Photosynthesis in Fescue

I. HIGH RATE OF ELECTRON TRANSPORT AND PHOSPHORYLATION IN CHLOROPLASTS OF HEXAPLOID PLANTS

ROGER W. KRUEGER AND DONALD MILES
Division of Biological Sciences, University of Missouri, Columbia, Missouri 65211

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ABSTRACT

Chloroplasts isolated from tall fescue, Festuca arundinacea Schreb., showed high rates of electron transport, comparable to rates observed for spinach chloroplasts. Chloroplasts were well coupled and rates of electron transport from water to methyl viologen (photosystem II and I) were increased two to five times when ADP and inorganic phosphate or methylamine (uncoupler) were added to the reaction mixture. Ratios of P:2e for photosystem II plus I were found to be near 1.2. Electron transport rates from water to p-phenylenediamine or 2,6-dichlorobenzoxoquine (photosystem II) were over 300 micromoles O₂ per hour per milligram chlorophyll, while P:2e ratios were found to be over 0.5. The highest rates of electron transport were found in electron flow from diaminodurene to methyl viologen (photosystem I) and P:2e ratios remained near 0.5.

Light intensity saturation curves for photosystem II and I, as well as the photosystems independently, resembled curves for spinach, with saturation of electron transport in photosystem I and photosystem II separately occurring at 35% of the available light intensity (6000 microeinstein per square meter per second). Photosystem II and I in sequence were saturated at about half this light intensity.

Tall fescue (Festuca arundinacea, Schreb.) represents an intriguing and underdeveloped model system for studying the relationship between photosynthesis and physiology. Fescue is a forage crop and yields are measured as vegetative growth, unlike other plants such as soybeans and maize which translocate photosynthetic products into seed production. Thus, growth can be directly correlated to the magnitude of the photosynthetic apparatus and directly applied to agronomic use. The advantage for the use of tall fescue in photosynthetic work is the ability to analyze photoreactions as affected by gene dosage. The commonly cultivated tall fescue is a hexaploid (42 chromosomes) (23) but can be found in a ploidy series ranging from tetraploid to decaploid (3). Contrasting tall fescue genotypes in this genetic series have been examined for RuBP\(^{2}\) carboxylase activity (16) and CER rates (24, 25) and results show increased nuclear chromosome number correlating with increased photosynthetic activity.

This report, as a preliminary study of the relationship between genome content and biochemical aspects of photosynthesis, presents a characterization of the photosynthetic electron transport system of the hexaploid tall fescue. Comparisons are made with the reported photosynthetic activities in isolated spinach chloroplasts and isolated maize tissue, two systems that have been examined extensively.

Because ploidy level has been shown to affect leaf morphology (19), isolation of envelope-free chloroplasts can eliminate many variations due to physical differences; thus, direct comparison of genotypes can be made. The isolation procedure for fescue appeared difficult because the cell walls are highly siliclated (27) and vegetative growth is coarse. Yet, when young, soft, actively growing tissue was harvested, little starch is isolated with the chloroplasts. Rates of electron transport and phosphorylation from this tissue are high and consistent.

The results presented here show that optimal conditions for electron transport in fescue chloroplasts are similar to spinach chloroplasts. Measurements of changes in O₂ concentration for PSI were 300+ μmol O₂/h-mg Chl, for PSI were 1,000+ μmol O₂/h-mg Chl, and for both photosystems in sequence were 30+ μmol O₂/h-mg Chl coupled and 180+ μmol O₂/h-mg Chl uncoupled.

MATERIALS AND METHODS

Plant Material. Tall fescue (Festuca arundinacea Schreb var. KY-31) was grown from seed in the growth chambers at 25 C during a 14-h day (400 μE m\(^{-2}\) s\(^{-1}\)) and 20 C during a 10-h night. All plants were fertilized biweekly with a commercial fertilizer 23-19-17 (RapidGro).

Isolation of Chloroplasts. Fully expanded leaves were excised from the upper 10 to 15 cm of young, actively growing plants. Five g. fresh weight, of leaf material was washed two times with distilled H₂O and cut with shears into 1 to 2 mm segments. These segments were ground at 0 to 4 C in 25 ml of medium containing 0.33 mM sorbitol, 50 mM Tricine (pH 8.0), 1 mM MgCl₂, 1 mM MnCl₂, 1 mM EDTA, 5 mM β-mercaptoethanol, and 0.1% BSA. A chilled mortar and pestle. The suspension was filtered through one layer of Miracloth and centrifuged at 4 C to 150g for 2 s and stopped to remove large debris. The supernatant was poured into a chilled tube and sedimented at 1,500g for 10 min. The pellet was resuspended in 2 to 4.0 ml of 0.4 μM sucrose, 20 mM Tricine (pH 8.0), 10 mM NaCl and 0.1% BSA. Chl content and Chl a/b was determined by dissolving 0.1 ml of chloroplast suspension in 80% acetone (v/v) as described by Arnon (1) and measuring A at 645 and 663 nm with a Perkin-Elmer 552 spectrophotometer. Total Chl content per mg fresh weight was measured by cutting a 4-mm disk from a young, fully expanded leaf, weighing and homogenizing in 5.0 ml of 80% acetone with a TenBroeck ground glass homogenizer and quantitating Chl as described above.

