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Delayed fluorescence in Photosystem I enhanced by phylloquinone (vitamin K-1) extraction with ether

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Time-resolved fluorescence emission spectra were measured in ether-extracted Photosystem I particles, which are depleted of phylloquinone (vitamin K-1) and about 95% of antenna chlorophylls. A long-lived fluorescence decay component having a 53 ns (80 ns at 110 K) decay life-time and peaking at 693 nm (701 nm at 110 K) was found to exist in addition to the 675–680 nm band which shows a 6 ns decay time. Yield of the long-lived component increased when P-700 was pre-reduced by ascorbate and 2,6-dichlorophenolindophenol or by dithionite, and decreased when P-700 was pre-oxidized by ferricyanide, or by the addition of vitamin K-3, which is known to suppress the charge recombination between P-700$^+$ and A_0^- in these ether-extracted Photosystem I particles. These results suggest that the long-lived (delayed) fluorescence is emitted from P-700 in the charge recombination reaction between P-700$^+$ and A_0^- and support the idea that vitamin K-1 is functioning as A_1 in the Photosystem I reaction center.

Introduction

In the PS I reaction center of green plants, an electron is now assumed to be transferred from the reaction center P-700 chlorophyll *a* molecule to the electron acceptor intermediate A_0, which is probably also a chlorophyll *a* molecule peaking at 690 nm [1,2] (687 nm in the ether-extracted PS I particles; see Itoh, S. and Iwaki, M., unpublished data). The electron is then passed to the primary stable electron acceptor A_1, probably vitamin K_1 [3–5] which is present at about 2 molecules per P-700 [5–7], and then to the iron-sulfur centers F_X, F_A and F_B [8–10]. Although the turnover of A_1 is not well characterized [3,4], its reduction by A_0^- is assumed to proceed with a half-time of 20 ps [1,2]. Recently, extraction of vitamin K-1 by ether treatment [5] or by hexane-methanol treatment [11] of PS I particles was shown to increase the charge recombination between P-700$^+$ and A_0^- by blocking the rapid oxidation of A_0^-. Measurement of the absorption changes at 820 nm [12] or at 660–720 nm (Itoh, S. and Iwaki, M. unpublished data) indicates that the charge recombination reaction proceeds with a half-time of 40 ns.

In this study we report the effects of extraction of vitamin K-1 with ether and addition of vitamin K-3 on the time-resolved emission spectrum and decay kinetics of fluorescence in the ether-extracted PS I particles. Although PS I delayed fluorescence has not been measured except under extremely low redox potential conditions [13,14],

Abbreviations: PS I, Photosystem I; PS II, Photosystem II; DCIP, 2,6-dichlorophenolindophenol.

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we show that the charge recombination enhanced by the extraction of vitamin K-1 produces PS I-delayed fluorescence even under ordinary redox potential conditions. The mechanism of delayed fluorescence seems to be similar to that in reaction centers of photosynthetic bacteria [15,16] or in PS II [17].

Materials and Methods

PS I particles were obtained by treating spinach chloroplasts with digitonin as described previously [5]. Extraction of vitamin K-1 was performed by treating the freeze-dried PS I particles with diethyl ether containing an 80% saturating amount of water [5]. This procedure is essentially the same as that used for the preparation of P-700-enriched particles described previously [18,19]. This treatment extracted all the vitamin K-1 present at about 2 molecules per P-700, and also extracted all the carotenoids and 95% of the antenna chlorophylls, but the light-induced oxidation of P-700 [5,18–20] and photoreduction of FeS centers [5,11,20] remained.

Time-resolved fluorescence spectra and fluorescence decay kinetics were measured with an apparatus equipped with a photodiode array in combination with a gated multichannel plate (SMA system, Princeton Instruments, Princeton). Samples in a liquid nitrogen cryostat (Oxford DN704) were excited by a Nd-YAG frequency-doubled laser flash of 532 nm, 10 ns FWHM (Quanta-Ray, DCR2-10) at an intensity of 3.5 mJ on the cuvette surface (this excites about 50% of P-700 when assayed by nanoseconds absorption change; see Itoh, S. and Iwaki, M. unpublished data). The fluorescence, emitted at a right angle to the excitation, was diffracted in a monochromator (Jobin Yvon 450) and focused onto the gated multichannel plate (opening gate time of 10 ns in this study) which was combined with a photodiode array (Princeton Instruments, IRY-512). This system allowed us to obtain the time-resolved fluorescence emission spectra (512 channel for 60 nm emission wavelengths), with a half emission band-width of 1 nm and decay kinetics with a half-decay time longer than 3.5 ns (see a profile of excitation laser in Fig. 2C). In most cases time-resolved fluorescence emission spectra were obtained as averages of 64–256 scans of the excitation with a 0.5–1 Hz laser repetition rate.

