

Effect of Temperature on Electron Transport Activities of Isolated Chloroplasts

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ABSTRACT

Temperature-induced changes in electron transport activities of chloroplasts isolated from chilling-sensitive and chilling-resistant plants have been examined. Using methylviologen as electron acceptor, temperature-induced changes occurred in the photosystem II plus photosystem I activities of chloroplasts isolated from chilling-resistant spinach (*Spinacia oleracea* L.) and pea (*Pisum sativum* L. cv. Alaska) plants. The changes occurred at approximately 17 C for spinach and 15 C for pea. A temperature-induced change, at approximately 13 C, in photosystem I activity using methylviologen was also observed for pea chloroplasts. These results extend earlier work and indicate that temperature has a general effect on the functioning of thylakoid membranes.

Chloroplasts isolated from chilling-sensitive bean plants (*Phaseolus vulgaris* L. cv. Blue Lake 141) show a temperature-induced change in ferricyanide reduction at approximately 12 C. These results with spinach, pea, and bean support the view that the presence of temperature-induced changes in chloroplast activity assayed *in vitro* is not correlated with chilling sensitivity.

The effect of temperature on the structure and function of the energy transducing membranes of plant mitochondria and chloroplasts has received increased attention in recent years. Much of this attention has focused upon understanding the chilling-sensitivity or chilling-resistance of different plant species at the molecular level (for reviews see Refs. 7, 13, 14, 15).

An increase in E_a^2 for respiratory enzymes and a decrease in lipid fluidity have been observed to occur below a temperature, typically between 10 to 15 C, for mitochondrial membranes isolated from chilling-sensitive plants but not for those from chilling-resistant plants (7, 14, 15). While the presence or absence of temperature-induced changes in mitochondrial membranes correlates with the chilling-sensitivity or resistance, respectively, of the plant species from which the mitochondria were isolated, the results obtained using chloroplast membranes do not show such a straightforward correlation (5, 6, 10, 11, 17).

We have recently reported that chloroplast membranes isolated from both chilling-sensitive and chilling-resistant plants show temperature-induced changes in E_a for electron transport when a rate limiting step(s) has been eliminated or circumvented (10, 11). A change occurred not only in the chilling-temperature range but also at a higher temperature for the chloroplasts isolated from

each plant. Since DCIP and ferricyanide, the principal electron acceptors used in the previous reports, are capable of accepting electrons at various points along the electron transport chain (18), it is conceivable that the results could be explained in terms of shifts in sites of reduction of the electron acceptors rather than a specific effect of temperature on an electron transport component(s). In addition the previous work did not investigate possible temperature effects on PS I reactions. This report shows that under certain conditions temperature-induced changes in E_a for PS II plus PS I electron transport activity can be observed using MV as electron acceptor with chloroplast membranes isolated from pea, a chilling-resistant plant. Also reported are temperature-induced changes in E_a for PS I activity of pea chloroplasts, for PS II plus PS I activity of chloroplasts from chilling-resistant spinach plants and for ferricyanide reduction by chloroplasts from chilling-sensitive bean plants.

MATERIALS AND METHODS

Plant Material. Pea plants (*Pisum sativum* L. cv. Alaska) were grown in Vermiculite in a growth chamber at 20 ± 1 C. Grow-lux fluorescent tubes and incandescent bulbs provided light of approximately 30 w/m² for 12 h/day. Bush snapbeans (*Phaseolus vulgaris* L. cv. Blue Lake 141) were grown similarly in a constant temperature plant growth room at 25 ± 1 C under light of approximately 55 w/m². Spinach (*Spinacia oleracea* L.) was obtained at a local market. Leaves were harvested from 11-day-old pea plants and primary leaves were harvested from 10-day-old bean seedlings.

Chloroplast Isolation. Chloroplasts were isolated (at 0–4 C) in a medium of 50 mM Sørensen's phosphate buffer (pH 7.4), 50 mM NaCl and 0.5% (w/v) BSA. DTT at 5 mM and 2% (w/v) Polyclar AT were also included in the medium used for isolating bean chloroplasts. Leaf segments were blended for four periods of 5 s each using speed setting three of a domestic seven speed blender (Oster Corp. Model 847). Chloroplasts were isolated from the homogenate as previously described (11).

Assays. Electron transport activities were measured polarographically using two Clark-type O₂ electrode assemblies. Saturating actinic red light was obtained by filtering light from a 500-w slide projector through a Schott RG-630 filter and a 500-ml boiling flask which served as a heat filter and to focus the actinic light on the reaction vessel. The basic reaction mixture consisted of 50 mM Sørensen's phosphate buffer (pH 7.4), 50 mM NaCl, and 0.005% (w/v) BSA. PS II plus PS I assays contained in addition 167 μM MV, 1 mM Na azide, 10 to 20 μg Chl ml⁻¹, and where indicated 60 mM methylamine (titrated to pH 7.4). Reaction mixtures for assay of PS I also contained 2 mM sodium ascorbate, 60 μM DCIP, and 40 μM DCMU. Ferricyanide reduction was measured in the basic reaction mixture which contained in addition 0.67 mM K-ferricyanide, 60 mM methylamine (pH 7.4), and 20 μg Chl ml⁻¹.

