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Metabolic adaptation of *Zostera marina* (eelgrass) to diurnal periods of root anoxia

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Abstract

The temperate seagrass *Zostera marina* L. is common in coastal marine habitats characterized by the presence of reducing sediments. The roots of this seagrass grow in these anoxic sediments, yet eelgrass is highly productive. Through photosynthesis-dependent oxygen transport from leaves to roots, aerobic respiration is supported in eelgrass roots only during daylight; consequently, roots are subjected to diurnal periods of anoxia. Under anoxic root conditions, the amino acids alanine and γ-amino butyric acid accumulate within a few hours to account for 70% of the total amino acid pool, while glutamate and glutamine decline. Little ethanol is produced, and the pool size of the organic acid malate changes little or declines slowly. Upon the resumption of shoot photosynthesis and oxygen transport to the roots, the accumulated γ-amino butyric acid declines rapidly, glutamate and glutamine pools increase, and alanine declines over a 16-h period. These adaptive metabolic responses by eelgrass to diurnal root anoxia must contribute to the successful exploitation of shallow-water marine sediments that have excluded nearly all vascular plant groups. A metabolic scheme is presented that accounts for the observed changes in organic and amino acid pool sizes in response to anoxia.

Introduction

Seagrasses, a functional grouping of about 50 species, are the only angiosperms that are successful in shallow-water coastal marine habitats characterized by the presence of reducing sediments (Fenchel, 1977). Seagrass beds play an important role in coastal ecology. The dense leaf canopy provides a structural haven from predation for larval and juveniles fishes and for many invertebrates. The beds enhance recycling of inorganic nutrients from sediments to benthic and water-column communities by transporting nutrients through the seagrass (McRoy and Barsdate, 1970) and by stimulating the regeneration of organically fixed nutrients (Iizumi *et al.*, 1982; Short, 1983). This latter process is partially due to oxidation of the seagrass rhizosphere (Oremland and Taylor, 1977; Iizumi *et al.*, 1980; Smith *et al.*, 1984), with a subsequent increase in microbial activity. Seagrasses also actively increase deposition and binding of sediments (Thayer *et al.*, 1977) through effective baffling of water currents. The low-current microenvironment and the stabilized sediments around seagrass shoots may increase settlement and survival of benthic fauna.

The seagrass *Zostera marina* (eelgrass) is distributed along a depth gradient in most locales. The lower depth limit of the beds appears to be set by light, since reductions in light intensity (Backman and Barilotti, 1976; Dennison and Alberte, 1982) or daily light period (Dennison and Alberte, 1982) markedly reduce their growth and productivity; low light-limited growth has been observed in the closely related seagrass *Heterozostera tasmanica* (Bulthuis, 1983). In spite of light limitation at depth, eelgrass plants seem to be not only adapted but also restricted to the shallow subtidal zone, for they only occur occasionally in the intertidal zone and grow poorly there (Harrison, 1982). Indeed, eelgrass shoots exhibit morphological and photosynthetic adaptations to the light regime of their subtidal habitat (Dennison and Alberte, 1982). The present study sought to determine if roots of *Z. marina* possess metabolic mechanisms, which permit them to grow in reduced sediments under diurnally anoxic conditions.

Materials and methods

Plant material

Healthy *Zostera marina* L. plants were collected in August 1983 from the Great Harbor, Woods Hole, Massachusetts,

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USA population studied previously (Dennison and Alberte, 1982). Plexiglas cores (15 cm diameter × 13 cm deep) containing intact roots and rhizomes were removed and transferred into flower pots and transported to Chicago in ice-packed coolers within 40 h. Plants were maintained in large, aerated aquaria filled with synthetic sea water (30‰ S) at 18 °C on a 16 h L:8 h D regime. Light intensity was $> 100\ \mu E\ m^{-2}\ s^{-1}$ (PAR, photosynthetically active radiation), which is above that required for photosynthetic saturation in *Z. marina* (Dennison and Alberte, 1982).

