Plant Physiol. (1982) 69, 724–727
0032-0889/82/69/0724/04/$00.50/0

Stability and Synthesis of Phospholipids during Desiccation and Rehydration of a Desiccation-Tolerant and a Desiccation-Intolerant Moss[1]

Received for publication May 27, 1981 and in revised form November 23, 1981

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ABSTRACT

The fatty acid composition of the phospholipids from the desiccation-tolerant moss *Tortula ruralis* (Hedw.) Gaertn, Meyer and Scherb and the desiccation-intolerant moss *Cratoneuron filicinum* has been determined. No changes in composition occur in either moss as a consequence of rapid drying, but, after slow drying, there is a decline in some unsaturated fatty acids. Upon rehydration of *T. ruralis* after slow drying, these acids decline further; however, within 105 minutes, they regain the same levels as those in undesiccated controls. A smaller and more transient decline occurs after rapid desiccation. Most phospholipid unsaturated fatty acids decrease during rehydration of *C. filicinum*, and their levels are not recovered. After both rapid and slow drying of *T. ruralis*, acetate and glycerol are incorporated into the phospholipid fraction, although *de novo* synthesis, alone, might not account for the increase in unsaturated fatty acids upon rehydration. Very little acetate or glycerol is incorporated during rehydration of *C. filicinum*. Loss of unsaturated fatty acids from the phospholipids of *T. ruralis* does not appear to be associated with increased lipoxygenase activity. Furthermore, there is little correlation between the extent of peroxidation of fatty acids due to desiccation and changes in the phospholipid fraction.

It is generally accepted that desiccation of a cell will lead to a structural alteration in membrane components; if an organism is to survive, the integrity of its membranes must be reconstituted during early rehydration (11). Normal metabolism cannot be resumed until membrane-associated enzymes are returned to their correct positions, from which they are likely to have been removed during desiccation (12). The reorientation of the membrane components may be monitored, to some extent, by following the leakage of solute from a tissue upon rehydration. Such studies (6, 8) on the desiccation-tolerant moss *Tortula ruralis* suggest that rapid drying leads to more severe disruption of membranes than does slow drying, since leakage upon rehydration of rapidly dried material is at least twice that from slowly dried material. It has been suggested (3, 8) that this increased leakage may be due to peroxidation of membrane lipids, occurring during both the dehydration and the rehydration phases. Peroxidation of lipids would lead to a loss of unsaturated fatty acids, since the double bond is the site of the peroxidation process.

In the present study, we have used a desiccation-tolerant moss, *Tortula ruralis*, and a desiccation-intolerant moss, *Cratoneuron filicinum*, to follow the relative levels of the phospholipid fatty acids after desiccation and rehydration. We also have used radioactive precursors to monitor the synthesis of phospholipids upon rehydration. We conclude that there is a poor correlation between lipid peroxidation (8) and changes to the unsaturated fatty acids of phospholipids.

MATERIALS AND METHODS

Plant Material. *Tortula ruralis* (Hedw.) Gaertn, Meyer, and Scherb was collected from a spruce forest west of Calgary, stored, and prepared for experiments, as previously described (6). *Cratoneuron filicinum* was collected from the banks of Heart Creek, Alberta, stored, and prepared for experiments, as described previously (2) in a report in which this moss was misnamed *Hygrohypnum luridum*.

Administration of Desiccation. Slow desiccation was achieved using atmospheres of high RH by the method of Malek and Bewley (9), while rapid drying was achieved using activated silica gel, as described by Dhindsa and Bewley (6).

Phospholipid Fatty Acid Analysis. Phospholipid fatty acids were analyzed as detailed previously (13), with minor modifications. Moss (250 mg fresh weight) was homogenized in propan-2-ol. The sample was processed as before, until after it was loaded onto the silica gel column and washed in 1% acetic acid in chloroform. The column was washed with acetone prior to the elution of the phospholipid fraction in methanol, as before (13). After the GLC program was completed, the maximum temperature (225°C) was held for 6 min.

