Short Communication

Metabolism As a Function of Water Potential in Air-Dry Seeds of Charlock (Sinapis arvensis L.)

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ABSTRACT

A new method is described for studying the metabolism of air-dry seeds. An initial pulse of $^{14}$CO$_2$ was supplied to seeds maintained in air at controlled low water potentials for 6 months. Seeds were also infiltrated with $^2$-$^{14}$C-acetate and with $^{14}$C-L-leucine at 0 C, redried rapidly at 0 C, and maintained at controlled low water potentials for 4 to 6 weeks. The metabolism of the air-dry seeds was a function of the water content of the tissues, which was in equilibrium with the water potential at the seed surface. The fixation of $^{14}$CO$_2$ and the utilization of $^2$-$^{14}$C-acetate increased exponentially with water content. The incorporation of $^{14}$C-L-leucine into protein increased linearly with water content. Metabolism was not reduced to a low rate except in air-dry seeds at the lowest water potentials (-1716 to -762 bars) with 4 to 6% water. m

During the last phase of seed development and dehydration, structural changes occur in the embryo axis and storage tissues associated with loss of water and reduction in rate of metabolism. The cells and organelles show signs of shrinkage and possibly alteration of membrane structure and permeability. The rough endoplasmic reticulum is reduced to a small number of crescent layers in the region of organelles and the plasmalemma. The cytoplasm contains free ribosomes, but few or no free polysomes can be detected. The mitochondria have a simplified structure with an electronopaque matrix and few cristae (1, 7, 8, 11, 17). The respiration rate of air-dry seeds has been measured manometrically in a respirometer and is of the order of 100 μl h$^{-1}$ g$^{-1}$ dry weight ($Q_{O_2} = Q_{CO_2}$). The rate increases exponentially with water content, with critical values for acceleration above the water status of air-dry seeds (1, 2, 12, 15). Evidence for in vivo protein synthesis in air-dry seeds has been published by Chen (4, 5). Seeds of Avena fatua L. exposed to traces of $^{14}$C-ethanol vapor at 23 C for 1 week incorporated a significant amount of radioactivity into ethanol-insoluble residues, from which labeled amino acids were released by hydrolysis with 6 N HCl at 105 C for 18 hr or by incubation with pronase. Marcus (10) was unable to detect protein synthesis in dry wheat embryos after exposure for 19 min to $^{14}$C-leucine. Extracts prepared from viable air-dry wheat and rye embryos contain all the components necessary for protein synthesis, and have the capacity to polymerize amino acids in vitro. Dry wheat embryos may be used as the basis of an efficient cell-free system for translation of mRNA (13, 14). Protein synthesis in air-dry seeds apparently occurs using stored mRNA (9, 10) (Payne and Gordon, personal communication). The results of this communication show that the rate of mitochondrial activity and protein synthesis in air-dry seeds is a function of the water content of the tissues, which is in equilibrium with the water potential of the atmosphere at the seed surface.

MATERIALS AND METHODS

Supply of $^{14}$C to Seeds at Low Water Potentials. Charlock (Sinapis arvensis L.) seeds were surface sterilized in 96% ethyl alcohol for 5 min and dried for 1 hr under UV light. Samples of 50 seeds were placed in thin-walled polypropylene cups which were suspended with nichrome wire from the rubber caps of small glass vials under sterile conditions. The water potential at the seed surface was maintained constant in air above aqueous solutions of H$_2$SO$_4$ (Table 1). Fifty μCi $^{14}$CO$_2$ generated from NaH$^{14}$CO$_3$ (8 C mol$^{-1}$) were supplied to the seeds in the gas phase. It was calculated that differences due to the solubility of CO$_2$ in the H$_2$SO$_4$ were negligible (0.6%) (6). The gas phase initially contained 2.164% CO$_2$. Measurements of the rate of loss by diffusion through the rubber caps showed that the amount of $^{14}$CO$_2$ present after 0.5, 1, 6, and 12 hr was 0.74, 0.57, 0.52, and 0.41 of the initial $^{14}$CO$_2$. Effective exposure therefore lasted for 2 to 3 days, and was followed by exposure for air to 6 months at the same low water potentials, at 25 C.

Samples of 50 sterile seeds and seed parts (embryos, seed coats) were also immersed in 2 ml of deionized distilled H$_2$O containing 5 μCi 2-$^{14}$C-acetate (3.2 C mol$^{-1}$) or 5 μCi $^{14}$C-L-leucine (278 Ci mol$^{-1}$) plus a drop of 0.5 μg ml$^{-1}$ each of penicillin, streptomycin, and mycelin for 24 hr at 0 C. The seeds and seed parts were redried above 40% H$_2$SO$_4$ at 0 C and equilibrated at low water potentials for 6 weeks following 2-$^{14}$C-acetate treatment and 4 weeks following 14C-L-leucine treatment at 25 C. Vapor pressures above various concentrations of H$_2$SO$_4$ are only slightly affected by temperature (0 to 75 C) (16).

Analytical Procedures. The seeds supplied with $^{14}$CO$_2$ or 2-$^{14}$C-acetate were ground in anhydrous diethyl ether, and the ether-soluble substances separated by centrifugation at 1800g for 10 min. The residue was re-extracted with 80% ethyl alcohol for 1 hr at 65 C. The ethanol-soluble substances were further fractionated by passing through a column (10 x 10 mm) of cation exchange resin (Dowex AG 50W-X8 hydrogen form) and of anion exchange resin (Dowex AG 1-X8 formate form) (3). The residue from the second extraction was resuspended in deionized distilled H$_2$O. Aliquots of each extract were dried on ground glass planchettes with and without 0.1 M H$_2$PO$_4$ for 1 hr at 70 C, and $^{14}$C estimated with a Nuclear-Chicago gas flow detector. The seeds, seed coats, and embryos supplied with $^{14}$C-
L-leucine were ground in ice-cold 0.2 m NaCl with 10⁻⁴ m L-leucine, and the supernatant separated from the residue by centrifugation at 12,000g for 15 min. The residue was resuspended in deionized distilled H₂O. Aliquots were precipitated immediately with cold 15% trichloroacetic acid, or after incubation with and without pronase (1 mg ml⁻¹, pH 8, for 30 min at 37 °C). The precipitates were washed and dried on Millipore filters before estimation of ¹⁴C with a Nuclear-Chicago gas flow detector.

