PROTON TRANSLOCATION IN THE SLOW RISE OF THE FLASH-INDUCED 515 nm ABSORBANCE CHANGE OF INTACT CHLOROPLASTS

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1. Introduction

The light-induced absorbance change around 515 nm (ΔA_{515}) in chloroplasts has been attributed mainly to the electrochromic response of pigments embedded in the thylakoid membranes [1]. This change in absorbance, related to the energized state of the thylakoids, starts with a fast (~ns) rise and relaxes with a slow (~100 ms) decay [2,3]. Recently, in intact isolated chloroplasts a biphasic rise, has been established [4–9]. The slow phase of the rise in ΔA_{515} has been explained in various ways. The most common view is that the slow rise of ΔA_{515} is connected with the reduction of cytochrome *f* and to an inward translocation of protons [5,8,10].

This paper provides additional, direct evidence to support this idea by analysing the kinetics of the flash-induced ΔA_{515} in a medium where ^1H_2O was replaced by ^2H_2O. Furthermore, from an analysis of the temperature dependence of the kinetic components of ΔA_{515}, we conclude that slow rise and decay represent different proton translocation processes as shown by their different activation energies.

2. Materials and methods

Intact chloroplasts were isolated from mesophyll protoplasts of maize [11]. The integrity of plastids, as assayed by oxygen evolution rates in the presence of ferricyanide before and after osmotic shock, was routinely 90–95%. The suspending medium [12] contained 0.4 M D-sorbitol, 10 mM NaCl, 5 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM EDTA, 0.4% bovine serum albumin and 50 mM Hepes dissolved either in ^1H_2O or ^2H_2O (99.7%). The ^1H_2O medium was set to pH 7.50, while the ^2H_2O medium was adjusted to pH 7.34 where electrostatic equivalence [13] was established. Chloroplasts (0.1 ml), 25 µmol chl/ml, were incubated in 5 ml suspending medium at 0°C in the dark for 10 min prior to measurements. Flash-induced ΔA_{515} was measured in a single-beam spectrophotometer [7]. Actinic flashes of saturating intensity with durations of 3 µs at half-peak emission were filtered through Schott RG 630 filters. Averages of 50–100 traces were taken at a flash frequency of 1 flash/s. The temperature of the samples was regulated by a thermostat operating on the Peltier effect. The temperature of the chloroplast suspension was monitored regularly and was controlled to an accuracy of ±0.1°C.

3. Results and discussion

Fig.1 shows a representative example of the time course of ΔA_{515} of intact chloroplasts at 5°C and 25°C in ^2H_2O and ^1H_2O containing media.

It can be seen that the amplitude of the fast ($\ll$ms) rise was unaffected either by the presence of ^2H_2O or by temperature, as expected from the photochemical nature of the primary charge separations occurring in the reaction centers [1].

In contrast, the slow phase of the rise and the decay were considerably decelerated in ^2H_2O and at lower temperatures. This kinetic isotope effect demonstrates the participation of protons in determining the kinetics of ΔA_{515}.

The slow (~100 ms) decay of ΔA_{515} is clearly

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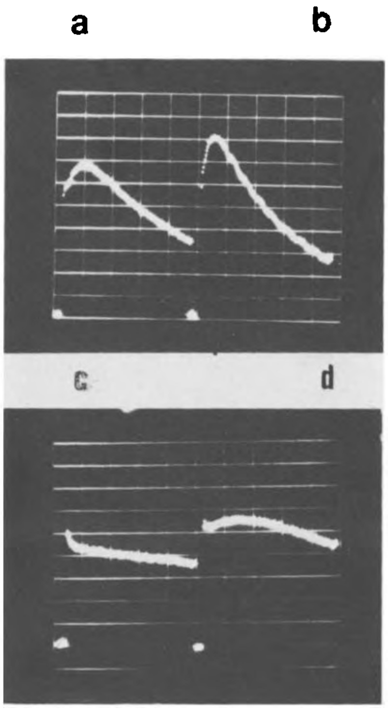

Fig.1. Oscilloscope display of ΔA_{515} for intact chloroplasts suspended in ^2H_2O (a,c) and ^1H_2O (b,d) containing media measured at 25°C (a,b) and at 5°C (c,d). Vertically the screen represents ΔA of 5×10^{-3}, horizontally a sweep of 400 ms. An av. 100 transients were collected. Time resolution was 1 ms.

connected with a proton efflux via the ATP synthetase [1].

As to the slow rise of ΔA_{515}, the kinetic isotope effect provides direct evidence of the involvement of proton translocation in the processes underlying this event. This may be correlated with proton uptake occurring via the plastoquinone pool [5,10]. The protons entering the membrane may either contribute to the electrochromism directly by a charge effect [5,6] or indirectly by changing the conformation of the field detecting pigment–protein complexes [9].