Lipoxygenase Assay. Lipoxygenase (linolenate oxygen oxidoreductase, EC 1.13. 11.12) was assayed, as described by Oelze-Karow and Mohr (10). Four-hundred-mg samples of moss were homogenized in ice-cold 100 μM phosphate buffer (pH 7). The homogenate was centrifuged at 34,000*g* for 20 min, the supernatant being the enzyme extract. The assay was performed at pH 7 and standardized against commercially prepared soybean lipoxygenase (Sigma Chemical Co.).

Incorporation of Precursors into Phospholipids. Moss samples (250 mg fresh weight) were rehydrated in distilled H_2O for 3 min, surface-dried, and transferred to 660 nCi of labeled precursor in 1 ml of distilled H_2O ([2-^{14}C]acetic acid, sodium salt diluted to 4 nCi mmol^{-1}; or [U-^{14}C]glycerol, diluted to 15 nCi mmol^{-1}; both from New England Nuclear). The moss samples were incubated with the precursor for 15 to 105 min and then washed once with 1% nonradioactive precursor in distilled H_2O and twice with distilled H_2O. Samples were homogenized as for fatty acid analysis, and the propanol solutions were taken to dryness *in vacuo*. They were redissolved in 5 ml chloroform:methanol (2:1, v:v), and 100-μl aliquots were removed to give the total uptake values. The chloroform:methanol solution was extracted against 1% nonradio-

[1] Supported by Natural Sciences and Engineering Research Council Grant A6352 and G0154.

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active precursor in distilled H_2O, the mixture being centrifuged at 1,000*g* for 5 min to break the emulsion. The aqueous phase was discarded, and the chloroform solution was taken to dryness *in vacuo*, redissolved in 1% acetic acid in chloroform, and added to the silica gel column. The phospholipid fraction from the column (methanol eluant) was collected and split into two aliquots. One was counted in a Beckman LS 8,000 liquid scintillation spectrometer, and the other was taken to dryness prior to lipid phosphorus determination.

Polar Lipid Phosphorus Determination. The dried polar lipid sample was suspended in 1 ml distilled H_2O, and 0.5 ml 10 N sulfuric acid was added. The assay was performed as described by Bartlett (1), except that a prepared Fiske-SubbaRow reagent was used (Sigma Chemical Co.).

RESULTS AND DISCUSSION

Phospholipid Fatty Acids. The phospholipid fatty acids of *T. ruralis* were found to consist of palmitic, palmitoleic (trace), oleic, linoleic, linolenic, eicosatrienoic, and eicosatetraenoic acids by cochromatography with authentic standards. Table I shows the amounts of these fatty acids (relative to palmitic acid) in a phospholipid fraction at various times after rapid and slow drying. Rapid or slow drying led to only minor alterations in the relative amounts of the fatty acids. However, upon initial rehydration after rapid drying, there was a slight loss of unsaturated acids. This was recovered to control levels by 75 min after rehydration. After slow drying, there was a greater loss of unsaturated acids, and recovery took longer, resulting in reduced unsaturated acid levels through 75 min after the onset of rehydration.

C. filicinum phospholipids contain palmitic, oleic, linoleic, linolenic, eicosatrienoic, and eicosatetraenoic acids. There was little change in the fatty acids upon dehydration at either speed (Table II). However, in both cases, upon subsequent rehydration, a reduction in unsaturated acids was noted. Up to 105 min after rehydration, there was no recovery of these acids.

