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Seasonal Changes in the Structure and Function of Mitochondrial Membranes of Artichoke Tubers

ACYL FATTY ACID COMPOSITION AND THE EFFECT OF GROWTH CONDITIONS

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ABSTRACT

Changes in the temperature response, fluidity, function and the acyl fatty acid composition, were determined for a mitochondria-rich membrane fraction from Jerusalem artichoke (*Helianthus tuberosus* L.) tubers during dormancy for a crop which matured in midsummer. The temperature of both the upper and lower limits of the membrane lipid transition decreased during dormancy from 26 C and 1 C to 4 C and −5 C, respectively. This was similar to the changes observed with crops maturing in late autumn. The order parameter of a spin label intercalated into the membrane lipids decreased from about 0.6 to 0.5 during dormancy and returned to the original value before sprouting, showing that membrane fluidity increased during dormancy. The activation energy of succinate oxidase of tuber mitochondria was generally high at middormancy when membrane lipids were more fluid and decreased as the membranes became more rigid at the end of dormancy. The fatty acid composition of the membrane lipids did not alter significantly during dormancy. The results indicate that neither decreasing day length nor low soil temperature during tuber maturation is essential for the initiation of the membrane changes necessary for tubers to avoid low temperature injury during dormancy. The increase in membrane fluidity during dormancy could not be accounted for by an increase in the proportion of unsaturated fatty acids in the membrane lipids.

Mitochondrial membranes of dormant tubers of Jerusalem artichoke (*Helianthus tuberosus* L.) differ in a number of respects from the same membranes of newly matured tubers or tubers approaching the end of dormancy (3). Immediately after tuber maturity the temperature of both the upper (T_f)[1] and lower (T_s) boundaries of the order-disorder transition of the membrane lipids decreased (3) and it was proposed that this alleviates the adverse effects of the subzero temperatures experienced by tubers during winter dormancy in the soil (3).

This hardening to low temperature has been observed with other plants. With some, the proportion of unsaturated fatty acids in the membrane lipids increased (7) and this was assumed to have increased membrane fluidity. With other plants such as wheat seedlings, increased unsaturation of membrane fatty acids was found not to be essential for hardening of the tissue (4). Our studies were made to determine if there was any alteration in the fluidity of the artichoke membrane lipids, concomitant with lowering temperature limits of the transition during dormancy, and whether there was any alteration in fatty acid composition during this change.

The stimulus for the initiation of the changes in tuber membranes is not known. The decrease in both T_f and T_s first occurs about the time of tuber maturation and therefore could be induced by the environmental conditions experienced by the plant during the latter part of the growing season. Hamner and Long (8), for example, demonstrated that tuberization of artichokes, a complex physiological event, is initiated by decreasing photoperiods. The membrane changes might therefore be initiated by decreasing photoperiods experienced at the end of the growing season in late autumn. Other environmental factors of the growing season such as temperature and water availability are known to influence the timing and duration of dormancy in potato tubers (2) and their influence in artichoke tubers cannot be dismissed. The validity of an hypothesis which ascribes the initiation of the decrease in T_f and T_s in dormant tubers to the decreasing photoperiod can be readily tested by growing artichokes in a "reversed" season, *i.e.* in winter and spring to produce tubers in summer.

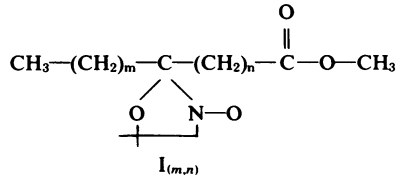
As shown in this paper tuberization does occur when plants are grown during winter, and the mitochondrial membrane lipids of these tubers display changes in T_f and T_s similar to those previously observed in membranes of summer-grown tubers (3). In addition the fluidity of the membrane lipids, measured by esr spectroscopy, increased during dormancy but there was no significant change in the proportion of unsaturated fatty acids in the membrane lipids.

MATERIALS AND METHODS

Preparation of Tubers. Artichokes (*H. tuberosus* L.) were grown in a glasshouse in 1976 to 1977 where tubers formed in December (midsummer), reached maturity and were harvested in February (late summer) at the completion of vegetative growth. Tubers were washed, dipped in a suspension of fungicide (Benlate, Dupont [Australia] Ltd.,) and dried before packing into covered trays of dry Vermiculite and storing at 4 C.

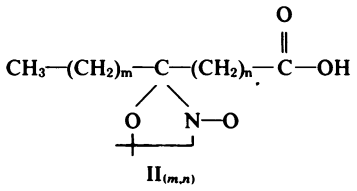
Preparation of Mitochondria and Measurement of O_2 Uptake. Mitochondria were isolated from tubers and succinate oxidase activity determined as previously described (3).

Electron Spin Resonance Spectroscopy. Nitroxide analogs of fatty esters, I(*m*,*n*) and fatty acids, II(*m*,*n*) were used as spin labels.