Results

Effects of redox conditions and temperature

Fig. 1A shows the time-resolved fluorescence emission spectra (taken at each 10 ns after the time of peak excitation from the laser) of the ether-extracted PS I particles in the presence of ferricyanide (about 90% of P-700 was oxidized in the dark). At the initial time range of the laser excitation (at −20 ns), an apparent fluorescence emission peak was observed at about 680 nm. At the time just after the excitation laser peak (at 0–20 ns), the peak wavelength shifted to about 675 nm. At the time range later than 30 ns the 675 nm band disappeared and a new band peaking at 693 nm became dominant. This band was observed until 150 ns. Fig. 1B shows the time-resolved fluorescence spectra under the P-700-reduced conditions (reduced by the ascorbate and DCIP couple). At −20 ns, a peak at 693 nm was apparent as well as the peak around 680 nm. At 0–20 ns, a peak around 675 nm became dominant. At times longer than 10 ns, the 693 nm peak again became dominant. Thus the reduction of P-700 seemed to increase the 693 nm band which shows a fast rise and a fast decay phase followed by a very slow decay phase. On the other hand, the 675 nm band is almost insensitive to the change of the P-700 redox state. There may also be another 680 nm component which shows a relatively fast rise and decay, but this was not distinguished clearly. If dithionite was used as a reductant, in the place of the ascorbate and DCIP couple, almost the same kinetics and the intensity of fluorescence were obtained at 273 K (not shown).

Similar studies were also done at 110 K with dithionite (Fig. 1C). At this temperature the time-resolved fluorescence spectra showed, at least, two types of fluorescence bands; the first one peaking at 701 nm rises rapidly and probably exhibits a fast decay rate, followed by a very slow multi-exponential decay and was detectable until 500 ns. The second one peaks at about 680 nm, with both a fast rise and fast decay kinetics. It is interesting that the peak position of the longest wavelength band significantly shifted from 693 nm to 701 nm