It has been suggested that the cause of desiccation damage is lipid peroxidation occurring soon after rehydration (8), which results in a loss of unsaturated fatty acids. More damage (using malondialdehyde production as an indicator of the extent of peroxidation) was observed on rehydration after rapid drying than was observed after slow drying (8). However, recovery from rapid drying occurred with fewer changes to the unsaturated fatty acids of the phospholipid fraction than did recovery from slow drying (Table I). Similarly, it took slow-dried samples longer to recover to control values than it took rapid-dried samples. There is no obvious correlation between the changing fatty acid composition of the phospholipid fraction upon rehydration and the extent of membrane leakage. It appears likely that the increased peroxidation associated with rehydration (8) has some other site of action, possibly the abundant oil droplets that appear in the cytoplasm of some mosses (14) (including *T. ruralis* [5]). These oil droplets, thus, may afford some protection to the essential phospholipids. In desiccation-intolerant *C. filicinum*, there were few changes in fatty acid composition during desiccation at either rate. Upon subsequent rehydration, there was a reduction in the proportion of unsaturated fatty acids. The lack of recovery during the experimental period may be because damage to *C. filicinum* membranes was too extensive to repair or because the repair mechanisms *per se* were damaged. To investigate further the effect of desiccation and rehydration on any such repair mechanism, phospholipid synthesis was followed during rehydration.

Table I. *Relative Amounts of Fatty Acids in the Phospholipid Fraction of T. ruralis upon Rehydration after Rapid- or Slow-Drying*

	16:0	16:1	18:1	18:2	18:3	20:3	20:4	P[a]
	relative to palmitic acid							*μg Pi·g fresh wt*$^{-1}$
H[b]	100	T[e]	37	35	112	39	27	81.6
RD[c]	100	T	38	36	115	36	26	82.8
R15[d]	100	T	36	37	98	29	19	74.4
R45	100	T	31	35	99	27	18	83.2
R75	100	T	34	37	114	39	26	87.6
R105	100	T	37	36	113	34	23	85.2
SD[c]	100	T	34	38	118	38	22	86.0
S15[d]	100	T	33	37	99	29	20	64.8
S45	100	T	24	24	71	25	14	70.8
S75	100	T	36	32	84	24	14	79.6
S105	100	T	38	37	116	39	24	81.2

[a] P, Total extractable lipid phosphorus.
[b] H, Hydrated, undesiccated moss.
[c] RD and SD, Rapid- and slow-dried moss, respectively.
[d] R and S, Followed by a figure, indicate number of min of rehydration following rapid or slow desiccation, respectively.
[e] T, trace amount.

Table II. *Relative Amounts of Fatty Acids in the Phospholipid Fraction of C. filicinum upon Rehydration following Rapid- or Slow-Drying*

See legend to Table I for explanation of symbols.

	16:0	18:1	18:2	18:3	20:3	20:4	P
	relative to palmitic acid						*μg Pi·g fresh wt*$^{-1}$
H	100	1.7	5.3	48.3	4.3	15.5	40.8
RD	100	1.7	4.8	47.2	4.5	13.7	38.8
R15	100	2.2	2.2	35.7	2.0	3.0	24.8
R45	100	1.6	1.6	34.4	1.6	3.1	22.4
R75	100	1.9	1.9	32.6	1.8	2.6	24.8
R105	100	1.7	1.8	30.4	1.8	1.8	18.0
SD	100	1.7	5.3	34.8	2.1	13.3	38.4
S15	100	1.8	2.9	35.7	1.8	8.8	23.2
S45	100	1.6	1.6	34.6	1.6	3.1	21.2
S75	100	2.1	0	36.2	0	0	20.4
S105	100	1.8	0	34.6	1.8	3.6	18.4

Lipoxygenase Activity. It is possible that the loss of unsaturated fatty acids shown in Tables I and II is due to the action of lipoxygenase rather than to that of peroxidation. Hydrated *T. ruralis* contains this enzyme (Fig. 1A); after desiccation at either rate, its level is reduced by about 70%. Upon subsequent rehydration, the enzyme rapidly returns to control values, in rapid-dried samples more quickly than in slow-dried. Desiccation of *C. filicinum* results in a reduction of lipoxygenase activity by about 20%; after rapid-drying, this level is not recovered in the rehydrated moss, while, in slow-dried samples, enzyme activity returns to, and exceeds, control values.

Thus, *T. ruralis* appears to have the ability to suppress its lipoxygenase activity upon desiccation while *C. filicinum* does not. This may be critical to the mosses' tolerance of the drying, since desiccation is purported to lead to a disruption of membrane organization to such an extent that compartmentation is not possible (11, 12). If high levels of a potentially hazardous enzyme, such as lipoxygenase, are released into the cytosol from a sequestering organelle or vesicle, then severe damage may result.

