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Article *in* Plant physiology · August 1987

DOI: 10.1104/pp.84.3.701 · Source: PubMed

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Plant Physiol. (1987) 84, 701-706
0032-0889/87/84/0701/06/$01.00/0

Cyanide-Resistant Respiration in Photosynthetic Organs of Freshwater Aquatic Plants

Received for publication January 7, 1987 and in revised form March 9, 1987

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ABSTRACT

The rate and sensitivity to inhibitors (KCN and salicylhydroxamic acid[SHAM]) of respiratory oxygen uptake has been investigated in photosynthetic organs of several freshwater aquatic plant species: six angiosperms, two bryophytes, and an alga. The oxygen uptake rates on a dry weight basis of angiosperm leaves were generally higher than those of the corresponding stems. Leaves also had a higher chlorophyll content than stems. Respiration of leaves and stems of aquatic angiosperms was generally cyanide-resistant, the percentage of resistance being higher than 50% with very few exceptions. The cyanide resistance of respiration of whole shoots of two aquatic bryophytes and an alga was lower and ranged between 25 and 50%. These results suggested that the photosynthetic tissues of aquatic plants have a considerable alternative pathway capacity. The angiosperm leaves generally showed the largest alternative path capacity. In all cases, the respiration rate of the aquatic plants studied was inhibited by SHAM alone by about 13 to 31%. These results were used for calculating the actual activities of the cytochrome and alternative pathways. These activities were generally higher in the leaves of angiosperms. The basal oxygen uptake rate of *Myriophyllum spicatum* leaves was not stimulated by sucrose, malate or glycine in the absence of the uncoupler carbonylcyanide-*m*-chlorophenylhydrazone (CCCP), but was greatly increased by CCCP, either in the presence or in the absence of substrates. These results suggest that respiration was limited by the adenylate system, and not by substrate availability. The increase in the respiratory rate by CCCP was due to a large increase in the activities of both the cytochrome and alternative pathways. The respiration rate of *M. spicatum* leaves in the presence of substrates was little inhibited by SHAM alone, but the SHAM-resistant rate (that is, the cytochrome path) was greatly stimulated by the further addition of CCCP. Similarly, the cyanide-resistant rate of O_2 uptake was also increased by the uncoupler.

The occurrence and properties of cyanide-resistant respiration has been studied in many plants species, but mainly in terrestrial plants. These studies have been made in several organs (*e.g.* male floral organs of the Araceae, fruits and tubers, leaves and roots of C_3, C_4, and CAM species, and callus), and at different levels of organization: isolated mitochondria and cells, and intact tissues (see reviews in Refs. 11-13 and 15). However, aquatic plants have received far less attention. There are some studies on the cyanide-resistant respiration of unicellular algae (9, 10, 16, 17, 19), but there is practically no information about this subject in multicellular aquatic plants.

The objective of this paper is to study the contribution of the cyanide-resistant alternative pathway to the respiration rate of intact photosynthetic organs (leaves, stems, and whole shoots) of several freshwater aquatic angiosperms, bryophytes, and algae. The techniques used for estimating the activities of the cytochrome and alternative pathways involve the use of the O_2 electrode and inhibitors, such as KCN and SHAM[1] (4, 13, 14). These techniques are especially suitable for plants living under water because the measurements are made by submerging tissue pieces in an aqueous solution.

MATERIALS AND METHODS

Plant Material. Most species of freshwater aquatic plants used in this work are found in Catalonia, a northeastern region of Spain. They were collected from their natural environment and transferred to the laboratory for measurements. The species used and the collection sites were the following: *Elodea canadensis* Michx (River Fluvia); *Myriophyllum spicatum* L. and *Potamogeton crispus* L. (Rivers Ter and Fluvia); *Potamogeton pectinatus* L. and *Ruppia cirrhosa* (Petagna) (Delta de l'Ebre lagoons); *Rhynchostegium riparioides* (Hedw.) Card. *Fontinalis antipyretica* Hedw. and *Cladophora glomerata* (L.) Kütz (River Muga); and *Cabomba caroliniana* A. Gray from an aquarium shop. The leaves and stems from the terminal part of the shoots of angiosperms were used separately in the experiments. The selected leaves included young and adult leaves, and generally were fully green. In the case of bryophytes and algae, the whole shoots were used. In all cases, the photosynthetic organs were selected after several hours in the light, to avoid substrate limitation of respiration.