Plant treatments

Just prior to experimental manipulations, whole plants were washed free of sediments, dead roots and rhizomes. Plants used for the anaerobic time course were held briefly in aerated sea water to ensure an oxygenated condition prior to exposure to anoxia. In contrast, plants used for the aerobic time course were held in nitrogen-bubbled seawater to ensure root oxygen deprivation prior to the experimentation. The plants for the time-course studies were submerged in separate 500-ml flasks containing filtered (Whatman # 1) synthetic sea water (30‰ S) and maintained at 18 °C (± 1 °C). Aerobically treated flasks (4 plants for each time point, 32 plants for the time course) were bubbled continuously with humidified air and maintained at photosynthesis-saturating light levels (PAR). Anaerobically treated flasks (3 plants for each time point, 24 plants for the time course) were bubbled with nitrogen gas (O_2 content < 5 ppm) and maintained in total darkness.

In order to investigate the production of ethanol under various conditions, additional plants were prepared as above and subjected to one of five aerobic or anoxic regimes: air-bubbled plants in light for 24 h; air-bubbled plants in darkness for 24 h; nitrogen-bubbled plants in light for 24 h; nitrogen-bubbled plants in darkness for 24 h; and nitrogen-bubbled plants in dark for 48 h. In other experiments, plants were maintained in intact rhizosphere/sediment cores, and ethanol levels were determined after the plants were subjected to one of four treatments: air-bubbled shoots in light for 16 h (normal daylight regime); air-bubbled shoots in darkness for 8 h (normal night regime); air-bubbled shoots in low light ($25\ \mu E\ m^{-2}\ s^{-1}$, PAR) for 3 d under the normal 16 h L:8 h D regime; and air-bubbled shoots in low light for 7 d as in the previous treatment. This low light condition is slightly above the photosynthetic compensation intensity for *Zostera marina* and well below photosynthetic saturation (Dennison and Alberte, 1982), permitting photosynthesis by shoots but greatly reducing oxygen transport to roots (Smith *et al.*, 1984).

Metabolite extractions

Ethanol. At the end of specific treatments, plants were separated into roots, rhizomes, and meristems. Portions of each tissue type were immediately frozen in liquid nitrogen (77 °K), weighed, ground in liquid nitrogen, and reweighed. Metabolically produced ethanol was obtained from the frozen tissues by fractional distillation.

Organic and amino acids. At the end of individual treatments, plants were removed from the incubation flasks, and roots from the first two or three nodes were quickly excised and plunged into liquid nitrogen. The roots were weighed, ground in liquid nitrogen, and reweighed. The frozen tissues were extracted twice in boiling ethanol [80% (v/v) followed by 60% (v/v)]. The extracts were combined, centrifuged (10 min at 1500 × g), and vacuum evaporated to dryness. The residue was redissolved in 60% (v/v) ethanol containing water-saturated polyvinylpyrrolidone to adsorb phenolic compounds (Gray, 1978), centrifuged (10 min at 1 500 × g), and evaporated to dryness. The final residue was redissolved in 60% (v/v) ethanol and stored at −20 °C until analyzed (1 to 10 d). Samples for α-keto acid analysis were redissolved in 20% (v/v) methanol. In some cases, additional plants were subjected to the same treatments, but roots were extracted with 6% (w/v) perchloric acid for metabolite analysis by enzymatic methods (Smith and ap Rees, 1979 a). Water samples were taken from the incubation flasks at the beginning and end of each plant incubation and subjected to the ethanolic-extraction procedure as above to examine for leakage of metabolites from the plants (Smith and ap Rees, 1979 b).

Quantification of metabolites

Ethanol. Ethanol distilled from plant tissues was assayed fluorometrically by endpoint difference, following reaction with the enzyme alcohol dehydrogenase (Sigma). The stoichiometric consumption of reductant by this reaction was determined with the fluorescent analog of NAD, 3-acetyl pyridine adenine dinucleotide (Witt, 1974).