Phospholipid Synthesis. To ascertain whether the recovery of unsaturated fatty acids in *T. ruralis*, as shown in Table I, was due to increased synthesis, the incorporation of labeled precursors into the phospholipid fraction was monitored: acetate into fatty acids; and glycerol into the triglyceride backbone of phospholipids. Undesiccated control moss incorporated [^{14}C]acetate into the

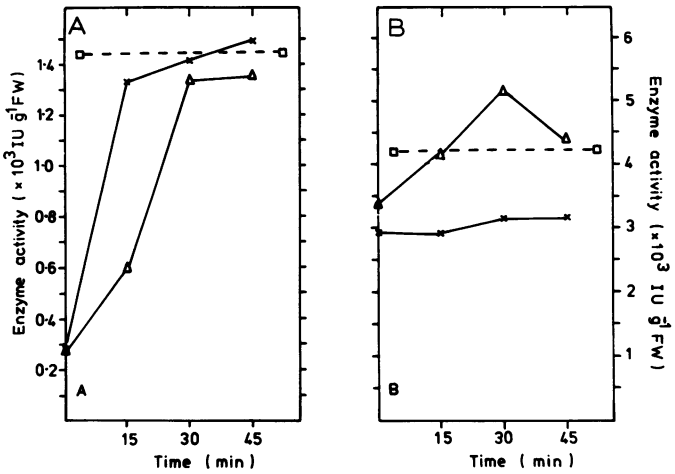

FIG. 1. Lipoxygenase activity in cf2*T. ruralis* (A) and *C. filicinum* (B) during rehydration after rapid (×) or slow (△) desiccation. □, Levels in undesiccated control moss.

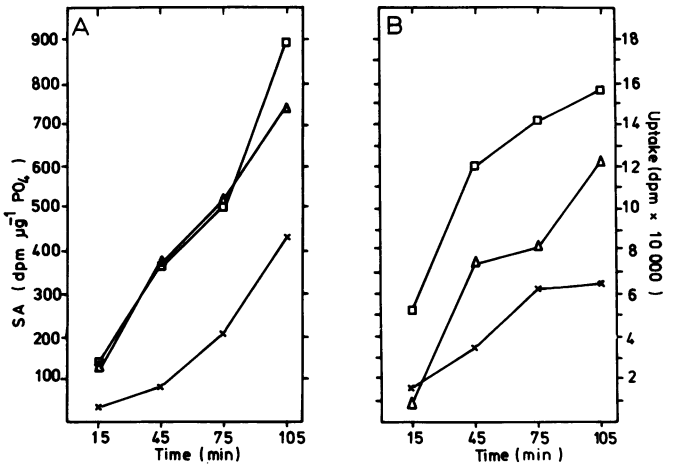

FIG. 2. Incorporation (A) and uptake (B) of [^{14}C]acetate into the phospholipid fraction of *T. ruralis* upon rehydration following rapid (×) or slow (△) desiccation. □, Incorporation and uptake by undesiccated control moss. Uptake is the total taken up into each 250-mg (predesiccation) fresh weight sample.

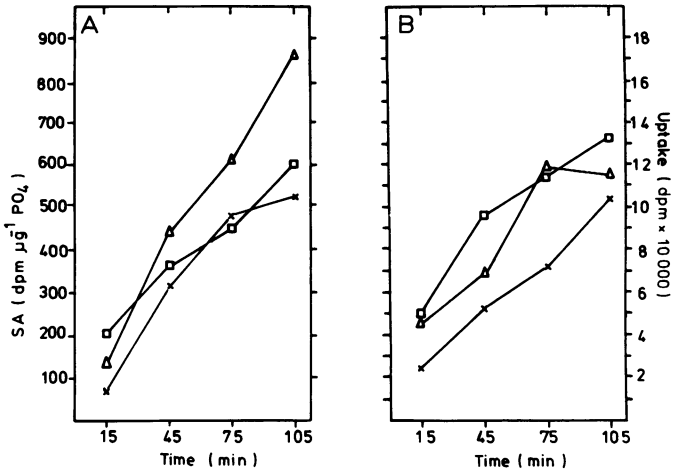

FIG. 3. Incorporation (A) and uptake (B) of [^{14}C]glycerol into the phospholipid fraction of *T. ruralis* upon rehydration followng rapid (×) and slow (△) desiccation. □, Incorporation and uptake by undesiccated control mss. Uptake is the total taken up into each 250-mg (predesiccation) fresh weight sample.

