has been recognized as being particular and different from others in a sense that the energy difference between P680* and $P680^+Pheo^-$ is relatively low ($0.04-0.12$ eV) [9–12]. This makes the primary photochemical reactions in this RC sensitive to changes in electric field strength (potential).

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1567-5394/02/$ - see front matter
PII: S1567-5394(02)00062-2

This sensitivity is due to a direct effect of the electric potential on the probabilities for radical pair recombination, photochemical trapping and for nonradiative recombination of $P680^+ Pheo^-$ plus $P680^+$-triplet formation, affecting the effective rate constants k_{-1}, k_p and k_d, respectively. Under assumed dynamic equilibrium between P680* and the radical pair, k_p and k_{-1} are related by the Boltzmann equation

$$k_p/k_{-1} = \exp(-(\varphi - \varphi_o)F/RT) = \exp(\psi_o - \psi), \qquad (1)$$

in which φ and φ_o (in mV) represent the difference in electric potential across the membrane section between P680 and Pheo and midpoint redox potential for P680* and Pheo, respectively. ψ ($=\varphi F/RT$) is a (dimensionless) relative potential and F/RT (at 20 °C) is about 25 mV/unit. The fluorescence yield Φ then can be described, as outlined in detail elsewhere [4], by the following relation

$$\Phi = 1/\left(1 + \frac{k_t}{k_f} + \frac{k_d}{k_f N}\exp(\Psi_o - \Psi) + \frac{k_e}{k_f N}\theta\exp(\psi_o - \psi)\right), \qquad (2)$$

in which θ is the fraction of semi-open and closed centers (the extreme values 0 and 1 correspond to 100% closed and open centers, respectively), N is the number of chlorophyll molecules per PS II reaction center (antenna size), and k_e, k_f and k_t are rate constants for electron transfer to the primary quinone acceptor Q_A and for fluorescence emission and for nonradiative losses of the antennas, respectively.

Fig. 1 shows the relation [$\Phi(\psi)$] between fluorescence yield and the electric potential for some discrete values of the fractional closure of the RC. The following parameter values were substituted in Eq. (2) for obtaining [$\Phi(\psi)$]:

$k_f = 3$, $k_d = 5$, $k_e = 100$, $k_t = 10$ (in 10^8 s^{-1}),
$N = 100$ and $\psi_o = 4$ ($\varphi_o = 100$ mV).

The figure and its $\Phi(\psi)$ curve give the following information with corresponding conclusions. (1) The fluorescence yield at constant photochemical quenching alters with a change in the electrical potential. (2) The magnitude of the change in fluorescence yield at a fixed change in the potential depends on the degree of RC closure, i.e. compare O–O′ and I–P for the extremes (open and closed), respectively. (3) Saturation of photochemistry does not necessarily result in saturation of the change in fluorescence yield.

Fig. 1 points to the fact that changes in chlorophyll fluorescence yield accompanying photochemical activity of the photosynthetic system can be the result of at least two mechanistically different processes. Therefore, we introduce the following definitions to discriminate between the two. A change in fluorescence yield at a constant potential in the vicinity of the reaction center is ascribed to (a change in) *photochemical quenching*. The change in fluorescence yield

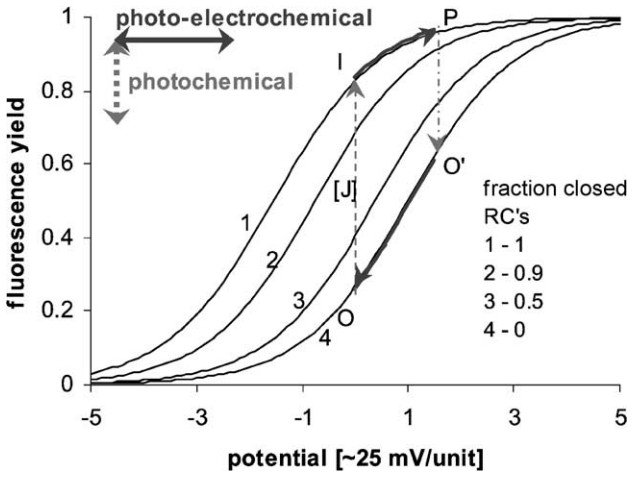

Fig. 1. The effect of potential ($\psi = \varphi F/RT$) on fluorescence yield (Φ) under conditions differing in the degree of RC closure (θ) is shown (Eq. (2); for a quantitative description see Ref. [4]). Fluorescence and photocurrent measurements (not shown) in chloroplasts indicate a lower time constant for lateral (photo-) potential propagation (~ 2 s^{-1}) than for full closure of RCs (~ 30 s^{-1}). As a consequence, release of photochemical fluorescence quenching (O–J–I phase) and photo-electrochemical stimulation (I–P phase) become distinguishable in $F(t)$ curve. I and P are fluorescence levels at saturated photochemistry in absence and presence of a photo-electrochemical effect, respectively; O and O′ are at ceased photochemistry (dark) in absence and presence of a photo-electrochemical effect, respectively.