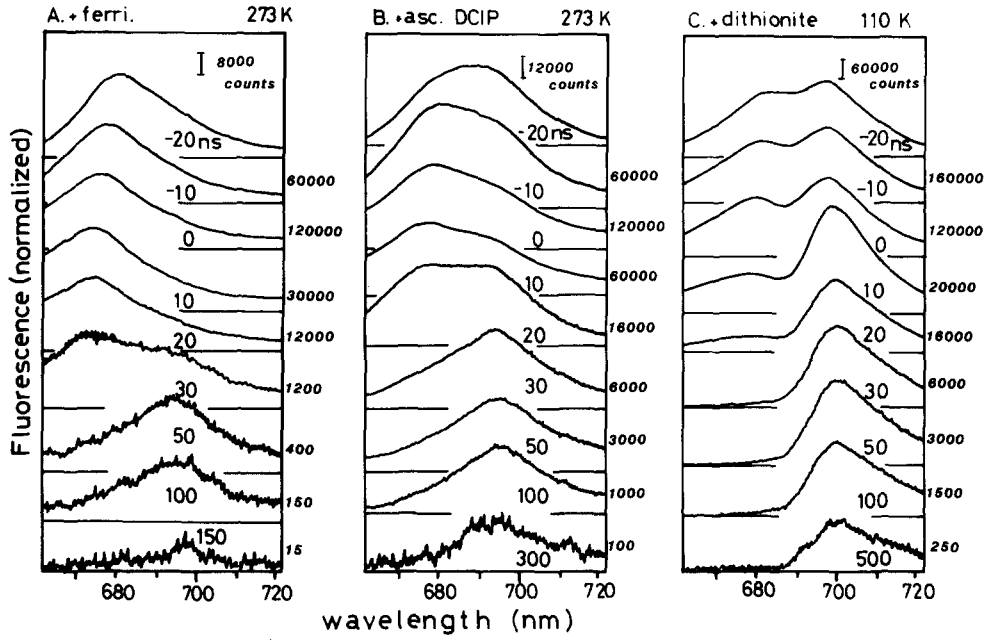

Fig. 1. Time-resolved fluorescence spectra of ether-extracted PS I-particles (P-700 enriched particles). (A) In the presence of 2 mM ferricyanide at 273 K. (B) In the presence of 10 mM ascorbate (asc.) and 20 μM DCIP at 273 K. (C) In the presence of 2 mM dithionite at 110 K. Reaction mixture contained 50 mM Tris-Cl buffer (pH 7.0), 60% glycerol and P-700-enriched particles having a chlorophyll/P-700 ratio of 10 at 3.0 μM P-700. Numbers shown in italics indicate the unit of vertical axis of each trace.

upon cooling. The extent of the shift was dependent on the temperature (not shown). The well-known 730 nm fluorescence band, which is significant both in prompt fluorescence [17] and in delayed fluorescence in untreated PS I particles at low temperature [13,14], was not detected in the

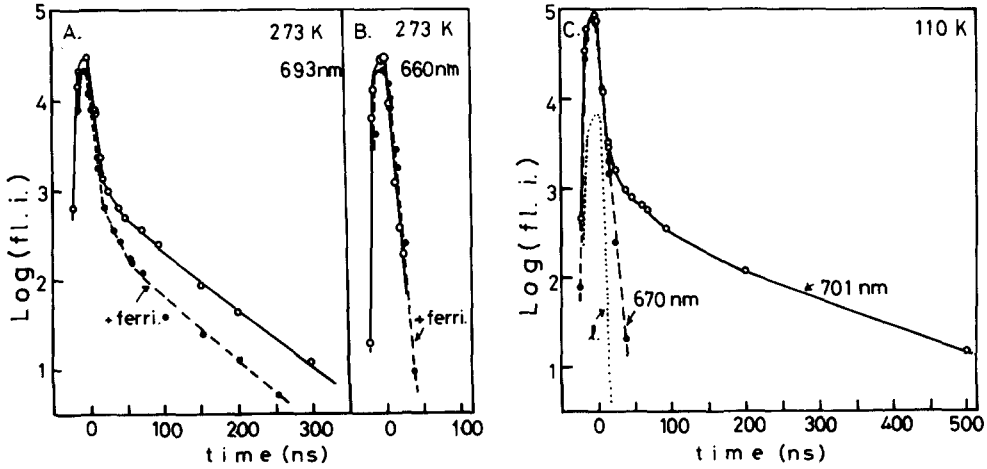

Fig. 2. (A and B) Decay kinetics of fluorescence at 693 (A) and 660 nm (B) at 273 K. Open circles, with 2 mM ascorbate and 20 μM DCIP. Closed circles with 2 mM ferricyanide. (C) Decay kinetics at 110 K in the presence of 2 mM dithionite. Open and closed circles represent decay kinetics at 701 and 670 nm, respectively. The dotted line indicates the profile of excitation laser measured under the same experimental set-up and shows the 3.5 ns time resolution of the present apparatus. Other conditions were similar to those in Fig. 1. fl.i., fluorescence intensity.

ether-extracted preparation, probably due to the extraction of longer-wavelength absorbing chlorophylls [20].

Fig. 2 shows the decay kinetics of the fluorescence intensity at different emission wavelengths after the laser excitation. At 660 nm (Fig. 2B) the fluorescence intensity decayed almost exponentially with an apparent ($1/e$) decay time of 6 ns. This component showed a similar intensity and decay kinetics in the presence or absence of ferricyanide. On the other hand, at 693 nm the fluorescence intensity decayed in two phases: one with a decay time of less than 3.5 ns and another with 53 ns when the decay time was estimated from the decay between 50 and 200 ns (also slower components seem to exist). More accurate decay kinetics of the fast phases will be studied elsewhere by the measurement using the picosecond excitation and detection system (Mimuro et al., unpublished results). In the presence of ferricyanide, the 693 nm fluorescence band, but not the 675 nm band, was significantly depressed. We observed that the addition of ferricyanide suppressed the slow 53 ns phase more significantly.