Measurements of Oxygen Uptake and Chl Concentration. Oxygen uptake rates were measured at 25°C, using an O_2 electrode (Rank Brothers, Cambridge, England) in an air saturated solution (the initial concentration of O_2 was considered to be 240 μm) containing 10 mM HEPES (pH 7.4, KOH) and 0.2 mM $CaCl_2$. The pH of 7.4 was selected because it is similar to the pH of the natural aquatic environment. Intact small leaves and short pieces (about 1 cm long) of stems (or whole shoots in the case of bryophytes and algae) were included in the measurement cuvette. The depletion of the O_2 concentration in the rapidly stirred solution of the closed cuvette was linear with time, except at low O_2 concentrations (less than 100 μM). Measurements were made in the dark between 240 and 120 μM O_2. A nylon net separated the plant material from the stirrer bar and the electrode. The respiratory substrates, inhibitors and uncouplers were prepared and used as described earlier (2, 6, 14). At the end of every O_2 uptake measurement, the leaves, stems or shoots were oven-dried at 60 to 70°C until constant weight. The fresh weight of these samples was also measured at the beginning of every experiment. Chl concentration was estimated according to Ziegler and Egle (21).

[1] Abbreviations: SHAM, salicylhydroxamic acid; CCCP, carbonylcyanide-*m*-chlorophenylhydrazone.

RESULTS

Rates of Respiration of Freshwater Aquatic Plants. The respiratory rates on a dry weight basis (V_t) of leaves of freshwater aquatic angiosperms varied widely among species, and were generally higher than those of the corresponding stems, except perhaps in the case of *C. caroliniana* (Table IA). The respiration rates of whole shoots of the bryophytes and algae studied ranged from 53 to 96 μmol O_2/g dry weight·h (Table I, B and C). The Chl content of the angiosperm leaves was also higher than that of the corresponding stems (Table IIA). The ratio Chl *a*/Chl *b* ranged from 3.00 to 3.25 in leaves of angiosperms, which are frequent values in terrestrial plants (5), and from 2.00 to 3.22 in stems (Table IIA). In general, this ratio was slightly higher in leaves than in the corresponding stems.

Effect of KCN and SHAM on Respiration Rates. The rate of respiration of leaves of freshwater aquatic angiosperms was slightly inhibited by KCN (Table IA). The percentage of cyanide-resistance ranged from about 54 to 91%. The exception was *C. caroliniana*, because KCN slightly stimulated leaf respiration (about 13%). In all cases, the addition of SHAM after KCN (or vice versa) almost fully suppressed O_2 uptake (the residual rate, V_{res}, was less than 7% of the initial rate). Similarly, stem respiration showed some cyanide-resistance, the percentage values ranged from 60 to 87%. The exceptions were the stems of *M. spicatum* and *P. crispus*. In the first case, cyanide-resistance was only 37%. In the second case, most stems of *P. crispus* were cyanide-resistant, but a few exceptional ones were almost fully cyanide-sensitive (see footnote to Table I). These stems belonged to a batch of *P. crispus* plants collected from different geographical sites than the other batches. The residual rate of O_2 uptake of stems was slightly higher than that of leaves, and ranged from zero to 26% (Table IA). The rate of respiration of whole shoots of freshwater bryophytes and algae was also cyanide-resistant, although the percentage of resistance was lower than that of angiosperms: it ranged from 25 to 50% of the initial rate of respiration (Table I, B and C). The exception was a batch of *C. glomerata*, which, similarly to some stems of *P. crispus* (see above), showed a very low cyanide-resistance (see footnote to Table I). The respiration rate of leaves, stems, and shoots of all freshwater aquatic plants studied was inhibited by SHAM alone; the percentage of inhibition ranging in most cases from 13 to 31% (Table I).