occurring at a constant photochemical activity of the electron transport system is ascribed to *photo-electrochemical control* of the primary reactions in the reaction center.

Evidence for photo-electrochemical control of the chlorophyll fluorescence yield has come from photocurrent and fluorescence measurements in isolated chloroplasts of *Peperomia metallica* and *Chenopodium album* [4]. These showed that, in the absence of PS II activity and after restoration of PS I activity, substantial changes in chlorophyll fluorescence yield with kinetics similar to those of photocurrents associated with PS I activity. The fluorescence changes were interpreted to be caused by changes in photo potentials generated by PS I and propagated laterally to PS II reaction center sites. This propagation, as yet, cannot be described analytically because of uncertainties about number and size of components in the internal electrical circuit of the thylakoid and its architecture. However, the close correspondence between the kinetics of the increase in lumenal resistance and increase in fluorescence is suggestive of a lateral propagation mechanism.

3. Photo-electrochemical control of chlorophyll fluorescence of PS II

The low energy equilibrium between the singlet excited and the charge-separated state of the PS II reaction center and the dependence of the antenna chlorophyll fluorescence yield on this equilibrium (Eq. (2)) lead us to the following hypothesis. The chlorophyll fluorescence yield of PS II in

green cells is under photo-electrochemical control that is exerted by the electric field in the vicinity of and sensed by the reaction center. The electric field can originate from the transmembrane electric potential or from single local charges or dipoles. Whichever the origin, photo-electrochemical control differs from photochemical quenching in a sense that it can in principle occur independent of chemical quenching of any sort. In some cases, but as yet not in all, the effect of electrochemical control and chemical quenching can be distinguished and resolved due to the difference in turnover and recovery time of the underlying reactions.

We will now give examples of cases in which we believe that the fluorescence yield is clearly and, for the major part, under photo-electrochemical control. (i) Changes in the dark fluorescence yield $[\Phi_F]_0$ caused by single turnover preexcitation(s). (ii) ns-fluorescence changes upon ps-excitation and possibly related to the first case. (iii) Increase in the fluorescence yield in the light in the 30- to 500-ms time range above the maximal level in the presence of DCMU (I–P rise). (iv) Recovery of the fluorescence yield in the dark after a 100-Hz train of 25–30 single turnover flashes or after a short saturating multi-turnover flash (F_m–F_o transition) and upon shutting off the actinic light under steady-state light conditions (F–F_o' transition).

3.1. Changes in F_o

It has been reported that the 15–25% increase in the dark fluorescence yield $[\Phi_F]_0$, which persists in a time domain of minutes after preexcitation of a dark-adapted system with single turnovers, is caused by an increase in the efficiency of radical pair recombination in open reaction centers [5] due to control by electric fields in the vicinity of the RC [13,14]. The likeliness and effectiveness of this kind of electrochemical interaction have come from measurements of flash-induced photocurrents in combination with measurements of (changes in) $[\Phi_F]_0$ in *P. metallica* [15–17]. These measurement showed for a single chloroplast a decrease in the electrical conductance of the thylakoid lumen and for a leaf a 15–25% increase in $[\Phi_F]_0$, both induced by a single turnover flash and with a dark recovery time of several minutes. The lumen conductance change has been interpreted to be caused by a contraction of the lumen, which will alter the proximity of membrane proteins and is likely to be accompanied by changes in energy transfer within and between light-harvesting complexes [17]. The change in k_{-1} has been interpreted to be due to an altered electrostatic interaction of single charges (or dipoles) in the close vicinity of the RC [5]. A 25% increase in $[\Phi_F]_0$ would, according to Eq. (2), suggest that the field responsible for this increase is equivalent to one associated with a local potential of about 7.5 mV. Reasonable arguments have been given that changes in any type of chemical quenching can be excluded for causing the increase in $[\Phi_F]_0$ of the kind as discussed here.