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2 Abbreviations: RuBP, ribulose 1,5-bisphosphate; PDₐ, oxidized-p-phenylenediamine; DCBQ, 2,6-dichloro-p-benzoquinone; DAD, diaminodurene; MV, methyl viologen; SOD, superoxide dismutase; MA, methylamine; CER, carbon exchange ratio; DBMIB, 2,5-dibromothymoquinone.
Electron Transport Measurements. All electron transport measurements were performed in a 5.8 ml water jacketed (25 °C) reaction chamber with a Clark-type O₂ electrode, YSI model 53, and recorded on a Beckman 10 inch strip chart recorder. The reaction mix consisted of 40 mM Tricine (pH 8.0 unless otherwise indicated), 60 mM NaCl, 4 mM MgCl₂ and 20 to 25 μg Chl of chloroplast suspension. The actinic light on the reaction chamber was 6 × 10² μE m⁻² s⁻¹ in the 400 to 700 nm wave band provided by a projection lamp for 1-min intervals. If measurements were run longer than 1 min, the light was passed through 5 cm of 0.2% CuSO₄ serving as a heat filter. Labeling of ATP in photophosphorylation was performed in the 5.8-ml chamber as described by Avron (2) and Saha and Good (17) with modification in extraction by using toluene-butanol. Sample counting was done by the Cerenkov method (4).

PDₐ and DCBQ were used as PSII electron acceptors. Both are known to be excellent lipophilic oxidants (6). For PSI activity, DAD was used as the electron donor. For PSI and PS I + II electron acceptors MV was used and O₂ uptake and measured instead of O₂ evolution with the Clark-type electrode.

Chemicals. General chemicals were from Sigma and Mallinkrodt with the exception of DAD and DCBQ (Eastman). PD₂, DAD, and DCBQ were re-crystallized and dissolved in water, 0.01 N HCl and ethanol (less than 1.0% in the final reaction mixture), respectively. PD₂ and DAD were made up immediately prior to use while DCBQ was kept at -10°C until use. ²⁵P was purchased from New England Nuclear.

RESULTS

Chloroplast isolation procedures and tissue selection were uniform as indicated by consistent Chl a/b ratios, 2.73 ± 0.05 sd. Total Chl content of leaf disks was 0.675 μg Chl/mg fresh weight.

Characterization of PSII electron transport in isolated tall fescue chloroplasts was first shown by using two lipophilic electron acceptors, DCBQ and PD₂ (Fig. 1A) over a range of concentrations. Optimal rates at pH 7.5 to 8.0 exceeded 350 μmol O₂/h-mg Chl, a much higher rate than maize tissue electron transport (7, 24) but comparable to isolated spinach chloroplasts (6, 14, 15).
FIG. 2. Properties of PSI in isolated fescue chloroplasts. Each experiment (A, B, C) was conducted on at least three different dates and points represent the mean of the trials. The 5.8 ml reaction mix and Chl concentration were the same as in Figure 1. Freshly prepared ascorbate and DAD were 0.5 mM and 1.0 mM concentrations, respectively, unless otherwise indicated. MV in A and C was 100 μM concentration, MA was 10 mM concentration. Buffers used in C were the same as in Figure 1. DCMU concentration was 8.6 μM and SOD concentration was greater than 300 units per reaction.

FIG. 3. Properties of PSII + I in isolated fescue chloroplasts. Each experiment (A, B, C) was conducted on at least three different dates and points represent the mean of the trials. The 5.8 ml reaction mix and Chl concentration were the same as in Figures 1 and 2. MV and MA (uncoupler) were used at 100 μM and 10 mM concentrations, respectively. Buffers were the same as listed in Figure 1.
Table I. PSII, PSI and PSII + I Electron Transport and Phosphorylation in Isolated Fescue Chloroplasts

Values expressed are means of three or more measurements performed on different preparations of chloroplasts. For reaction conditions see Figures 1, 2, 3 and "Materials and Methods"

<table>
<thead>
<tr>
<th>Reaction</th>
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<th>P:2e</th>
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<td>μg/h·mg Chl</td>
<td>μmol ATP/h·mg Chl</td>
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* 30 μM.

Figure 3 shows that maximal electron transport rates of the two photosystems in sequence H₂O to MV, were 30+ μmol O₂/h·mg Chl, almost identical to the rate recorded of H₂O to ferricyanide. Since the rates of the two systems were so low, titration of final acceptor MV and pH variations were performed on the uncoupled systems (+MA). Optimal conditions were near pH 8.0 and 100 μM MV resulted in rates 200+ μmol O₂/h·mg Chl. When the assay was coupled and run for longer than 2 min, 1.0 mM KCN was added to eliminate the nonlinear uptake of O₂ due to catalase activity. When ADP and Pi were added for the P:2e measurements (Table I), rate of O₂ uptake was increased to 100+ μmol O₂/h·mg Chl.