Estimation of the Activities of Respiratory Pathways. The results shown in Table I were utilized for calculating the activities of the cytochrome and alternative pathways, assuming that KCN and SHAM only inhibited these two pathways, respectively. The 'activity' of the cytochrome path (v_{cyt}), estimated by the rate of O_2 uptake resistant to SHAM minus V_{res}, was about 3 times higher in the leaves of angiosperms than in the stems (Table IIIA). The exception was *C. caroliniana*, because v_{cyt} was similar in both, leaves and stems. In the case of shoots of bryophytes and algae, the values of v_{cyt} were not very high, but were generally higher than those of angiosperm stems (Table III, B and C).

The 'capacity' of the alternative pathway (V_{alt}), which was estimated by the rate of O_2 uptake resistant to KCN (providing that KCN inhibited) minus V_{res} (however, see later for objections to this method), was about 2 to 3 times higher in the leaves of angiosperms than in the stems (Table IIIA). In the case of shoots of freshwater bryophytes and algae, V_{alt} presented very low values (Table IIIB). The green alga *C. glomerata* showed a considerable capacity of the alternative pathway (Table IIIC), but was lower than that of most angiosperm leaves. The alternative path was expressed in the absence of cyanide in all the species studied. The actual rate or 'activity' of this path (v_{alt}), estimated by the rate of O_2 uptake sensitive to SHAM alone, was normally higher in the leaves of angiosperms than in the stems, with the exceptions of *C. caroliniana* and *P. crispus* (Table IIIA). The estimation of both, v_{alt} and V_{alt} permitted a calculation of the fraction

Table I. *Effect of KCN (1 mM) and SHAM (4–8 mM) on O_2 Uptake Rates of Leaves, Stems, and Shoots of Several Freshwater Aquatic Plants*

Measurement temperature was 25°C. V_t is the rate of respiration on a dry weight basis in the absence of inhibitors, and the values of V_t shown are means ± SE of 7 to 11 measurements. The rates in the presence of inhibitors are means ± SE of 3 to 11 measurements.

Species	Tissue	O_2 Uptake			
		V_t	+Cyanide	+SHAM	+SHAM + Cyanide or +Cyanide + SHAM
		μmol/g dry wt·h			
A. ANGIOSPERMS					
Myriophyllum spicatum	Leaves	141.9 ± 5.3	70.6 ± 4.5	128.6 ± 8.8	9.2 ± 2.4
	Stems	54.8 ± 8.5	27.2 ± 13.7	36.6 ± 8.9	0
Potamogeton crispus	Leaves	113.9 ± 16.4	85.3 ± 13.0	89.2 ± 16.9	4.3 ± 2.0
	Stems[a]	71.7 ± 11.2	33.2 ± 7.7	49.9 ± 11.7	11.6 ± 3.5
Elodea canadensis	Leaves	108.8 ± 17.4	69.5 ± 11.3	79.9 ± 14.1	0
	Stems	31.8 ± 7.1	22.6 ± 9.3	24.7 ± 7.0	0
Ruppia cirrhosa	Leaves	67.1 ± 21.8	63.3 ± 25.6	56.9 ± 19.7	0
	Stems	33.8 ± 5.3	22.8 ± 4.1	29.2 ± 6.3	5.5 ± 2.1
Potamogeton pectinatus	Leaves	39.4 ± 2.3	25.6 ± 4.2	31.1 ± 3.9	2.0 ± 1.0
	Stems	13.3 ± 1.9	10.2 ± 3.0	10.5 ± 1.2	3.0 ± 0.5
Cabomba caroliniana	Leaves	30.3 ± 2.4	34.1 ± 3.7	22.6 ± 3.2	1.2 ± 0.5
	Stems	25.0 ± 2.8	18.6 ± 2.0	22.0 ± 4.8	1.1 ± 0.4
B. BRYOPHYTES					
Rhynchostegium riparioides	Shoots	52.8 ± 7.9	22.6 ± 2.8	41.4 ± 7.4	11.0 ± 1.2
Fontinalis antipyretica	Shoots	66.2 ± 2.0	15.9 ± 2.3	56.0 ± 2.9	5.5 ± 1.2
C. ALGAE					
Cladophora glomerata	Shoots[a]	96.0 ± 12.1	37.7 ± 3.9	57.9 ± 11.5	3.0 ± 1.1

