^{13}C NMR Studies of Lipid Mobilization Pattern in Germinating Groundnut (*Arachis hypogaea* L.) Seeds

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The products of lipid mobilization in groundnut (*Arachis hypogaea* L.) seeds as a function of time immediately after imbibition are monitored by ^{13}C NMR. Different parts of the embryonic axis, namely, the radicle, hypocotyl, and plumule, exhibit characteristic time dependent ^{13}C NMR spectra observed at 24-h intervals after imbibition. The various stages in the transformation of storage lipids present in different parts of the embryonic axis are clearly demonstrated. The transformaton of storage lipids is completed first in the radicle followed by the hypocotyl and finally the plumule. A mechanism of the transformation of the storage lipids is discussed.

INTRODUCTION

The major storage lipids in oil seeds are triglycerides. During germination consequent to imbibition they undergo hydrolysis by lipases, yielding glycerol and free fatty acids. The products of this hydrolysis are to a large extent converted into hexoses, although a part of glycerol and free fatty acids may be used for resynthesis of fats and membrane lipids (Bewley and Black, 1985; Bradbeer and Stumpf, 1959). This end product, hexose, is taken up by active transport into the growing embryonic axis. The mobilization of lipid degraded products from the cotyledons to the plumule during the growth of the latter is known (Bradbeer and Stumpf, 1959). However, very little is known about these events in the embryonic axis (tigellum), which consists of the radicle, hypocotyl, and plumule. Therefore, it was planned to monitor the above processes individually in the radicle, hypocotyle, and plumule in the germinating seed using ^{13}C NMR spectroscopy, and the results are reported here.

MATERIALS AND METHODS

Materials. Groundnut seeds of variety TMV-2 were chosen for the investigation. Excised tissues in wet condition were packed into 10 mm o.d. glass NMR tubes. The quantity was so chosen as to fill the sensitive volume in the coil. The ^{13}C NMR spectra were obtained using a Bruker AMX-400 FTNMR spectrometer. Acquisition time, relaxation delay, and number of scans for each of the samples were 0.59 s, 2 s, and 5000, respectively.

Experimental Conditions. For the first sample, corresponding to the zeroth day, the intact (entire) embryonic axis of the seed was used to obtain the ^{13}C NMR spectrum as the component parts of the axis, namely, the radicle, hypocotyl, and plumule, could not be separated. The embryonic axis and the whole seed on the zeorth day showed identical ^{13}C NMR spectra. However, subsequent to imbibition, the component parts of the axis could be excised, and then the spectra were obtained individually for them.

RESULTS AND DISCUSSION

The time-dependent ^{13}C NMR spectra of the radicle, hypocotyl, and plumule at 24-h intervals after imbibition are shown in Figures 1–3, respectively.

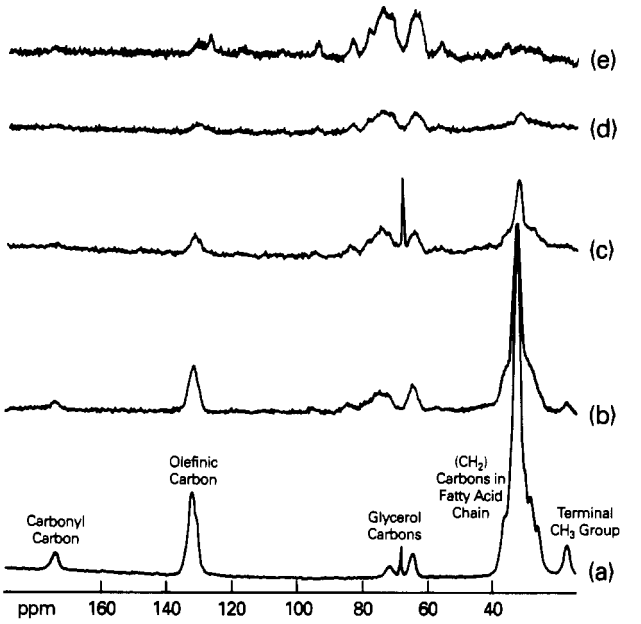

Figure 1. ^{13}C NMR spectra of the radicle as a function of time after imbibition: (a) prior to soaking; (b–e) 24, 48, 72, and 96 h, respectively, after imbibition. Spectrometer, AMX-400; temperature, 20 °C.

The spectra for the radicle, hypocotyl, and plumule corresponding to the zeroth day (Figures 1a, 2a, and 3a) were identical, as the different parts could not be separated out. The various peaks are characteristic of the different functional groups in the triglyceride molecule (Shoolary, 1973). The peak at about 15 ppm is for the terminal methyl group of the fatty acid chain. The large peak at about 35 ppm corresponds to the CH_2 groups in the fatty acid chain. The two broad peaks between 60 and 80 ppm are from the glycerol carbons. The peak at about 130 ppm is from the olefinic carbons, while the peak at 170 ppm is from the carbonyl carbon (Shoolary, 1973).