The reaction mixture (minus chloroplasts) was pre-equilibrated

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² Abbreviations: E_a : Arrhenius activation energy; DAD_{ox}: oxidized 2,3,5,6-tetramethyl-*p*-phenylenediamine; DCIP: 2,6-dichlorophenolindophenol; MV: methylviologen.

to the required temperature. The chloroplasts which had been stored at 0 C were added to the reaction mixture and incubated for 4 min prior to assay. The temperature of the reaction mixture was generally maintained to within ± 0.05 C during the course of each assay. Fall off in the activity of the chloroplast preparation stored at 0 C was approximately 10% and activities have not been corrected for fall off. Determinations of lines of best fit through the data points in the Arrhenius plots presented were determined using a least-squares regression analysis (2). Calculated E_a values were reproducible to within ± 4 kJoules mol^{-1} . Chl was determined according to the method of Arnon (1).

RESULTS

The Effect of Temperature on PS II plus PS I and PS I Activities of Isolated Pea Chloroplasts. Figure 1A shows the effect of temperature on PS II plus PS I activity of isolated pea chloroplasts in the presence of the uncoupler methylamine. This Arrhenius plot shows a change in slope at approximately 15 C; the E_a values for temperatures above and below the change in slope are given in Table I. An Arrhenius plot for the same activity except for the omission of methylamine from the reaction mixture is presented in Figure 1B. In this case a linear relationship is seen to exist between the log of the activity and the absolute temperature. The activation energy for the data in Figure 1B approximates that for temperatures above 15 C in Figure 1A (Table I).

Data for PS I activity of isolated pea chloroplasts using reduced

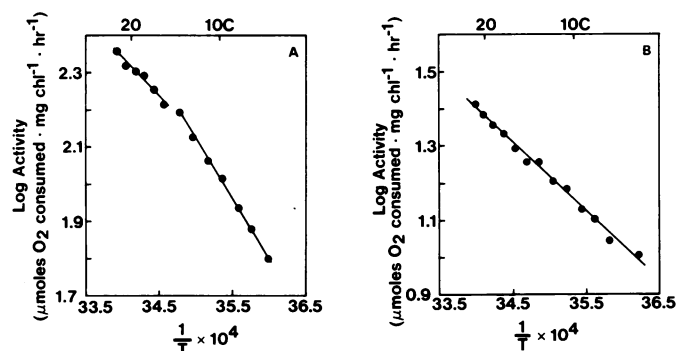


FIG. 1. Arrhenius plot of PS II plus PS I activities by isolated pea chloroplasts with MV as electron acceptor. Assays were conducted in the presence (A) or absence (B) of the uncoupler methylamine.

Table I. E_a for Electron Transport Activities in Various Temperature Ranges

The values for E_a were calculated from the data given in Figures 1-4.

Plant	Activity ^a	Methylamine	Temperature Range	E_a
			C	kJoules mol^{-1}
Pea	$\text{H}_2\text{O} \rightarrow \text{MV}$	+	<15	60.6
			>15	39.3
	$\text{H}_2\text{O} \rightarrow \text{MV}$	-	<21	35.5
			>13	31.1
$\text{DCIPH}_2 \rightarrow \text{MV}$	+	<13	50.8	
		>13	31.1	
$\text{DCIPH}_2 \rightarrow \text{MV}$	-	<21	49.9	
		>17	25.7	
Spinach	$\text{H}_2\text{O} \rightarrow \text{MV}$	+	<17	56.0
			>17	25.7
Bean	$\text{H}_2\text{O} \rightarrow \text{FeCN}$	-	<12	50.3
			>12	34.8

^a DCIPH_2 : reduced DCIP; FeCN, ferricyanide.

DCIP as electron donor and MV as electron acceptor are presented in Figure 2. For the data in Figure 2A the activity was assayed in the presence of methylamine. A change in slope of the Arrhenius plot occurs at about 13 C. The E_a values are given in Table I. Figure 2B shows the effect of temperature on PS I activity assayed in the absence of methylamine. A straight line fits the data and the calculated E_a is nearly identical to that for temperatures below 13 C in Figure 2A (Table I).

The Effect of Temperature on Electron Transport Activities of Isolated Spinach and Bean Chloroplasts. An Arrhenius plot for PS II plus PS I activity of isolated spinach chloroplasts using MV as electron acceptor in the presence of methylamine is presented in Figure 3. A change in E_a is observed at about 17 C. The magnitude of the change in E_a is slightly greater than that for the same activity of pea chloroplasts (Table I).