[a] There were some stems of *P. crispus*, belonging to the same batch of plants, and a batch of *C. glomerata* which presented a respiration very sensitive to cyanide. In both cases, these anomalous results were taken into account only for calculating the values of V_t and V_{res}, the residual respiration.

Table II. *Chl Content of Freshwater Aquatic Plants*

The total Chl concentration, expressed per units fresh weight and dry weight, and the Chl *a*/Chl *b* ratio were measured in the leaves and stems of six species of angiosperms, and in the whole shoots of two bryophytes. The Chl content of the algae *Cladophora glomerata* was not determined.

Species	Tissue	Chl *a* + Chl *b*		Chl *a*/Chl *b*
		mg/g fresh wt	*mg/g dry wt*	*ratio*
A. ANGIOSPERMS				
Myriophyllum spicatum	Leaves	1.71	21.5	3.17
	Stems	0.38	6.7	3.22
Potamogeton crispus	Leaves	1.84	23.4	3.00
	Stems	0.11	1.6	2.76
Elodea canadensis	Leaves	1.70	24.5	3.25
	Stems	0.26	5.0	3.08
Ruppia cirrhosa	Leaves	1.06	6.7	3.08
	Stems	0.16	1.7	2.38
Potamogeton pectinatus	Leaves	0.40	3.4	3.00
	Stems	0.18	1.3	2.00
Cabomba caroliniana	Leaves	0.49	7.5	3.08
	Stems	0.16	4.0	2.87
B. BRYOPHYTES				
Rhynchostegium riparioides	Shoots	0.51	2.8	5.38
Fontinalis antipyretica	Shoots	0.93	5.1	2.71

Table III. *Estimation of the Activities of Respiratory Pathways in Leaves, Stems, and Shoots of Freshwater Aquatic Plants*

The values shown were calculated from the experiments described in Table I, and are means ± SE of 3 to 11 measurements. The values of V_t, the rate of O_2 uptake in the absence of inhibitors, are given in Table I. v_{cyt} is the activity of the cytochrome pathway, estimated by measuring O_2 uptake in the presence of SHAM. v_{alt} is the activity of the alternative pathway, estimated by measuring O_2 uptake sensitive to SHAM. V_{alt} is the capacity of the alternative pathway and is estimated by the rate of O_2 uptake in the presence of KCN. The parameter ρ is the fraction of the maximum capacity of the alternative pathway that is expressed, and is calculated by the ratio v_{alt}/V_{alt}. V_{res} is the rate of O_2 uptake resistant to a combination of both, KCN and SHAM, and it has been taken into account for calculating v_{cyt} and V_{alt}.