$I_{(m,n)}$

[1] Abbreviations: T_f: temperature of the upper limit of the lipid transition; T_s: temperature of the lower limit of the lipid transition; esr: electron spin resonance; Ea: Arrhenius activation energy.



$II_{(m,n)}$

Spectra were recorded and the transition temperatures determined using the motion parameter τ_0 as previously described (3). The order parameter, S, was calculated according to the method of Gaffney (5) using the spin label $II_{(11,4)}$.

Lipid Analysis. All procedures were performed using redistilled solvents and 0.01% butylated hydroxytoluene was added as an antioxidant. Total lipids were extracted twice with chloroform-methanol (2:1, v/v) and the nonlipid material removed from the combined extracts by partitioning against a solution of NaCl (0.73%, w/v). Fatty acid content was determined by GLC of methyl esters prepared from the total lipids (1).

RESULTS

Artichokes were grown in the "reverse" season, *i.e.* during the winter and spring and harvested in summer. A mitochondria-rich fraction was prepared from tubers stored at 4 C and changes in the structure and function of the membranes in this fraction at various times over a 20-week period are shown in Figure 1. The temperatures of the order-disorder transition, T_f and T_s, decreased from 26 C and 1 C to 4 C and −5 C, respectively, in the first 10 weeks of dormancy, and increased again before sprouting at about 20 weeks, a trend previously observed with tubers harvested in late autumn (3). However the minimum for T_f and T_s for membranes of this winter grown crop was maintained for only 2 weeks compared with the 12 and 15 weeks of summer crops (3). It has been proposed that lowering T_s and decreasing the temperature range of the transition is a mechanism adopted by artichoke tubers to avoid low temperature injury (3). This implies that the lipids adapt to maintain membranes in a fluid state at low temperatures. The order parameter, S of the acyl chains of membrane lipids, determined by esr spectroscopy, is a direct measure of membrane fluidity; a value of 1 represents a rigid chain and a value of 0, a very fluid chain (5). As shown in Figure 1B the value of S for label $II_{(11,4)}$ at 30 C decreased in the first 8 weeks of storage, coincident with a lowering of T_f and T_s and with the narrowing of the temperature range between T_f and T_s. After 9 weeks of storage S increased at the same time as T_f increased and the temperature range between T_f and T_s broadened. Thus, the membrane lipids were more fluid when T_f and T_s were at their minima.

No attempt was made to purify the mitochondria isolated by differential centrifugation. The preparations oxidized succinate at a rate of 100 to 200 nmol O_2/min·mg protein and exhibited a respiratory control ratio of between 2 and 4 showing that the preparation is rich in intact, active mitochondria. The Arrhenius activation energy, Ea, for succinate-dependent oxidase activity of mitochondria, from tubers at various times of storage from mid-dormancy, was measured to determine if mitochondrial function was affected by the changes in membrane structure. As shown in Figure 1A the Ea was relatively high, 53 kJ/mol, when the membrane lipids were more fluid during dormancy but decreased to 20 kJ/mol when the membranes became more rigid, about 4 weeks before sprouting. The coincidence of the over-all pattern of change in Ea (Fig. 1A) and membrane fluidity (Fig. 1B) indicates that the bulk of the mitochondrial membrane lipids are involved in the transition, indicated by T_f and T_s (Fig. 1C).

To seek an explanation for the alterations in physical properties of the membranes, an analysis was made of the fatty acids of the total membrane lipids at various stages during the season. Table I shows the fatty acid composition of the total lipids of the mitochondrial fractions from tubers at various times over the

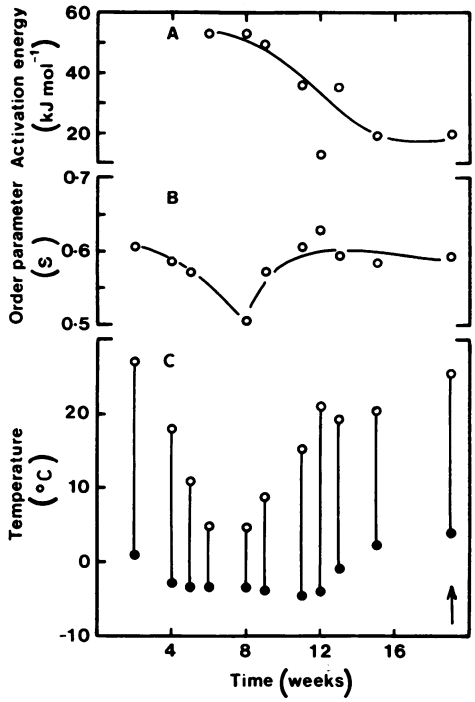


FIG. 1. Changes in the structural and functional properties of membranes of artichoke tubers during 4 C storage. Zero for the abscissa was the time at which the aerial portion of the plant had died and the tubers had reached maximum diameter. A: changes in Ea of succinate oxidase activity above T_f. B: changes in the order parameter, S, for the spin label $II_{(11,4)}$ at 30 C. C: changes in the temperature of T_f (○) and T_s (●) for the order-disorder transition. Time of sprouting is indicated by the arrow. Maximum experimental error in A is ±4 kJ/mol, in B ±0.005, and in C is ±0.5 C degrees. Ea values shown in A were obtained from the slope of the regression line for activity measured at 5 different temperatures above T_f.