In cold samples an additional kinetic component, a fast (<10 ms) decay could also be distinguished, which virtually disappeared when samples were rewarmed [7]. This type of fast decay of ΔA_{515} can be explained in terms of a discharge and/or a flow of ions across the leaks of the thylakoid membranes [14].

The three, experimentally resolved, kinetic components of ΔA_{515} (slow rise, fast and slow decay of absorbance) were separated by means of a stepwise deconvolution according to a linear combination of three exponentials:

$$\Delta A(t) = a_1 e^{-k_1 t} - a_2 e^{-k_2 t} + a_3 e^{-k_3 t}$$

in a procedure as illustrated in fig.2.

In the equation, a_1 and k_1 are amplitude and rate constant of the slow decay; a_2 and k_2 characterize the slow rise; a_3 and k_3 refer to the fast decay of ΔA_{515}.

Arrhenius plots for the three kinetic components of chloroplasts suspended in ^1H_2O and ^2H_2O containing media revealed different activation energies and isotope effects (fig.3).

The proton efflux via ATP synthetase, correlated with the slow decay of ΔA_{515}, proceeds with the same activation energy in the temperature range of 10–30°C irrespective of the ^2H_2O content of the medium ($\Delta E = 7.5 \pm 0.9$ kcal/mol for the ^2H_2O containing medium and 7.3 ± 0.4 kcal/mol for the ^1H_2O containing medium). The kinetic isotope effect, characterised by $(k^1H_2O)/(k^2H_2O)$ was 1.46 ± 0.02. This value is in agreement with the fact that the mass of hydrogen atom of water may be directly involved in

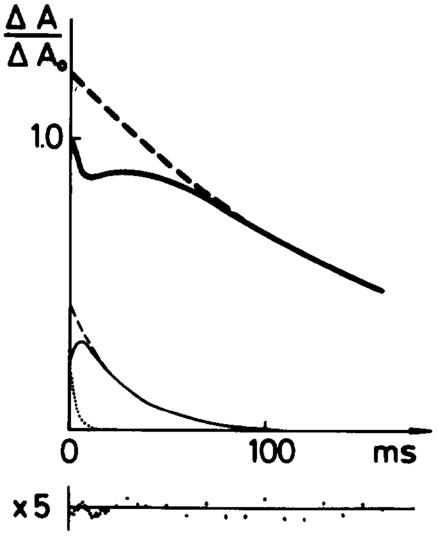

Fig.2. Deconvolution of an absorption transient ΔA_{515} of chloroplasts suspended in ^1H_2O at 15°C. Upper part: the steps of the procedure; solid heavy line, experimental curve; dashed heavy line, exponential for a_1 and k_1; solid light line, $a_1 e^{k_1 t} - \Delta A(t)$; dashed light line, exponential for a_2 and k_2; dotted line, $a_1 e^{k_1 t} - a_2 e^{k_2 t} - \Delta A(t)$ for a_3 and k_3. Further explanation in the text. Lower part: the error of fitting between the experimental and calculated curve.

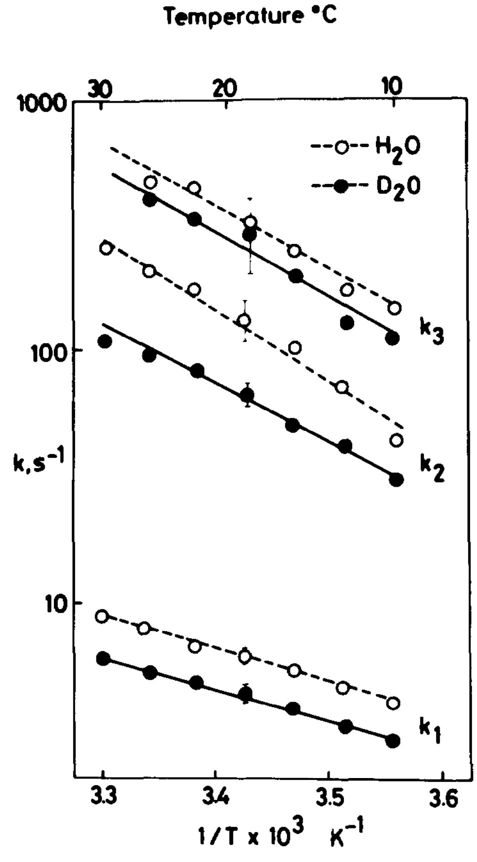

Fig.3. Arrhenius plots for the slow (10–100 ms) kinetic components of ΔA_{515} of intact chloroplasts: k_1, rate constants for the slow efflux of protons; k_2, for the slow uptake of protons; k_3, for the rapid decay of ΔA_{515}. The bars show the error in the determination of rate constants.

the underlying reaction [15]. Our values for the activation energy are low compared to the values which have been reported earlier [16,17] and in contrast to [17], our plot did not show any break-point. These discrepancies might be resolved by postulating a composite character of ΔA_{515} in conventional preparations of spinach chloroplasts also, which, as a rule contain appreciable amounts of intact plastids.