Species	Tissue	O_2 Uptake				ρ
		v_{cyt}	v_{alt}	V_{alt}	V_{res}	
		μmol/g dry wt·h				*ratio*
A. ANGIOSPERMS						
Myriophyllum spicatum	Leaves	117.0 ± 10.2	23.9 ± 8.3	63.8 ± 6.4	9.2 ± 2.4	0.37
	Stems	36.6 ± 8.9	3.5 ± 1.3	27.3 ± 13.7	0	0.13
Potamogeton crispus	Leaves	83.1 ± 19.7	19.9 ± 12.3	80.6 ± 15.8	4.3 ± 2.0	0.25
	Stems	29.6 ± 10.8	24.7 ± 12.8	22.7 ± 12.1	11.6 ± 3.5	1
Elodea canadensis	Leaves	79.9 ± 14.1	35.7 ± 9.1	69.5 ± 11.3	0	0.51
	Stems	24.7 ± 7.0	5.3 ± 3.0	22.6 ± 9.3	0	0.23
Ruppia cirrhosa	Leaves	56.9 ± 19.7	12.1 ± 3.7	63.3 ± 25.6	0	0.19
	Stems	21.4 ± 6.0	8.3 ± 3.9	18.9 ± 6.3	5.5 ± 2.1	0.44
Potamogeton pectinatus	Leaves	28.6 ± 2.4	5.7 ± 2.2	24.3 ± 2.9	2.0 ± 0.0	0.23
	Stems	7.6 ± 0.5	2.7 ± 2.1	7.2 ± 2.7	3.0 ± 0.5	0.38
Cabomba caroliniana	Leaves	21.4 ± 3.4	7.4 ± 1.4	33.0 ± 3.9[a]	1.2 ± 0.5	0.22
	Stems	19.5 ± 4.5	7.2 ± 3.1	18.6 ± 2.0	1.1 ± 0.4	0.39
B. BRYOPHYTES						
Rhynchostegium riparioides	Shoots	30.4 ± 5.7	7.3 ± 2.5	11.7 ± 2.2	11.0 ± 1.2	0.62
Fontinalis antipyretica	Shoots	47.8 ± 2.7	12.9 ± 1.7	12.4 ± 1.0	5.5 ± 1.2	1
C. ALGAE						
Cladophora glomerata	Shoots	54.9 ± 10.3	20.5 ± 7.7	34.8 ± 3.3	3.0 ± 1.1	0.59

[a] The value of V_{alt} was probably underestimated, because KCN stimulated the rate of O_2 uptake (Table I).

of the alternative path that was expressed ($\rho = v_{alt}/V_{alt}$). Only the stems of *P. crispus* and the shoots of *F. antipyretica* had a fully expressed alternative path ($\rho = 1$). The alternative path was expressed more than 50% of its capacity ($\rho > 0.5$) in the leaves of *E. canadensis* and in the shoots of *R. riparioides* and *C. glomerata*. In the remaining cases, the parameter ρ was less than 0.5. Thus, it seems that the expression of the alternative pathway does not seem to be correlated with its capacity in freshwater aquatic plants: the degree of expression of the alternative path was normally higher in organs with lower alternative path capacity, like shoots of bryophytes and algae, and some stems of angiosperms.

Effect of Substrates and the Uncoupler CCCP on Respiratory Pathways of Leaves of *M. spicatum*. The basal rate of O_2 uptake of intact leaves of *M. spicatum* was not significantly stimulated by a combination of respiratory substrates (sucrose, malate and

Table IV. *Effect of Respiratory Substrates and the Uncoupler CCCP on the O_2 Uptake Rate of Leaves of Myriophyllum spicatum*

The concentrations used were as follows: sucrose, 30 mM; malate, 30 mM; glycine, 30 mM; CCCP, 4 μM. Measurement temperature was 25°C. The values shown are means ± SE of 4 to 8 measurements.

Addition	O_2 Uptake
	μmol/g dry wt·h
Basal rate	108.3 ± 6.4
+ Sucrose + malate + glycine	112.0 ± 7.2
+ CCCP	215.4 ± 15.5
+ Sucrose + malate + glycine + CCCP	226.8 ± 19.3

Table V. *Effect of Respiratory Substrates, Inhibitors, and the Uncoupler CCCP on O_2 Uptake Rates of Leaves of Myriophyllum spicatum*

The concentrations of KCN and SHAM were 1 mM and 4 mM, respectively. The values shown are means ± SE of 4 measurements. For other details see Table IV.