It has previously been reported that isolated bean chloroplasts show a very low rate of the PS II plus PS I reduction of NADP (3). In the present work, the PS II plus PS I reduction of MV was extremely slow and the data yielded too much scatter to be meaningful when graphed as an Arrhenius plot (data not shown). However, in agreement with previous reports (3, 4) reasonable rates of ferricyanide reduction were obtained. An Arrhenius plot for photochemical activity by bean chloroplasts using ferricyanide as the electron acceptor in the presence of methylamine is presented in Figure 4. A change in E_a occurs at about 12 C. The E_a values are given in Table I.

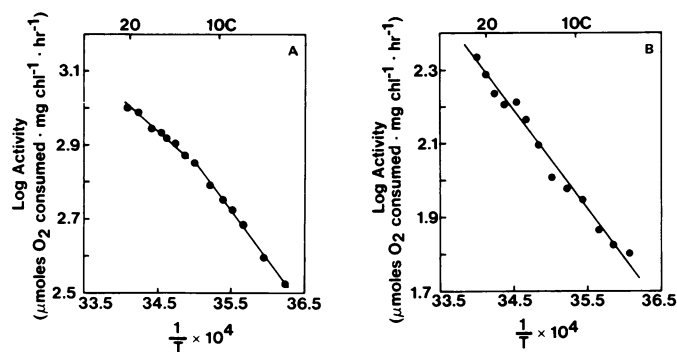


FIG. 2. Arrhenius plot of PS I activities by isolated pea chloroplasts with reduced DCIP as electron donor and MV as the electron acceptor. Assays were conducted in the presence (A) or absence (B) of the uncoupler methylamine.

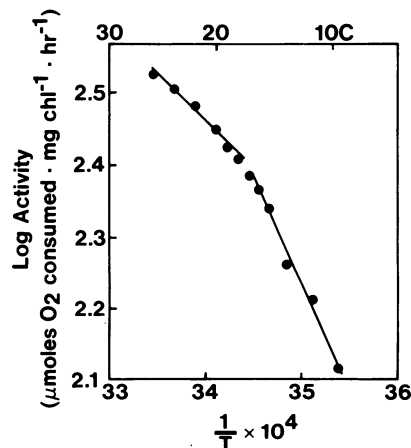


FIG. 3. Arrhenius plot of PS II plus PS I activities by isolated spinach chloroplasts with MV as electron acceptor. Reaction mixtures contained the uncoupler methylamine.

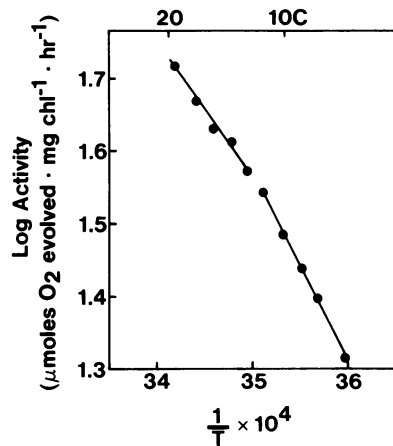


FIG. 4. Arrhenius plot of electron transport activities by isolated bean chloroplasts with ferricyanide as electron acceptor. Reaction mixtures contained the uncoupler methylamine.

DISCUSSION

The data presented show that temperature-induced changes in PS II plus PS I and PS I electron transport activities of isolated pea chloroplasts can be observed under certain conditions when MV is used as the electron acceptor. In agreement with the results of Shneyour *et al.* (16), who studied the PS II plus PS I and PS I reduction of NADP by pea chloroplasts, there was no change in activation energy when the assay mixture did not contain an uncoupler. However in agreement with our previous studies (10, 11), a distinct change in activation energy was observed as the temperature was varied when an uncoupler was present in the reaction mixture. In addition the temperature at which the change occurs agrees with that previously reported for DCIP photoreduction (10).

Shneyour *et al.* (16) did not observe temperature-induced changes in electron transport activities of chloroplasts isolated from chilling-resistant plants, however, changes were observed for chloroplasts isolated from chilling-sensitive plants when the enzyme ferredoxin-NADP oxidoreductase (EC 1.6.99.4) was involved in the assay. It was suggested that the presence of a temperature-induced change in activity resulted from an effect of a thermal transition of the thylakoid membrane lipids on the enzyme in the case of chloroplasts from chilling-sensitive plants whereas thylakoid membranes from chilling-resistant plants lacked such a membrane lipid transition (at least in the range of chilling-temperatures) and therefore showed no temperature-induced change in activity. The present work on chloroplasts isolated from chilling-resistant pea and spinach plants, and other reports (5, 8, 10, 11, 17) clearly show that neither are temperature-induced changes in electron transport activity assayed *in vitro* confined to chloroplasts isolated from chilling-sensitive plants nor is there a necessity for the involvement of the enzyme ferredoxin-NADP oxidoreductase in the assay. Additionally, Quinn and Williams (13) have recently reviewed the possible causal agents of temperature-induced changes in membrane function, and they have questioned the interpretation that such changes in function are due to temperature-induced changes in lipid ordering *per se*. At present it is impossible to state conclusively the direct cause of the temperature-induced changes in function.