3.2. Fluorescence changes in the ns time region upon ps-excitation

There are numerous reports, starting with the pioneering work of Mauzerall [18], which show that the chlorophyll fluorescence yield in chloroplasts and PS II preparations upon laser excitation increases biphasically [19]. The fast ns-phase goes parallel with the reduction of $P680^+$, which was taken as evidence that this increase reflects the release of photochemical quenching of $P680^+$. A subsequent second phase of the fluorescence rise in the μs time range has been attributed to release of triplet quenching [19]. There is as yet no direct experimental evidence for an alternative interpretation. However, similarities of the ns-fluorescence changes with those in $[\Phi_F]_0$, in particular with respect to size (i.e. 25 – 35% of F_o), saturation, 4 periodic oscillation and dark recovery, would not violate an alternative interpretation. In this conceptual view, the ns-fluorescence change would reflect a photo-electrochemical effect on the antenna fluorescence associated with a change in the local electric field at the RC site caused by charge displacement from

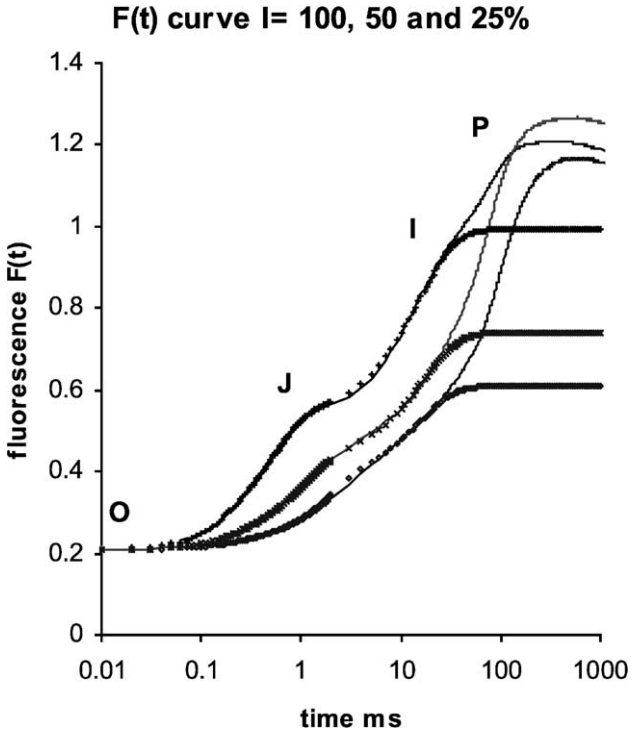

Fig. 2. Fluorescence induction $F(t)$ in *C. album* leaf upon illumination with 1-s light pulse of 100% (~ 600 W m^{-2}), 50% and 25% intensity (from top to bottom). Symbol curves are calculated with Three-State Trapping Model (TSTM), with fixed rate constants for electron transport at donor and acceptor sides, and excitation rate k_L proportionally variable with intensity [20]. The close correspondence of simulated and experimental curves indicates that the release of photochemical quenching (O–J–I) is completed after about 30 ms with approximately 100%, 67% and 51% full closure of RCs at intensities used. The final rise (I–P) in the 30–300-ms time range is attributed to photo-electrochemical stimulation of the fluorescence, which in confirmation with theory (see Ref. [4]) is supplementary to the release of photochemical quenching.