Sequential Additions	O_2 Uptake
	μmol/g dry wt·h
Experiment 1	
None	102.4 ± 8.4
+Sucrose + malate + glycine	107.2 ± 7.1
+SHAM	99.1 ± 7.1
+CCCP	156.5 ± 13.5
+KCN	10.2 ± 3.1
Experiment 2	
None	108.3 ± 6.4
+Sucrose + malate + glycine + CCCP	226.8 ± 19.3
+SHAM	169.2 ± 14.3
+KCN	6.8 ± 1.4
Experiment 3	
None	104.8 ± 9.4
+KCN	47.3 ± 4.2
+CCCP	68.7 ± 2.1
+SHAM	12.6 ± 1.1

glycine) in the absence of CCCP, but was greatly increased in the presence of both substrates and CCCP (Table IV). The respiration rate was greatly stimulated by CCCP alone, and the further addition of substrates stimulated very little O_2 uptake (Table IV). These results suggest that respiration was limited by a factor released by the uncoupler, but not by substrate availability.

The respiration rate in the presence of substrates was little inhibited by SHAM alone, but the SHAM-resistant rate was greatly stimulated by CCCP (Table V, Experiment 1; Fig. 1A). Similarly, the cyanide-resistant rate of O_2 uptake was also significantly increased by the uncoupler (Table V, Experiment 3; Fig. 1B). The addition of rotenone after KCN and CCCP slightly reduced the O_2 uptake rate (Fig. 1C). The same phenomena was observed in leaves of *P. crispus* (Fig. 1D).

The activities of respiratory pathways of leaves of *M. spicatum* were estimated from the results shown in Table V. The increase in the rate of respiration (measured in the presence of substrates) by the uncoupler CCCP was due to an increase in the activities of both the cytochrome and alternative pathways (Table VI). Surprisingly, the activity of the alternative path (v_{alt}) in the presence of CCCP (57.6 μmol O_2/g dry weight·h; see Table VI) was much higher than the capacity of this pathway measured by the conventional method: V_{alt} in the absence of CCCP was 34.7 μmol O_2/g dry weight·h, but V_{alt} in the presence of CCCP was about 56.1 μmol O_2/g dry weight·h (Table VI). It seems evident that, independently of the correct estimate of V_{alt}, the alternative pathway was used at full capacity only in the presence of CCCP, but not in the absence of this uncoupler (Table VI).

DISCUSSION

The use of photosynthetic organs of freshwater aquatic plants for respiratory studies, including the sensitivity of respiration to inhibitors, seemed to be justified for several reasons: (a) The rate of respiration of these organs was not apparently limited by O_2 availability in a wide range of O_2 concentrations (at least between 120 and 240 μM O_2). (b) The plant material was immersed in an aqueous solution during measurements, which is a normal situation for aquatic plants in their natural environment. (c) The fact that the tissues used in the experiments had thin cuticles and very few cell layers (20) facilitated the penetration of inhibitors, substrates and uncouplers into the cells; for instance, a combination of respiratory substrates and the uncoupler CCCP allowed the maximum expression of both the cytochrome and the alternative pathways in intact leaves of *M. spicatum* (Table VI). Normally, it is difficult to engage the alternative pathway of leaves of terrestrial plants to full capacity under similar conditions, perhaps due to diffusional problems (1, 3, 6, 13). (d) The responses of respiration to inhibitors, substrates and uncoupler were very fast, and the values of residual respiration were normally very low. Unexpected effects of inhibitors, like the stimulation of O_2 uptake by SHAM observed mainly in some root tissues (13, 14), were never observed in the case of photosynthetic organs of freshwater aquatic plants. Summarizing, the use of inhibitors (KCN and SHAM), substrates, and uncoupler gave similar results to those found in other photosynthetic tissues (13, 14), and these results were interpreted according to the currently accepted effects of KCN and SHAM on plant tissues (15, 18).