Malate. The organic acid malate was quantified by two methods. Initially, the enzymatic technique of Möllering (1974) was used to determine concentrations in the perchloric acid extracts of root tissues. The absence of interference by other compounds was checked by enzymatic analysis of the malate fractions obtained from HPLC. Confirmation of these results was obtained by high-performance liquid chromatography (HPLC) analysis of the ethanolic extracts. The enzymatic and HPLC analyses yielded comparable results. One half of the final root extract was evaporated to dryness and redissolved in HPLC-grade water. Aliquots, 10 μl, were injected into a Waters Associates (Milford, CT, USA) HPLC, and organic acids were separated on an Interactions (Palo Alto, CA, USA) ORH-801 organic acid column by elution with 0.01 N sulfuric acid at 35 °C (Woo and Benson, 1983). Organic acids were detected by absorbance at 210 nm; the limit of detection was 200 nmol injected and detector response was linear in the range of 200 to 1 000 nmol.

Amino acids. Amino acids were also analyzed by HPLC using a reversed-phase elution profile based on the solvents and gradient of Jones and Gilligan (1983). An IBM C_{18} (octadecylsilane) column (250×4.5 mm, 5.0 μm packing) was used. Amino acids were prederivatized with o-phthalaldehyde (OPA) as follows: 100 μl of the metabolite extract was reacted with 25 μl of fluoraldehyde (Pierce Chemical) reagent solution for 2 min, centrifuged for 1 min to sediment precipitates, and 75 μl was injected into the HPLC. The OPA-amino acid complexes were detected by absorbance at 340 nm with a limit of detection of 50 pmol of amino acid injected. Because the absorbance of the OPA-derivatized amino acids have different extinctions at 340 nm, separate extinction coefficients were determined for each amino acid, using authentic standards. The detector response at 340 nm was linear between 0.10 and 10 nmol for 26 protein and physiological amino acids with correlation coefficients ranging from $r^2 = 0.79$ to 0.98 (mean $r^2 = 0.91$). Reclamation of known amounts and combinations of amino acids through the extraction and detection procedure ranged from 66 to 101% and averaged 90% by linear regression. Retention times, over the 50-min gradient elution, for the individual OPA-derivatized amino acids were used to identify the peaks in the sample elution profiles. In all cases the retention times for standards and sample amino acids were consistent between individual runs and samples. To further verify the identity of sample peaks, internal standards of each amino acid were run in sample analyses.

α-keto acids. The α-keto acids were derivatized with 1,2-phenylene diamine following the procedures of Kieber and Mopper (1983), and separated by HPLC as described above for the amino acids. The detector response was linear between 0.2 and 10.0 nmol as determined on a range of standards. The α-keto acids were detected by absorbance at 340 nm.

Results

Analysis of water samples taken before and after 24-h aerobic and anoxic treatments showed that the amino acid content of the incubation water was < 3% of that extracted from the roots. Eelgrass roots are known to release amino acids (Wood and Hayasaka, 1981); however, since whole plants were used, the shoots also contributed to the dissolved organic compounds (DOC). Since DOC release by eelgrass leaves approached 2% (Kirchman *et al.*, 1984), it is likely that many of the amino acids present in the incubation water were lost by shoots rather than roots. Additionally, there was no substantial difference in the types and amounts of amino acids released during aerobic versus anaerobic treatments. Replicate analyses of four root segments between the apex and rhizome indicated that there was no significant difference in amino acid concentrations or patterns along the root axis (data not shown).

Typical amino acid elution patterns of root extracts from plants subjected to 24-h aerobic and anaerobic treatments are contrasted in Fig. 1. Under extended anaerobic conditions (24 h) two amino acids, alanine (1.53 ± 0.27 μmol g^{-1} fresh wt.) and γ-amino butyric acid (GABA) (1.57 ± 0.28 μmol g^{-1} fresh wt), accounted for 70% of the total free amino acid pool. Under aerobic conditions, glutamate (0.50 ± 0.09 μmol g^{-1} fresh wt) and glutamine (1.39 ± 0.10 μmol g^{-1} fresh wt) accounted for 42% of the amino acids, while alanine and GABA only comprised 13% of the free amino acids.