RESULTS AND DISCUSSION

Water Status of Seeds. When air-dry seeds were placed in atmospheres at controlled water potentials (ψ), the water content increased or decreased with time until equilibrium was reached (Table I). The change in water content was a hyperbolic function of time. The rate of water uptake increased with the deficit from equilibrium, which was not established under all conditions until after several days. The water content at equilibrium depended on the water potential at the seed surface; in air-dry seeds (surface potential, -1716 to -762 bars) it was 4 to 6%, in seeds at about the permanent wilting point of soils (surface potential, zero) it was 82.5%. When imbibed seeds were placed in a similar range of controlled water potentials, the rate of water loss increased rapidly with the gradient, and at low surface potentials, equivalent to the air-dry state, equilibrium was established in 1 day. The water content at equilibrium was the same after absorption or desorption, however, the resistance to movement of water vapor into air-dry seeds was much higher than that out of imbibed seeds. Seeds were supplied with ¹⁴CO₂ in the gas phase without interference with the equilibrium water status of the seeds. Seeds were infiltrated with ²-¹⁴C-acetate and ¹⁴C-L-leucine in the liquid phase and restored to the air-dry condition at 0 C, before equilibrating at low water potentials at 25 C.

Incorporation of ¹⁴CO₂, ²-¹⁴C-Acetate, and ¹⁴C-L-Leucine by Seeds at Low Water Potentials. Statistical analysis showed that there was a significant increase in metabolism with water content in the dry seeds (Fig. 1A). The amount of ¹⁴CO₂ incorporated was 104, 150, 191, 438, and 3156 cpm/50 seeds at -1716, -762, -204, -72, and -32 bars, water potential, respectively. There was an exponential increase in ¹⁴CO₂ fixation with increase in water content such that ln(c) = 1.64 + 0.077 w, r = 0.98, where c is ¹⁴C incorporated (cpm) and w is seed water content (%). Most of the ¹⁴C was incorporated in the residue, relatively more remained in the ethanol-soluble substances at the higher water contents, and a significant amount was in ether-soluble substances at the highest water content.

The utilization of ²-¹⁴C-acetate was also a function of water content. It may be assumed that the labeled acetate would enter the tricarboxylic acid cycle metabolism following conversion to acetyl-CoA. The increase in rate of respiration with water content resulted in increased loss of ¹⁴CO₂ from acetate, which was not measured. Some of the ¹⁴C was incorporated as an inverse function of water content in the tissues (c = 1623 - 47 w, r = 0.99) (Fig. 1B). After 6 weeks most of the ¹⁴C was in the ethanol-soluble substances; in dry seeds with 4 to 14% water, 93% of the ethanol-soluble ¹⁴C was in amino acids, and the remainder in organic acids and sugars. Part of the ¹⁴C was accumulated in the residue probably synthesized from labeled amino acids into proteins. A small amount was found in ether-soluble substances in moist seeds.

The resistance to penetration of amino acids into the seeds was high. At zero time, immediately after redrying at 0 C, the total uptake of ¹⁴C-L-leucine from a source of high specific radioactiv-

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Table 1. Equilibrium Water Contents of Seeds at Controlled Water Potentials

<table>
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<tr>
<th>H₂O Content (%)</th>
<th>Relative Humidity (%)</th>
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<th>Water Content (%)</th>
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<td>-a</td>
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</table>

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Fig. 1. Incorporation of ¹⁴CO₂, ²-¹⁴C-acetate, and ¹⁴C-L-leucine by seeds at controlled water potentials at 25 C. A: ¹⁴CO₂ (6 months); B: ²-¹⁴C-acetate (6 weeks). Symbols in A and B: ○: total; □: insoluble residue; △: 80% ethanol-soluble substances; Δ: ether-soluble substances. C: ¹⁴C-L-leucine (4 weeks). ○: total; □: insoluble residue; △: 0.2 m NaCl soluble substances. W/D = water content/unit absolute dry weight (cpm/50 seeds [0.1503 g dry weight]).

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metabolism of air-dry seeds is a function of the water content of the tissues, which is in equilibrium with the water potential of the atmosphere at the seed surface. Kinetic analysis of the incorporation of \(^{14}\)C CO\(_2\) and \(^{2-14}\)C-acetate indicates an exponential increase in rate of mitochondrial activity with increase in water content. The incorporation of \(^{14}\)C-L-leucine indicates a linear increase in rate of protein synthesis with water content. The data provide definitive evidence for the occurrence of metabolic reactions in air-dry seeds.

CONCLUSION

The metabolism of air-dry seeds is a function of the water content of the tissues, which is in equilibrium with the water potential of the atmosphere at the seed surface. Kinetic analysis of the incorporation of \(^{14}\)C CO\(_2\) and \(^{2-14}\)C-acetate indicates an exponential increase in rate of mitochondrial activity with increase in water content. The incorporation of \(^{14}\)C-L-leucine indicates a linear increase in rate of protein synthesis with water content. The data provide definitive evidence for the occurrence of metabolic reactions in air-dry seeds.

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LITERATURE CITED