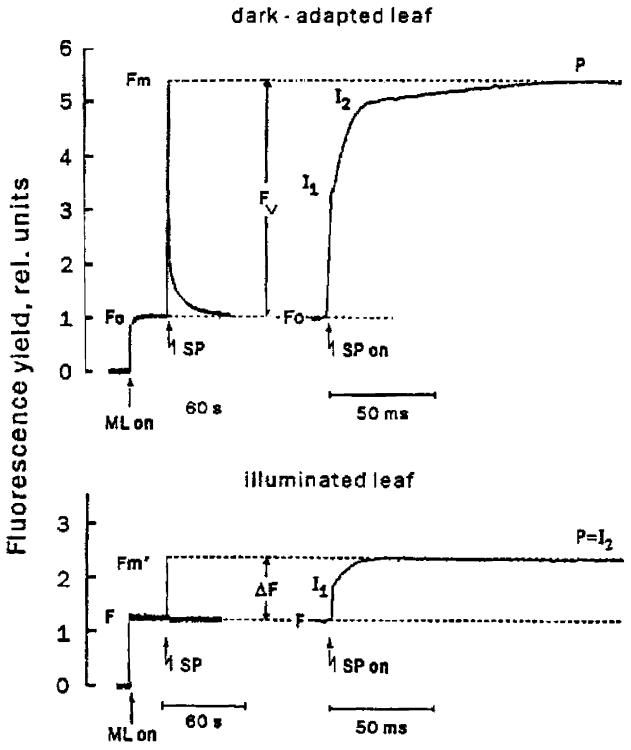

Fig. 3. Recordings and kinetic details of the polyphasic fluorescence rise upon onset of a multi-turnover saturating light pulse in a dark-adapted and illuminated leaf. In the text, the levels I1 and I2 are replaced with J and I, respectively. The difference in the kinetics of the recovery of the fluorescence yield after a multi-turnover saturating flash (SP) and in the rise kinetics in a dark-adapted and illuminated leaf are interpreted in terms of a difference in electrochemical effects (reproduced from Ref. [21]).

$P680^+$ to the secondary donor Y_Z The second μs-phase then would reflect the release of Y_Z^+ quenching associated with oxidation of the quencher by the oxygen-evolving complex [20].

3.3. Increase in the fluorescence yield in the light in the 30- to 500-ms time range (I–P phase)

The final slow phase of the light-induced increase in fluorescence before the decline starts in prolonged actinic illumination occurs in the 30- to 500-ms time range [21,22]. This phase is easily seen in intact leaves as a separate I–P phase and is above the maximal fluorescence level in the presence of DCMU [23]. It has been attributed to additional static quenching by plastoquinone [24]. It has been shown in Ref. [22] and illustrated in Fig. 2 that (i) the I–P phase as fraction of the total increase in the fluorescence yield (O–J–I–P phase) increases when the actinic light intensity is lowered. In addition, (ii) the kinetic pattern of the I–P phase shows a relatively steep rise in the time range above 100 ms. These data support the hypothesis that the I–P rise is a reflection of photo-electrochemical stimulation of the fluorescence yield supplementary to photochemical quenching during the O–J–I rise. In confirmation with the theory (see Fig. 1), the electrochemical component of the fluorescence rise increases when the chemical quenching mechanism becomes limited. This of course is only true as long as the electric potential responsible for the electrochemical fluorescence stimulation has not changed much, which apparently is the case due to a high turnover rate of PS I.

3.4. Recovery of the fluorescence yield in the dark after illumination (F_m–F_o, F_m'–F and F–F_o' transitions)

The dark recovery of the fluorescence yield after a saturating light pulse will follow, as illustrated in Fig. 1, the P–O′–O pattern within the context of our hypothesis. If the recovery time of the chemical quenching (P–O′ phase) is different from that of the electrochemical control, then this should show up in the light-off kinetics. And it does. Fig. 3, reproduced from one of Schreiber's publications [21], shows that the F_m–F_o decline in the dark after a saturating light pulse of minimum 200-ms duration (left-hand upper trace) has a slow phase, which is 30–40% of the total variable yield. Its time of completion of a few tens of seconds is comparable with recovery times of changes in electrical conductance of the thylakoid lumen measured in *Peperomia* chloroplasts [4,15,16]. The slow recovery phase of the variable fluorescence is absent (left-hand lower curve)