Figure 4 shows light saturation curves for each photosystem as well as for the two systems in sequence. Separately, the photosystems saturated at about 35% available actinic light intensity (6,000 μE/m²/s), but in sequence, electron transport was saturated at almost 15% available light.

Results presented in Table I show P:2e for each photosystem to be between 0.5 and 0.55. This ratio corresponds to accepted values of 0.4 to 0.6 (5, 13, 15). When the water to MV assay was performed in the presence of ADP and Pi, rates of electron flow were about one-half the maximal uncoupled (+MA) rates.

DISCUSSION

Although tall fescue has been a forage crop in much of Central and Eastern America for over 100 years, only recently have plant physiologists discovered its value in biochemical research. Its growth is favored by moist soils and cool temperatures; thus, leaves become coarse by mid-summer. Probably because of this anatomical response, no photosynthetic studies involving isolated chloroplasts from tall fescue have been reported. Yet, yields can be correlated to photosynthetic activity (11) more easily than with crops such as maize and soybeans. Since various levels of polyploidy exist in tall fescue, a model system for better understanding the relationship between photosynthesis and nuclear chromosome number might be created.

The results presented show the extremely high rates of electron transport and excellent rates of phosphorylation that are obtainable if soft, young tissue is harvested at the end of a dark cycle. Isolation is not difficult if plants are maintained with high moisture, adequate nutrients, and cool temperatures, not exceeding 25°C (12, 22). However, we found poor rates of electron transport when older tissue was used to isolate chloroplasts. A possible explanation for these rates may lie in the poor physical condition of the isolated thylakoids due to the increased starch content. Wilhelm and Nelson (26) also found poor rates of CER in older leaves of tall fescue, regardless of genotype. Comparison of the maximum rates in fescue to those reported by Oort and Izawa (14, 15) and Hardt and Kok (7) indicate that fescue chloroplasts are capable of performing equal to spinach chloroplasts. Unlike spinach, fescue can be harvested for study and new tissue will regrow quickly.

When PSII was examined, it was found that while both lipo-philic acceptors of PSII, DCBQ, and PD₆₆, gave excellent rates of electron transport under optimal conditions, rates decreased as the DCBQ concentration was increased beyond the optimum. This decline may be due to the inhibition of electron flow by reduced DCBQ (Guikema, personal communications) but confirmation of this decline can be found in work presented by Trebst et al. (22) where 100 μM DCBQ was found to inhibit NADP reduction as well as O₂ evolution.

When PSII electron transport was assayed in the presence of DBMIB (Table I), only partial inhibition of electron transport and phosphorylation occurred but P:2e ratios were increased from 0.47 to 0.53 if comparison is made to the corresponding control assay. In addition, the control experiment (ethanol:ethylene glycol without DBMIB, see Table I) showed a small decrease in electron transport and phosphorylation.
transport, phosphorylation and P:2e. The partial inhibition of PSII electron transport has been published (8, 21), indicating a partial reduction of PDH by PSI.

pH optimum for PSII was one-half pH unit lower than PSI as well as the two photosystems in sequence. Also, the effect of the uncoupler MA on the water to PDH assay was the shifting of the optimum rate to a lower pH, similar to the results presented by Saha et al. (18) and Trebst and Reimer (21).

PSI assay exhibited a high electron transport rate but when P:2e measurements were made in the presence of DCMU and SOD, ratios were consistently found to be near 0.5. Rates of cyclic phosphorylation, using pycocyanine, were the same as the noncyclic assays, corresponding almost identically to results found with spinach (28). The control rate of cyclic phosphorylation presented by Lilley and Walker (10) of 547 μmol ATP/h·mg Chl was similar to the DAD to MV PSI assay, 554 μmol ATP/h·mg Chl.

The assay for PSII plus PSI coupled to phosphorylation (Table I, Fig. 2B) exhibited a very low rate of electron flow, probably due to the rate-limiting step between plastoquinone and Cy f (8). When the reaction was uncoupled (Fig. 3B), the rate was increased over 4-fold, lending further evidence to support the suggestion that the associated phosphorylation step is the rate-limiting reaction (8).

The next research step is to apply this characterized system to varying genotypes of tall fescue. Previous work has shown that photosynthetic activity varies with genotype in tall fescue (25, 26). Randall et al. (16), using the same genotypes as above (25, 26), have correlated increasing ploidy to increasing RuBP carboxylase activity. Other work with this ploidy series indicates that net photosynthesis rates increased with ploidy level (9). Because an increase in chromosome number usually adversely affects physiology of higher plants (19), this model system in fescue could lead to an explanation of gene dosage effects or to specific factors, i.e. chromosomes, that directly affect the photosynthetic activity without the influence of diverse or altered leaf morphology.

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