For the slow rise of ΔA_{515}, related to an inward translocation of protons, activation energies were 12.5 ± 1.5 kcal/mol and 16.4 ± 1.9 kcal/mol for the ^2H_2O and ^1H_2O containing media, respectively. The difference between the slopes and the high values of standard deviation could be brought about by break points, not resolved in these measurements. The kinetic isotope effect for this phase of ΔA_{515} varied from 1.4–2.1. The value of the activation energy obtained for the ^1H_2O medium agrees well with the 15.2 kcal/mol determined for the slow proton uptake using a bromocresol purple probe [16]. This figure is not very far removed from the 11 ± 1 kcal/mol activation energy required for the reduction of cytochrome *f* [18], which is probably involved in the proton uptake generating the slow rise of ΔA_{515} [5,6,10].

The accuracy of the values obtained for k_3 did not permit calculation of activation energies but a temperature dependence may be clearly observed. In the fast phase of ΔA_{515} decay only a very slight ^2H_2O effect can be observed. This is in agreement with the view that the underlying phenomenon, the unspecific discharge of the membrane potential, does not necessarily involve the participation of protons. The amplitude a_3 characterising discharges was not appreciably affected by temperature over the range investigated. This means that the perceptibility of this kinetic component in the cold samples results from the suppression of the slow rise of absorbance and not from a net increase of the decay brought about by discharges.

In conclusion, this work provides direct experimental evidence for the involvement of proton translocation in the slow rise of ΔA_{515}, a characteristic feature of the energization of intact chloroplast. In addition, we drew the attention to the fact that the time course of ΔA_{515} has, in all cases, a composite character, therefore it is necessary to separate the individual components.

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References

[1] Witt, H. T. (1979) Biochim. Biophys. Acta 505, 355–427.
[2] Witt, H. T. (1955) Naturwiss. 42, 72–73.
[3] Wolff, Ch., Buchwald, H. E., Ruppel, H. and Witt, H. T. (1969) Z. Naturforsch. 24b, 1038–1041.

[4] Horváth, G., Droppa, M., Mustárdy, L. A. and Faludi-Dániel, Á. (1978) Planta 141, 239–244.

[5] Velthuys, B. R. (1978) Proc. Natl. Acad. Sci. USA 75, 6031–6034.

[6] Crowther, D., Mills, J. D. and Hind, G. (1979) FEBS Lett. 98, 386–390.

[7] Horváth, G., Niemi, H. A., Droppa, M. and Faludi-Dániel, Á. (1979) Plant Physiol. 63, 778–782.

[8] Slovacek, R. E., Crowther, D. and Hind, G. (1979) Biochim. Biophys. Acta 547, 138–148.

[9] Schapendonk, A. H. C. M., Vredenberg, W. J. and Tonk, W. J. M. (1979) FEBS Lett. 100, 325–330.

[10] Bouges-Bocquet, B. (1977) Biochim. Biophys. Acta 462, 371–379.

[11] Bialek, G. E., Horváth, G., Garab, Gy. I., Mustárdy, L. A. and Faludi-Dániel, Á. (1977) Proc. Natl. Acad. Sci. USA 74, 1455–1457.

[12] Reeves, S. G. and Hall, D. O. (1973) Biochim. Biophys. Acta 314, 25–30.

[13] Kalinichenko, L. P. and Lobyshev, V. P. (1976) Studia Biophys. 58, 235–240.

[14] Junge, W. and Witt, H. T. (1968) Z. Naturforsch. 23b, 244–254.

[15] Kihara, T. and McCray, J. A. (1973) Biochim. Biophys. Acta 292, 297–309.

[16] Yamamoto, Y. and Nishimura, M. (1977) Plant Cell Physiol. 18, 293–301.

[17] Graeber, P. and Witt, H. T. (1974) Proc. 3rd. Int. Congr. Photosynth., Rehovot (Avron, M. ed) pp. 951–956, Elsevier/North-Holland, Amsterdam, New York.

[18] Whitmarsh, J. and Cramer, W. A. (1979) Biophys. J. 26, 223–234.