These results indicate that in the ether-extracted PS I particles there are at least two (but probably more) distinct fluorescence bands peaking at 675 and at 693 nm, respectively. The 675 nm band is rather insensitive to the redox state of P-700. On the other hand, the 693 nm band seemed to be emitted only when P-700 was reduced before the flash excitation. Its 53 ns decay time, which is slower than the intrinsic life time of the excited state of chlorophyll *a*, suggests that this phase is the delayed fluorescence produced in the charge recombination in the PS I reaction center. The decay time (53 ns for $1/e$ and 37 ns for $1/2$) agrees almost with the reported half-time of the ($P\text{-}700^+ A_0^-$) biradical state measured spectroscopically in this preparation (40 ns) [12]. At 10 K the yield of this fluorescence band increased about twice (compare the data in Figs. 2A and C). The observation that its decay time increased to 80 ns (estimated from the decay between 50 and 200 ns) at 110 K also agrees with the reported characteristics of the charge recombination reaction in this preparation [12]. Ferricyanide suppressed the slow decay phase also at this temperature (data not shown). The increase of the intensity around 0 ns on cooling seems to be due to the increase of phases with decay times faster than 3.5 ns (i.e., faster than the time resolution of the present system), with intensity peaks around 680–700 nm as can be estimated from the time-resolved fluorescence spectra in a shorter time range in Fig. 1C (e.g., at −20 ns). The 675 nm band, on the other hand, showed a decay time of 6 ns also at 110 K.

The 693 nm fluorescence band has been observed in previous studies under continuous illumination [5,21,22] and has been claimed to be delayed fluorescence from the antenna chlorophyll *a* [21,22] or from P-700 [5] judging from the dependency of its yield on the redox state of P-700. The results in Figs. 1 and 2 clearly indicate that a portion of this band is indeed delayed fluorescence and shows the characteristics as expected for that induced by the charge recombination. This also explains the observation that under the continuous illumination the 693 nm fluorescence band has been seen only in the ether-extracted PS I particles [5,21,22] in which the charge recombination probability is significantly increased due to the lack of vitamin K-1.

Amplitudes of 693 nm and 701 nm slow decay phases at 273 K and 110 K, obtained by the extrapolations to 0 ns, amounted to be only 3.1% and 4.1% of the total intensities at 0 ns (calculations from Fig. 2), respectively. An integrated intensity of the slow phase at 273 K (calculated by using 53 ns decay time) was estimated to be about 8% of the total integrated fluorescence intensity (including the faster phase) at 693 nm. At 110 K the slow phase amounted to be about 17% of the total integrated intensity at 701 nm. Therefore, the 693 (701) nm fluorescence band measured under the continuous illumination seems to be mainly the faster decay phase of the 693 (701) nm band in Figs. 1 and 2.

Effects of addition of vitamin K-3

It has previously been shown that re-addition of vitamin K-1 or vitamin K-3 suppresses the charge recombination (in about a half of reaction centers) in nanosecond time range and results in the increased stability of $P\text{-}700^+$ in the microsecond time range [5,12]. Fig. 3A shows the effects of addition of vitamin K-3 on the fluorescence decay kinetics at 693 nm at 273 K under the ascorbate-

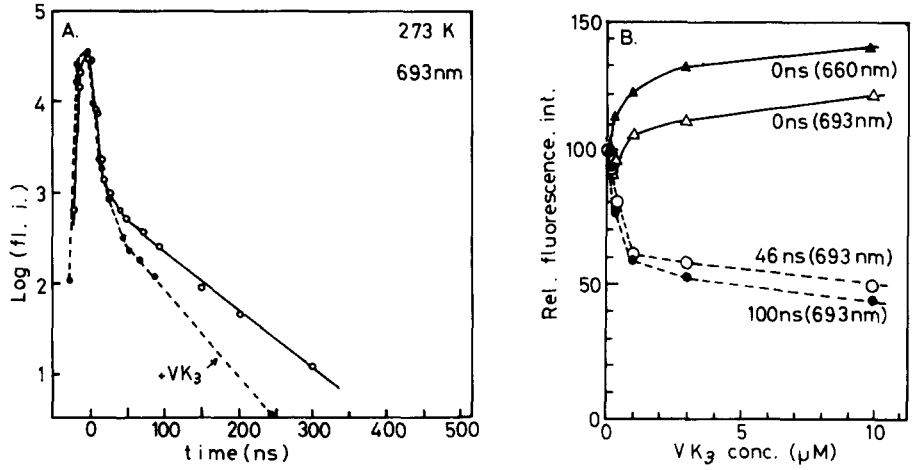

Fig. 3. (A) Effect of vitamin K-3 on the fluorescence decay kinetics. Closed and open circles, with and without 20 μM vitamin K-3, respectively. (B) Effects of concentration of vitamin K-3 on the intensities of fluorescence at 0 ns (at 660 and 693 nm), 46 ns and 100 ns (at 693 nm) at 273 K. Reaction mixture contained 50 mM Tris-Cl buffer (pH 7.0), 10 mM sodium ascorbate, 20 μM DCIP, 60% glycerol and the ether-extracted PS I particles (10 chlorophylls/P-700) at 3.0 μM P-700. fl.i., fluorescence intensity.