Previous studies employing higher plant chloroplasts have demonstrated temperature-induced changes for activities involving PS II such as delayed light emission (6), and the photoreduction of various electron acceptors such as DCIP (5, 10, 11), ferricyanide (8, 11, 17), and DAD_{ox} (11). The temperature-induced effects on delayed light emission and photoreduction of DAD_{ox} suggest that there is a specific temperature effect on a reaction associated with

PS II. Temperature-induced effects on DCIP and ferricyanide photoreduction are less conclusive since these compounds are capable of accepting electrons at various sites along the electron transport chain (18). It is conceivable that the temperature-induced changes observed resulted from a shift in the site of the reduction of these compounds rather than a temperature effect on a component(s) of the electron transport chain. From the present work, the latter would appear to be the case since any shift in site of MV reduction would be most unlikely. However it can not be ruled out that a temperature-induced membrane conformational change is regulating the accessibility of MV to its reductant in the membrane. The present results are consistent with our earlier conclusion that under conditions which significantly increase the rate of electron transport (*e.g.* the use of an uncoupler) some rate-limiting step with constant E_a is eliminated or circumvented which allows temperature-induced changes in electron transport activity to be observed (11). The results of the PS I assays indicate that not only are reactions associated with PS II affected by temperature but that a reaction(s) associated with PS I is also affected. The data of Neumann *et al.* (9, Fig. 9) clearly indicate a temperature-induced change at about 20 C in photophosphorylation accompanying electron transport from reduced DCIP to MV in the presence, but not absence, of an uncoupler for a subchloroplast fraction from romaine lettuce, a chilling-resistant plant. A temperature-induced change at about 18 C has also been reported for *N*-methylphenazonium methosulfate cyclic photophosphorylation by a subchloroplast fraction from spinach (8). It appears that at least several and perhaps all of the components of the electron transport chain are affected similarly by temperature and thus result in a general temperature-induced effect on membrane function. At present this conclusion must be restricted to higher plant photosynthetic lamellae in view of the report that not all photosynthetic electron transport activities in the prokaryote *Anacystis* are affected similarly by temperature (12).

With the exception of assays employing DAD_{ox} as the electron acceptor, the activation energies for the reactions we have studied show remarkable consistency. They are in general between 50 to 60 kJoules mol⁻¹ for coupled reactions and for uncoupled reactions at low temperatures below the change in slope of the Arrhenius plots (10, 11; Table I). For uncoupled reactions above the change in slope at low temperature they are between 25 to 40 kJoules mol⁻¹ (10, 11; Table I). However the activation energy for the PS II plus PS I reduction of MV in the absence of an uncoupler is much lower than expected, being only about 36 kJoules mol⁻¹ (Fig. 1B, Table I). Whether or not it is significant that this latter value lies within the range for uncoupled reactions above the change in slope at low temperature is not clear.

It was recently reported that chloroplasts isolated from bean and spinach show a temperature-induced change in delayed light emission in the range of what are considered chilling temperatures whereas pea chloroplasts did not show such a change (6). This report prompted the selection of plant species used in the present study. With regard to electron transport activities for these plants, it has previously been reported that bean chloroplasts show a temperature-induced change with NADP as the electron acceptor but not when DCIP was used (16). However as shown here, a temperature-induced change is observed for ferricyanide reduction by bean chloroplasts when methylamine is present in the reaction mixture. Arrhenius plots for DCIP reduction by spinach chloroplasts (5) and for ferricyanide and MV reduction by digitonin-treated spinach chloroplast membranes (8) have shown temperature-induced changes. The present report confirms this change for spinach chloroplasts with MV as the electron acceptor in the presence of methylamine. With regard to spinach and bean chloroplasts, there seems to be general agreement between the delayed light emission and electron transport assays. Under conditions which have previously been shown to be required for demonstrat-

ing temperature-induced changes in electron transport activity for chloroplasts isolated from either chilling-sensitive or chilling-resistant plants, it is clear from this and previous work (10) that pea chloroplasts show temperature-induced changes in the range of chilling temperatures. Why such changes in electron transport activity do not correlate with the results of the delayed light emission by pea chloroplasts needs further study as there does not appear to be any fundamental difference between the electron transport data for uncoupled spinach, bean, and pea chloroplasts.

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