storage period. Linolenic acid (18:3), linoleic acid (18:2), and palmitic acid (16:0) were the major fatty acids found at all stages of dormancy and these are the most abundant fatty acids found in storage tissue of other plants (9). Considering the errors in estimating the fatty acid composition (*i.e.* ±1%) the change in the proportion of unsaturated fatty acids throughout dormancy is not considered significant.

DISCUSSION

Jerusalem artichokes are normally grown through summer and autumn. However, as reported in this paper, when the plants are grown through winter and spring, reaching maturity in the summer, they also produce tubers which remain dormant for up to about 18 weeks when stored at 4 C. The production of tubers during a period of increasing daylength is contrary to the results of other workers (8) who show that tuberization is not initiated if plants are exposed to increasing photoperiods. The summer-grown tubers are physiologically competent in that they sprout after a dormancy period and their membrane lipids undergo changes (Fig. 1) which are essentially similar to the changes observed with tubers from a crop grown through summer and autumn, a period of decreasing photoperiods (3). On the basis of these observations, tuberization and the subsequent changes in the membrane lipids are evidently not controlled by photoperiod in this variety of artichoke.

The factors involved in the initiation of the changes in membrane properties are not known. Some insight into this question can be gained by comparing the different environmental conditions experienced by the summer and autumn crops and the time course of the membrane changes. With a crop grown during

Table I. *Fatty Acid Composition of Membrane Preparations*

Storage	Fatty Acids					Total Unsaturation
	16:0	18:0	18:1	18:2	18:3	
weeks			*(mol %)*			
2	21	2	5	64	8	77
4	21	1	5	64	9	78
5	21	1	5	64	9	78
6	20	1	5	66	8	79
8	20	1	5	66	8	79
9	20	1	5	65	9	79
12	20	1	6	65	8	79
13	22	1	5	64	8	77
15	22	1	4	65	8	77
19	23	1	4	64	8	76

winter and spring, tubers develop on plants whose vegetative parts are exposed to increasing temperatures rather than the decreasing temperatures experienced by plants growing during autumn. The more rapid change in membrane properties and the brief time these changes were maintained in tubers from the winter-spring-grown crop compared with tubers from a summer-autumn crop, points to the possibility that factors produced in the aerial portion of the plant and translocated to the tubers in some way regulate the membrane changes in the tubers. In addition, it is of interest to note that tubers which matured in the "reverse" season, *i.e.* during spring and summer, were not exposed to declining soil temperature as were tubers maturing during autumn, yet their membrane lipids increased in fluidity during storage at 4 C. This suggests that transfer to storage at 4 C initiates the change in membrane fluidity.

The decrease in the order parameter for the spin label $II_{(11,\ 4)}$ from about 0.6 to 0.5 represents an increase in fluidity equivalent to the increase observed in sarcoplasmic reticulum membranes by increasing the temperature by about 20 C degrees (10). Thus, even though exposed to lower temperatures during winter the membranes of artichoke tubers are maintained at a relatively constant fluidity. The Ea of succinate oxidase in general decreases from the high values at maximum membrane fluidity to relatively low values as the membrane lipids become more rigid (Fig. 1). A similar relationship between Ea and membrane fluidity has been observed with mitochondria from sheep and rat liver where fluidity was altered by feeding a diet rich in polyunsaturated fatty acids (13).

These changes in membrane lipid fluidity cannot be accounted for in terms of a change in the unsaturation of the acyl fatty acids of membrane lipids (Table I). For many organisms, maintenance of cellular integrity, at low temperatures, depends on an increase in membrane fluidity and a common feature of growth at low temperatures appears to be an increase in the unsaturation of membrane lipid fatty acids (11, 12). However, it has been shown that increased lipid unsaturation is not essential for the adaptation of wheat seedlings to low temperatures (4). A similar conclusion can be drawn from the observation that the same percentage of unsaturated fatty acids are present in the polar lipids of several species of *Passiflora*, which exhibited various degrees of tolerance to chilling, consistent with a lowering of T_s (14). More specifically, Galliard *et al.* (6) found no change in lipid unsaturation for potato tubers stored at 5 C. The lack of change in lipid unsaturation in artichoke tubers during 4 C storage is therefore not unusual and indicates that some other mechanism is operating to account for the changes observed in membrane fluidity. That these changes occur even when the tubers reach maximum development during summer indicates that decreasing photoperiod and/or decreasing soil temperature are not factors influencing these changes.

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