Time-course examinations of amino acid levels under anaerobic conditions (Fig. 2) demonstrated that alanine and GABA increased rapidly (within 2 h of anoxic treatment) and that glutamate and glutamine pools decreased markedly in the same time period. The fluctuations in pool sizes during the first hour of treatments reflected both

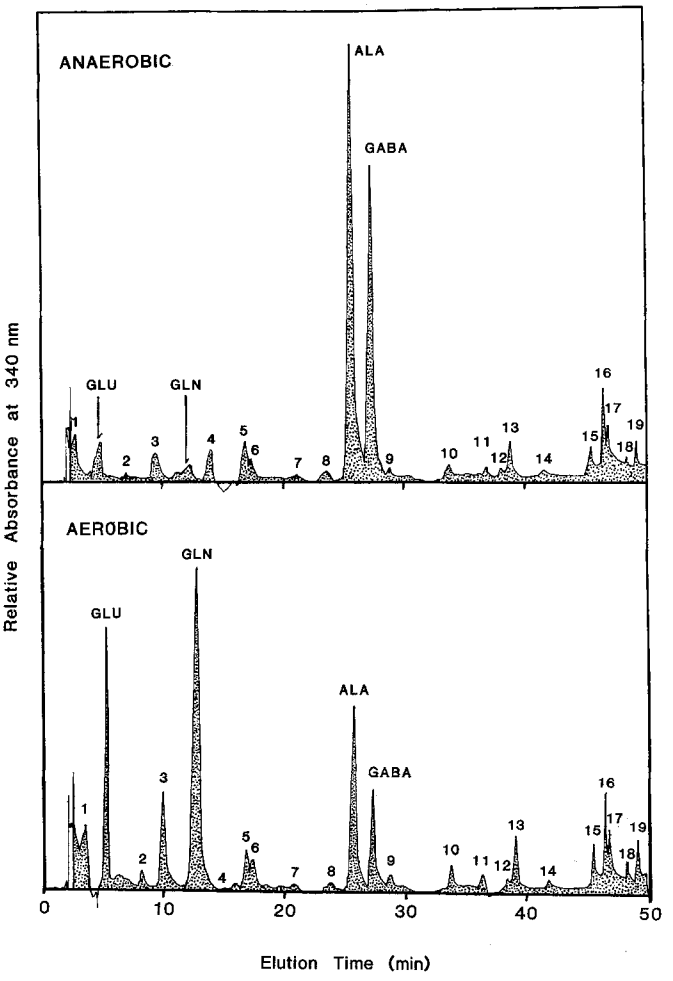

Fig. 1. *Zostera marina.* Typical HPLC elution patterns of amino acids extracted from roots following 24-h anaerobic and 24-h aerobic treatments (0.1 absorbance full scale). The amino acids glutamate (GLU), glutamine (GLN), alanine (ALA), and γ-amino butyrate (GABA) are indicated by their abbrevations. Other amino acids include: (1) aspartate (2) asparagine (3) serine (4) histidine (5) glycine (6) threonine (7) citrulline (8) β-alanine (9) tyrosine (10) α-amino butyrate (11) methionine (12) valine (13) tryptophan (14) phenylalanine (15) isoleucine (16) leucine (17) hydroxylysine (18) ornithine (19) lysine

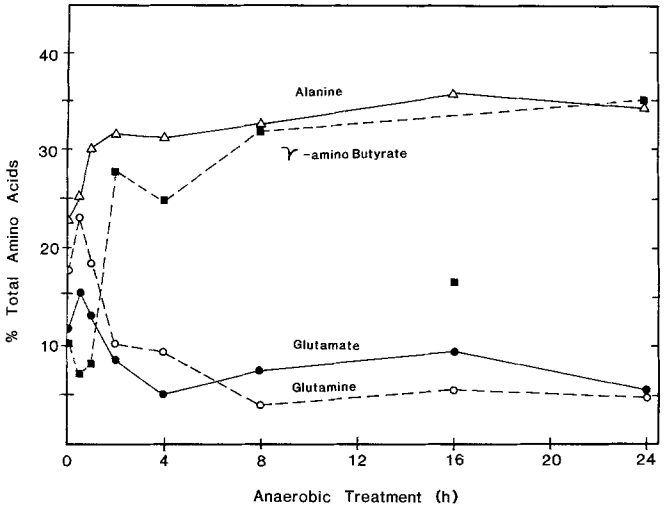

Fig. 2. *Zostera marina.* Changes in alanine, γ-amino butyrate, glutamate, and glutamine levels in roots of whole plants subjected to 24-h anaerobic time course (3 plants per time point, 24 plants for the treatment)