The effects of KCN and SHAM suggested that photosynthetic organs of freshwater aquatic plants have a significant alternative pathway capacity (with only two exceptions—see text and Table I), and that this pathway is active in all cases studied, although the fraction expressed of the maximum capacity is variable (Table III). A careful examination of the results suggest that there was no correlation between the capacity and activity of the alternative path on a dry weight basis: shoots of bryophytes and the algae *C. glomerata* and stems of some angiosperms presented higher values of the parameter ρ than tissues with higher alternative pathway capacity, like some leaves of angiosperms (Table III). These data agree better with the model of distribution of electrons through the cytochrome and alternative pathways proposed by Bahr and Bonner (4) than that proposed by De Troostemberg and Nyns (7).

The use of substrates (sucrose, malate, and glycine) and the uncoupler CCCP suggested that the respiration of leaves of *M. spicatum* was limited by the adenylate system, and not by substrate availability, given that O_2 uptake was greatly stimulated by CCCP in the absence of substrates, but was not stimulated by substrates in the absence of CCCP (Table IV). A similar mechanism of regulation of respiration was found in high carbohydrate leaves of wheat (1), bean (3), and *Lolium perenne* (6). However, in wheat leaves with low carbohydrate levels, the respiration was stimulated by sugars but not by CCCP (1, 2), suggesting that substrate supply to the mitochondrion was the main limiting factor under these conditions.

The uncoupler CCCP greatly stimulated the expression of the cytochrome pathway (estimated by a SHAM-resistant and KCN-sensitive O_2 uptake), suggesting that the activity of this pathway was significantly restricted under normal conditions (Tables V and VI; Fig. 1A). A similar situation has been observed in leaves of terrestrial plants (1, 3, 13), and has been interpreted in terms of a limitation of the cytochrome pathway by the adenylate system.

Interestingly, the cyanide-resistant and SHAM-sensitive rate of O_2 uptake, which is considered to be an estimate of the capacity of the alternative pathway, was also significantly stimulated by CCCP (Table V, Fig. 1, B–D). This result is more difficult to