The lipid transformation in the case of the radicle starts after 24 h, as indicated by changes in the NMR spectrum of 1-day-old germinating seed when new lines start appearing in the region around 70 (±10) ppm, which correspond to hexoses (Chen et al., 1979). By 48 h, these lines grow, and, at the same time, there is a reduction in intensity of the CH_2 and CH_3 peaks. By day 3 (72 h) the lipid proportion is reduced to almost nil, and the hexose

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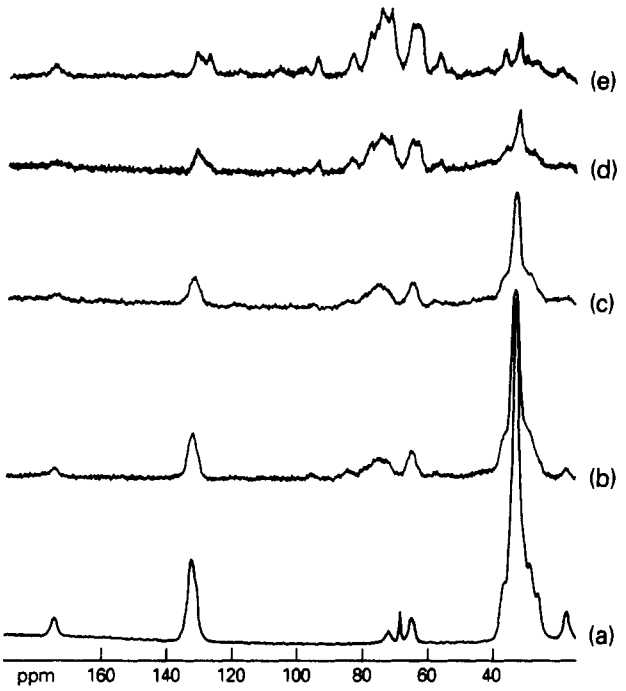

Figure 2. Same as in Figure 1, but the spectra are for the hypocotyl.

lines completely develop. This is the limiting period for the conversion of fat into hexoses in the radicle during the germination period. Beyond this period, no lipid is present in the radicle. In the case of hypocotyl the degradation of fat also starts at 24 h, but the process continues up to 96 h, beyond which practically no lipid is observed. The presence of triglycerides beyond 72 h suggests that digestion and transportation of triglycerides are slower in the hypocotyl than in the radicle.

In the case of the plumule, even though fat degradation starts after 24 h, the degradation is much slower (least), and a residual lipid component can be seen even in the 96-h (fourth day) sample. The degradation of lipids is complete only beyond this period.

The radicle is the first part of the embryonic axis responding to imbibition and begins to grow. With the beginning of germination, the stored lipids in the radicle are degraded rapidly and completely by 72 h (Figure 1d) and are converted into hexoses. In the hypocotyl, which grows subsequently, the storage lipids are transformed completely into hexoses by 96 h (Figure 2e). The complete loss of lipids in the hypocotyl almost coincides with the completion of its growth. The plumule, which is the last part of the embryonic axis to show signs of growth, still has lipids in it at 96 h (Figure 3e), and only later are the stored lipids in it completely exhausted. Thus, the rate of lipid mobilization corresponds to the rate of growth in the individual parts of the embryonic axis, most rapid in the radicle, followed by the hypocotyl, and least in the plumule.

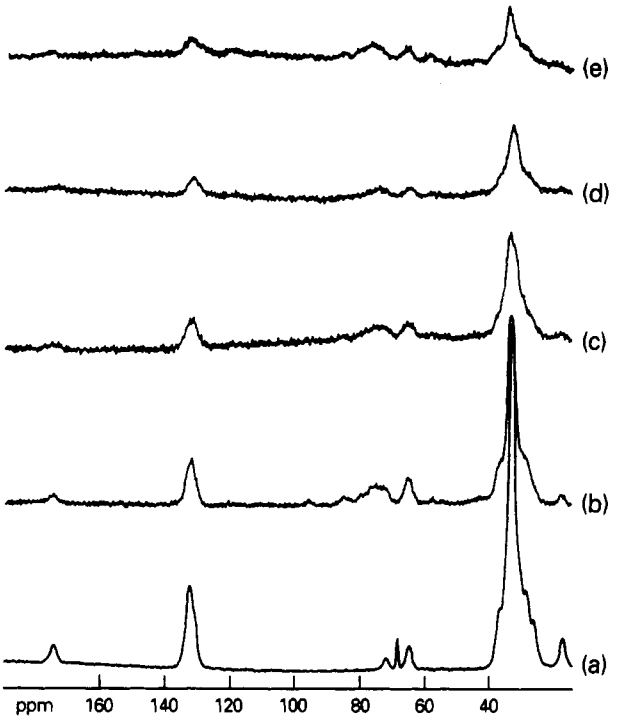

Figure 3. Same as in Figure 1, but the spectra are for the plumule, recorded only up to 96 h after imbibition.

CONCLUSIONS

The differential rates of fat degradation into hexoses in various parts of the embryonic axis of the germinating groundnut seed are reflected in their time-dependent ^{13}C NMR spectra. This serves as a useful technique in plant physiological studies.

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