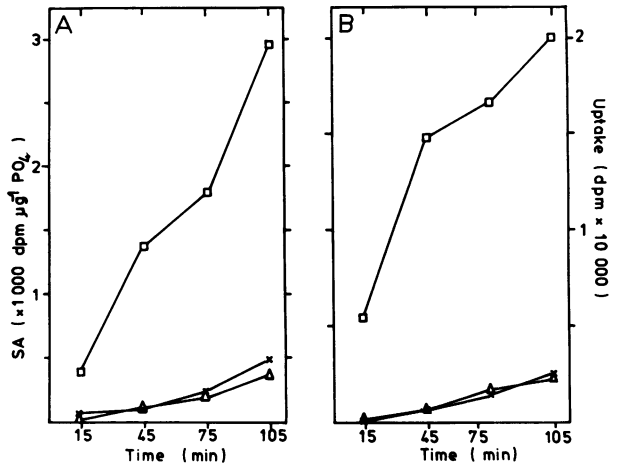

FIG. 4. Incorporation (A) and uptake (B) of [^{14}C]acetate into the phospholipid fraction of *C. filicinum* upon rehydration following rapid (×) and slow (△) desiccation. □, Incorporation and uptake by undesiccated control moss. Uptake is the total taken up into each 250-mg (predesiccation) fresh weight sample.

phospholipid fraction essentially linearly with time for the first 105 min of rehydration (Fig. 2A). Upon rehydration after slow-drying, a very similar pattern was noted. After rapid-drying, there was a greatly reduced rate of incorporation during the early stages of rehydration, which showed progressive recovery, so that, by 75 to 105 min, the incorporation rate was similar to that in control samples (Fig. 2A). The uptake of [^{14}C]acetate also was reduced following desiccation at either rate (Fig. 2B), but slow-dried samples recovered quickly, so that, after 15 min of rehydration, the rate of uptake was similar to that in control values (although the total label taken up was somewhat less, suggesting that slow-dried samples were synthesizing at a faster rate or that they had a smaller endogenous pool of precursor). Rapid-dried samples did not recover so quickly and did not regain control rates of uptake throughout the course of the experiment.

The incorporation of [^{14}C]glycerol into the phospholipid fraction was also linear in hydrated *T. ruralis* (Fig. 3A). Incorporation was reduced upon initial rehydration after desiccation at either rate. However, by 45 min, both recovered; rapid-dried samples incorporated as much [^{14}C]glycerol as did controls, while slow-dried samples exceeded this. The uptake of [^{14}C]glycerol was reduced little by slow drying and somewhat more by rapid-drying (Fig. 3B).

In *C. filicinum*, control samples incorporated [^{14}C]acetate into phospholipids linearly with time (Fig. 4B). Both rapid- and slow-dried samples showed a pronounced reduction in incorporation rates, which did not recover during the experimental period. This pattern was reflected in the uptake values (Fig. 4B). The incorporation of [^{14}C]glycerol into the phospholipids of *C. filicinum* was reduced by a prior desiccation treatment at either rate (Fig. 5A). Uptake of the precursor also was reduced (Fig. 5B), but neither the uptake nor the incorporation of the glycerol was reduced as much as those of acetate.