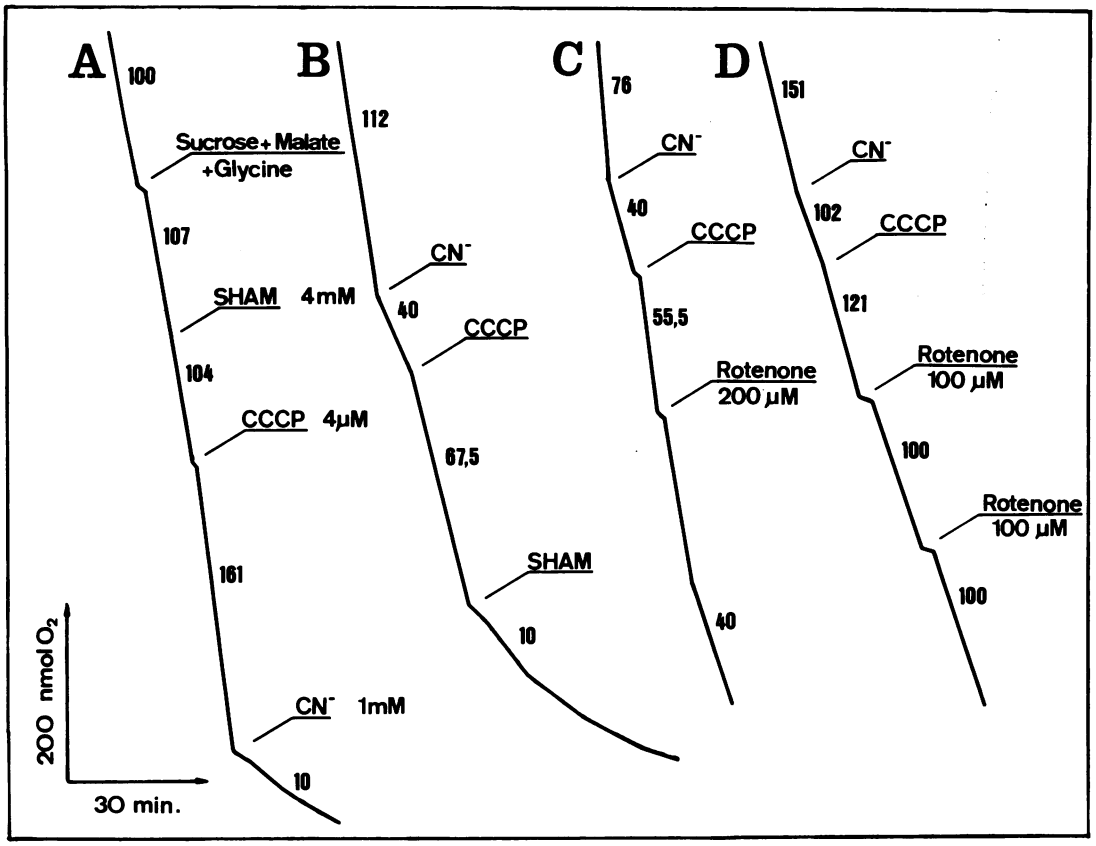

FIG. 1. Effect of the uncoupler CCCP on the SHAM-resistant and KCN-resistant O_2 uptakes rates of leaves of freshwater aquatic angiosperms. Typical O_2 electrode traces are shown: A, B, and C, *Myriophyllum spicatum;* D, *Potamogeton crispus.* The dry weights of tissue in each experiment were: A, 16 mg; B, 18 mg; C, 37 mg; D, 9 mg. Rates on traces are expressed as µmol O_2/g dry weight·h.

Table VI. *Estimation of Respiratory Pathways in Leaves of Myriophyllum spicatum in the Absence and in the Presence of CCCP*

In both cases, the cytochrome and alternative pathways were estimated in the presence of respiratory substrates (sucrose, malate, and glycine). The values shown were calculated from the results described in Tables IV and V. For symbols and definitions see Table III. V_{res} = 9.8 ± 1.0 µmol O_2/g dry wt·h (n = 16) and was unaffected by CCCP.

Parameter	O_2 Uptake	
	− CCCP	+ CCCP
	µmol/g dry wt·h	
V_t	112.0 ± 7.2	226.8 ± 19.3
v_{cyt}	88.9 ± 6.1	154.4 ± 12.9
v_{alt}	8.1 ± 3.7	57.6 ± 5.0
V_{alt}	34.7 ± 3.3	56.1 ± 1.8

understand in terms of an energy restriction of the alternative pathway in the presence of cyanide, than the energy restriction of the cytochrome pathway in the presence of SHAM, because it could be expected that cyanide would produce a larger decline in ATP levels in the cell than SHAM. However, the fact that the rate of O_2 uptake in the presence of CCCP sensitive to SHAM alone was much higher than the 'capacity' of the alternative estimated in the absence of CCCP (Table VI) suggests that, at least, a SHAM-sensitive oxidase system is involved in this CCCP dependent increase of O_2 uptake in the presence of cyanide. If this system is the alternative pathway, it might by hypothesized that in leaves of *M. spicatum* and *P.* crispus, the phosphorylation site I might be involved in the control of the alternative pathway, even in the presence of cyanide. The inhibitory effects of rotenone, an inhibitor of electron transport through the phosphorylation site I, on the rate of O_2 uptake of intact leaves in the presence of cyanide and CCCP (Fig. 1, C and D) would support this interpretation. However, it must be taken into account that the effects of rotenone on the respiration of intact tissues have not been sufficiently investigated. Some unpublished results (G Burgos, JL Araus, J Azcón-Bieto) obtained with leaves of *Fatsia japonica*, a C_3 plant, also suggest the existence of a control of the alternative pathway *in vivo* by the energy status of the cell.

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