DCIP-reduced conditions. Addition of vitamin K-3 slightly increased the short wavelength fluorescence bands at 0 ns (see Fig. 3B) but decreased the slow decay phase at 693 nm. Magnitude of the slow phase extrapolated to 0 ns was depressed to be 1/2. An apparent acceleration of the rate of the slow phase was also detected. However, this acceleration effect cannot totally interpret the decrease of the slow phase observed at 0 ns. It may be due to the suppression of the very slow component. A decrease of yield at 693 nm at 0 ns was also seen in Fig. 3B at low vitamin K-3 concentrations (at 0.1 and 0.3 μM). 10 μM vitamin K-3 was found to be sufficient for the suppression of the slow phase (Fig. 3B). This concentration range is almost the same as that known for the suppression of the charge recombination [5]. That almost the same ratio of suppression was observed both at 46 and 100 ns (Fig. 3B) also indicates that vitamin K-3 decreased the yield of the slow phase. Vitamin K-3 does not seem to be acting as a quencher of the excited state of chlorophyll *a* in this concentration range, since this quinone had a rather enhancing effect on the yield of 660 nm fluorescence. A small enhancing effect of 660 nm fluorescence may be due to some nonspecific structural effect on the organization of the chlorophylls which are uncoupled with respect to the excitation energy transfer to P-700.

Discussion

PS I delayed fluorescence in millisecond and submicrosecond time ranges was reported by Shuvalov [14] and by Sonneveld et al. [13], respectively. The 50 ns delayed fluorescence detected in this study seems to be almost identical to the 100ns component reported by Sonneveld et al., although fluorescence emission bands detected in the latter study was at somewhat longer wavelengths in the latter work due to the abundance of antenna chlorophylls in their preparation. The 100 ns component was measured at times longer than 150 ns after the laser flash excitation and was estimated to be produced in the charge recombination in PS I reaction center judging from its sensitivity to magnetic field [13]. The 50 ns life time of the component measured in this work, however, directly shows the correlation with the charge recombination rate between $P\text{-}700^+$ and A_0^- measured by absorption spectroscopy [12].

In the previous studies [13,14], PS I-delayed fluorescence was detectable only under extremely low redox potential conditions in the presence of

dithionite, under which conditions A_1 as well as FeS centers are assumed to be reduced. However, the yield of 50 ns delayed fluorescence was high without dithionite in the ether-extracted preparation used in this work. Use of dithionite in the place of the absorbance and DCIP couple gave almost the same yield of flash-induced delayed fluorescence in a 20–100 ns time range. This situation can easily be explained by assuming a block of rapid oxidation of A_0^- by FeS centers after the extraction of vitamin K-1. Loss of a large amount of antenna chlorophylls in this preparation does not seem to explain the result, since similar long-lived fluorescence peaking at 693 nm can be detected in the PS I particles treated with dry ether which extracts all the vitamin K-1 but only 50% of antenna chlorophylls (Ref. 5 and Itoh, S. and Iwaki, M. unpublished data).

These results as well as the effect of vitamin K-3 support the idea that vitamin K-1 is functioning as the electron acceptor (A_1) adjacent to A_0 and stabilizing the reducing power, and that its extraction blocks the oxidation of A_0^- and results in the enhanced charge recombination producing the delayed fluorescence, although some contradictory evidence against this idea has been reported elsewhere [11,23,24]. The 50 ns delayed fluorescence measured here can be estimated to be directly produced in the charge recombination between P-700$^+$ and A_0^- with no participation of the triplet state, which has a longer life-time of about 100 μs in this preparation [5,12].