Thus, the synthesis of phospholipids is variously suppressed on rehydration after rapid and slow desiccation; the desiccation-tolerant *T. ruralis* recovers more completely than does the desiccation-intolerant *C. filicinum*. The reappearance of the unsaturated fatty acids may not be due solely to increased *de novo* synthesis, however, since there is a poor overall correlation between their reappearance (Table I) and the incorporation pattern of acetate (Fig. 2). Perhaps the reappearance of the unsaturated acids is accounted for by transacylation between the abundant triglycer-

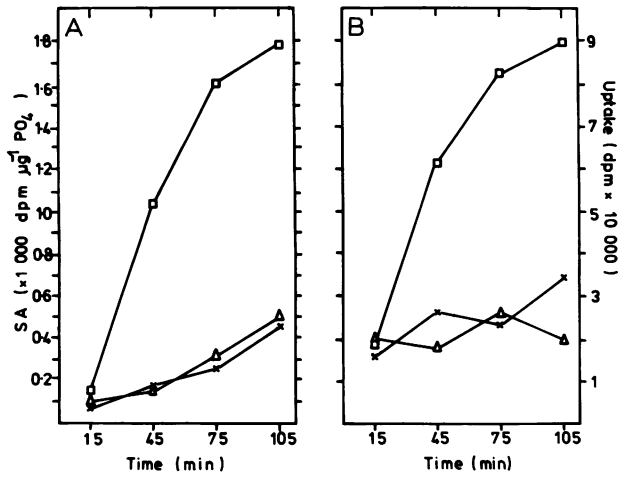

FIG. 5. Incorporation (A) and uptake (B) of [^{14}C]glycerol into the phospholipid fraction of *C. filicinum* upon rehydration following rapid (×) and slow (△) desiccation. □, Incorporation and uptake by undesiccated control moss. Uptake is the total taken up into each 250-mg (predesiccation) fresh weight sample.

ides found in the oil bodies of *T. ruralis* and the phospholipids. Additionally, there is little evidence to suggest that there is increased specific synthesis of fatty acids upon rehydration; in fact, glycerol utilization appears to be stimulated more than that of acetate. It is not clear why this should be so. It is possible that the sites of utilization of the two precursors differ to such an extent that one is affected more severely by desiccation.

It should be noted that uptake, as well as incorporation of radioactive precursors, is initially reduced upon rehydration; *T. ruralis* recovers while *C. filicinum* does not. In both mosses, the uptake of acetate is suppressed more than that of glycerol. This might be attributed to the ionic nature of the former, making it less able to diffuse across a lipid bilayer. It is likely that the uptake by control moss requires membrane protein components for facilitated and/or active transport, and any reduction in these proteins as a consequence of desiccation would be expected to lead, in turn, to reduced uptake. Whatever the cause of this reduced uptake, it is unlikely to be due to any shortage in ATP availability, since this compound returns to control levels within minutes of rehydration after rapid- or slow-drying (4). A more likely cause of reduced uptake is that essential carrier proteins are displaced from their correct positions in the membrane during the initial rapid inrush of water upon rehydration (12). Since *T. ruralis* is able to reverse leakage of low mol wt cell constituents within 1 h (7), this moss can minimize, and recover quickly from, any membrane disruption, allowing normal metabolism to recommence (3). *C. filicinum*, however, is unable to accommodate desiccation or to prepare for rehydration, leading to severe membrane damage. No reversal of solute leakage is seen in this intolerant moss (6, 8).

The observed changes in the levels of the phospholipid fatty acids during desiccation and rehydration of *T. ruralis* and *C. filicinum* cannot be correlated with the reported changes in malondialdehyde levels (8), nor can they be correlated with the different extents of damage to these mosses. It seems unlikely that peroxidation has a major role to play in desiccation tolerance or intolerance in these two species. Instead, it seems likely that the increased leakage upon rehydration after rapid-drying of *T. ruralis* is due to a greater disruption of the membrane transport system (*i.e.* the protein component of the membrane), which takes longer to recover than it does following slow-drying. In slow-dried moss, the system comes into operation more quickly and counteracts the continuing leakage, so that, initially, it is reduced and, later, reversed. *C. filicinum* is unable to accommodate desiccation, and its membranes are severely and irreversibly damaged.

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