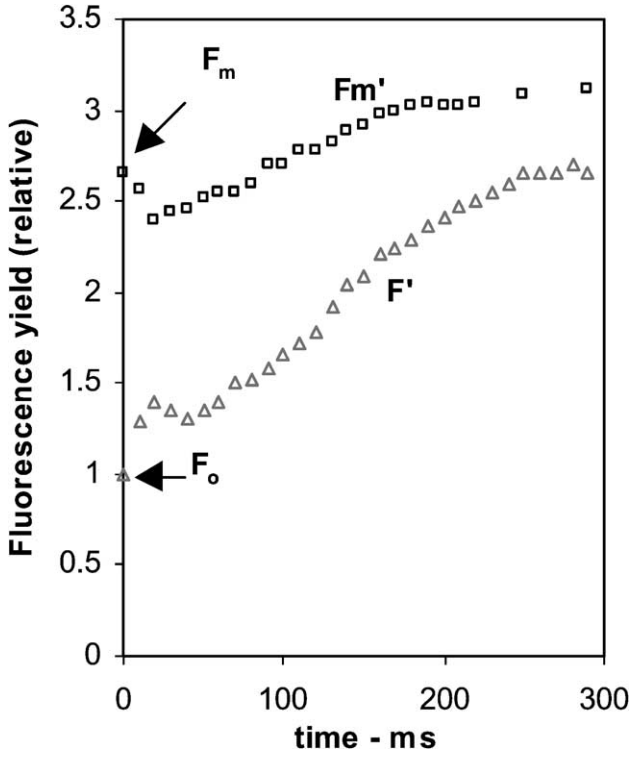

Fig. 4. Time course of the fluorescence yield in a 100-Hz train of 30 single turnover flashes of 150-s duration in *Chlorella* cells. Fluorescence signals were collected at the end (upper curve) and 10 ms after each single turnover flash (lower curve), respectively. Data of lower curve indicate a low light requiring substantial increase in the fluorescence yield in the dark, which is independent of (a release of) photochemical quenching. See further text (data reproduced from Ref. [26, Fig. 6] with kind permission of authors).

in steady-state actinic light ($F_m'-F$ decline), when as expected, the electrical events have come to a saturated steady-state equilibrium. In this case, the slow component in the dark recovery of the fluorescence yield shows up in light-off response ($F-F_o'$ response) after shutting off the actinic light (data not shown, but see, for instance, Ref. [25]). Taken together, the multiphasic recovery kinetics of the variable fluorescence yield are in agreement with our hypothesis that part of this yield is under control of photo-electric events.

4. Conclusion, controversies and outlook

Available data strongly suggest that the chlorophyll fluorescence yield of photosystem II is, in addition to regulation by variable photochemical quenching, under control of photo-electric events. We call this photo-electrochemical control. Evidence in support of this hypothesis has come from the response of the dark fluorescence yield $[\Phi_F]_0$ to single turnover flashes [5,17] and from photocurrent and fluorescence measurements in chloroplasts under conditions at which photochemical activity of PS II is inhibited and electrochemical activity of PS I was promoted [4]. A closer look to some theoretical aspects and to kinetics of particular fluorescence responses under various conditions gives support to the hypothesis as well. For instance, supporting evidence of a slowly recovering light-induced increase in fluorescence yield related to photo-electrochemical stimulation rather than to release of photochemical quenching can be obtained from elegant experiments with single turnover flashes done in Falkowski's group [26]. Fig. 4 shows fluorescence values collected at the end (F_m') and 10 ms after each excitation (F'), respectively, in a 100-Hz sequence of 30 single turnover flashes of 150-s duration. The figure clearly shows after the fifth flash a cumulative increase in each subsequent flash of a slowly recovering dark-fluorescence component up to a more than 2.5-fold value of F_o, saturated after 25 flashes. A similar but smaller increase is also observed in F_m. Contrary to the interpretation given by the authors, this increase in F_o (F') an F_m' cannot be ascribed to the release of quenching concomitant with PQ reduction. The 10-ms dark interval between flashes and the number of flashes do not warrant an accumulation of PQH_2. The figure also nicely shows and confirms the 20–30% increase in F_o after the first flash [5] and the (additional) 4 periodic oscillatory pattern in the first four flashes [27,28].

So far, the effect of electric potential on fluorescence yield could only be studied under conditions at which the chloroplast membranes were subjected to artificially imposed potentials [13,14]. Simultaneous fluorescence and photocurrent measurement in isolated chloroplasts under voltage clamp so far failed. They need perfection with respect to the limit of the potential that externally can be imposed across the thylakoid membrane and to the time resolution. It is felt that there is need for experimental confirmation of the suggestion, based on the hypothesis, that the ns-fluorescence increase in ps-excitation is a response to a change in electrical field strength at the site of the reaction center associated with charge transfer from $P680^+$ and Y_Z. If experimentally confirmed, the ns-fluorescence response is unlikely due to or associated with (a release of) photochemical quenching. Its association with charge transfer then would mean that, contrary to current views, Y_Z^+ is a quencher of PS II fluorescence. This would be in agreement with observations and conclusions that the increase in fluorescence yield is limited at the donor side of PS II by the rate of oxidation of the oxygen-evolving complex [20].

If our hypothesis is confirmed and found to be correct the photo-electrochemical component of the fluorescence yield should be incorporated in fluorescence parameters like F_v'/F_m' (Genty factor) that routinely is used as a linear parameter of the actual photochemical efficiency of PS II. There are several reports that show strong deviations of this parameter value at low actinic intensities with the actual rate of electron transport [29]. Research in which the F_m' value by subtraction is corrected for an electrochemical component is currently in progress (Kromkamp, personal communication). This would help to clarify an as yet unresolved problem.

Acknowledgements

This work was supported by a grant to AAB by the Dutch Ministry of Agriculture, Nature Management and Fisheries.

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