The emitter of the 675 nm fluorescence, which has a life-time of 6 ns, seems to be a chlorophyll *a* uncoupled with respect to the energy transfer to P-700, since its yield and decay time neither depend on the redox state nor on the temperature. The emitter of the 693 (701) nm fluorescence, on the other hand, seems to be P-700 itself, which is re-excited in the charge recombination reaction, since there is no absorption band of chlorophyll which can be an emitter of this long-wavelength fluorescence band other than P-700, in the wet-ether extracted PS I particles used in this study [19,25]. Absorption peak of P-700 in its reduced form has recently been estimated to be at 690 and 694 nm at room and at 90 K, respectively [25], i.e., shorter than the apparent maximum (695 nm) of the reduced-oxidized difference spectrum of P-700. The estimated absorption peak wavelength of P-700 well correlates with the emission peak of delayed fluorescence measured in this study. The faster phase of 693 (701) nm band, whose kinetics cannot be precisely followed, was also enhanced by the extraction of vitamin K-1. This fluorescence may consist of prompt fluorescence of P-700 and probably delayed fluorescence induced by nanosecond charge recombination. This point will be discussed elsewhere in the analysis of fluorescence kinetics in a picosecond time range (Mimuro, M. et al., unpublished results).

Acknowledgments

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References

1 Nuijs, A.M., Shuvalov, V.A., Van Gorkom, H.J., Plijter, J.J. and Duysens, L.N.M. (1986) Biochim. Biophys. Acta 850, 310–318.
2 Shuvalov, V.A., Nuijs, A.M., Van Gorkom, H.J., Sit, H.W.J. and Duysens, L.N.M. (1986) Biochim. Biophys. Acta 850, 310–318.
3 Brettel, P., Sétif, P. and Mathis, P. (1986) FEBS Lett. 203, 220–224.
4 Mansfield, R.W. and Evans, M.C.W. (1986) FEBS Lett. 203, 225–229.
5 Itoh, S., Iwaki, M. and Ikegami, I. (1987) Biochim. Biophys. Acta 893, 508–516.
6 Takahashi, Y., Hirota, K. and Katoh, S. (1985) Photosynth. Res. 6, 183,–192.
7 Schoeder, H.E. and Lockau, W. (1986) FEBS Lett. 199, 23–27.
8 Sétif, P., Hervo, G. and Mathis, P. (1981) Biochim. Biophys. Acta 683, 257–267.
9 Malkin, R. and Bearden, A.J. (1978) Biochim. Biophys. Acta 505, 147–181.
10 Evans, M.C.W. (1982) in Iron-Sulfur Proteins (Spiro, T.G., ed.), Vol. 4, pp. 249–284, John Wiley & Sons, New York.
11 Sétif, M., Biggins, J. and Ikegami, I. (1987) Biochim. Biophys. Acta 894, 146–156.
12 Ikegami, I., Sétif, M. and Mathis, P. (1987) Biochim. Biophys. Acta 894, 414–422.
13 Sonneveld, A., Duysens, L.N.M. and Moerdijk, A. (1981) Biochim. Biophys. Acta 636, 39–49.
14 Shuvalov, V.A. (1976) Biochim. Biophys. Acta 430, 113–121.

15 Schenck, C., Blankenship, R. and Parson, W.W. (1982) Biochim. Biophys. Acta 680, 44–59.
16 Woodbury, W.W., Parson, W.W., Gunner, M.R., Prince, R.C. and Dutton, P.L. (1986) Biochim. Biophys. Acta 851, 6–22.
17 Lavorel, J. and Étienne, A.-L. (1977) in Bioenergetics of Photosynthesis (Govindjee, ed.), pp. 183–221, Academic Press, New York.
18 Ikegami, I. and Katoh, S. (1975) Biochim. Biophys. Acta 376, 588–592.
19 Ikegami, I. and Itoh, S. (1986) Biochim. Biophys. Acta 851, 75–85.
20 Ikegami, I. and Itoh, S. (1987) Biochim. Biophys. Acta 893, 517–523.
21 Ikegami, I. (1976) Biochim. Biophys. Acta 449, 245–248.
22 Ikegami, I. and Ke, B. (1984) Biochim. Biophys. Acta 764, 80–85.
23 Palace, G.P., Frankel, J.E. and Warden, J.T. (1987) FEBS Lett. 245, 58–62.
24 Ziegler, K., Lockau, W. and Nitschke, W. (1987) FEBS Lett. 217, 16–20.
25 Ikegami, I. and Itoh, S. (1987) Biochim. Biophys. Acta 934, 39–46.