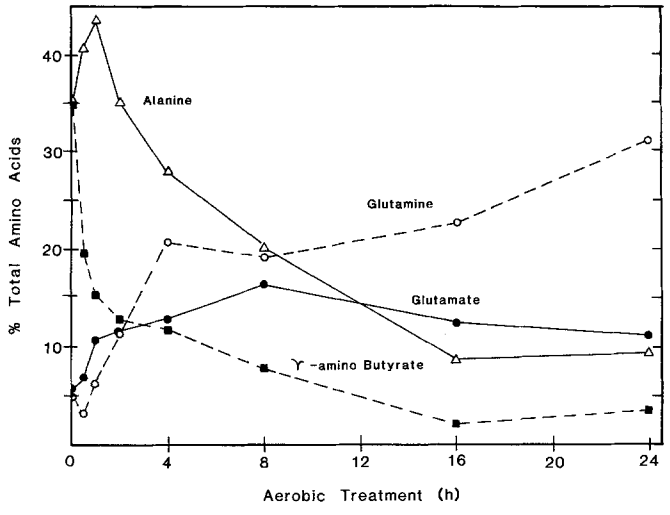

Fig. 3. *Zostera marina.* Changes in alanine, γ-amino butyrate, glutamate, and glutamine levels in roots of whole plants subjected to 24-h aerobic time course (4 plants per time point, 32 plants for the treatment)

between-plant variability and the time lag (15 to 30 min) between the start of the anaerobic treatment and actual root anoxia (Smith *et al.*, 1984). Between-plant variation was such that within-treatment standard errors averaged 3.4% for the amino acids considered here (3 plants per treatment). Results are expressed as percent of total free amino acids, since amino acid concentrations of roots were quite variable from plant to plant (mean $4.42 \pm 3.00\,\mu$mol (g fresh wt)$^{-1}$). However, there was no significant difference in total free amino acid content on a weight-corrected basis between aerobic ($N=32$ plants) and anaerobic ($N=24$ plants) treatments.

In a nearly reciprocal pattern (Fig. 3), GABA declined markedly within 1 h of aerobic treatment, while alanine declined more gradually after 2 h. Glutamate and glutamine accumulated quickly, and the amount of glutamine surpassed that of glutamate within 2 to 4 h. Alanine and GABA continued to decline over an extended period (16 h) of aerobiosis. Between-plant, within-treatment standard errors averaged 2.7% for the amino acids considered (4 plants per treatment).

Concentrations of ethanol in tissues of plants from intact sediment cores remained low in all treatments, even those in which roots received very little or no oxygen for 3 to 7 d (due to low shoot photosynthesis under below-saturation light levels, see Table 1). However, when whole plants (free of sediments) were maintained under anaerobic conditions (darkness and nitrogen bubbling), ethanol levels increased in roots, rhizomes and shoots. Ethanol levels in the meristem and rhizome tissues were 5 to 8 times greater than those in roots after 24 h of such treatment (Table 1). However, after 48 h of shoot darkness and bubbling of roots and shoots with nitrogen, the ethanol levels in the meristem declined, probably due to leakage of ethanol from the tissues.

Our initial enzymatic analyses of malate concentrations in perchloric acid extractions of roots indicated no significant difference between the 24-h anoxic treatment (4 plants) and the 24-h aerobic treatment (3 plants) (Table 2). In addition, HPLC analyses of the ethanol extractions from the 24-h aerobic time-course treatments indicated that there was no significant change in malate with time. During the anoxic time-course treatments malate appears to have decreased very gradually in a linear fashion (data

Table 1. *Zostera marina.* Ethanol content of eelgrass tissues following aerobic and anoxic treatments (minimum of two plants per treatment). Different letters (a–d) indicate significantly different mean concentrations ($p < 0.05$)

Plant treatment	Ethanol content (μmol g fresh wt^{-1})		
	Roots	Rhizomes	Meristem
Intact sediment cores, air-bubbled shoots			
16 h of light*	0.09 a	0.12 a	0.08 a
8 h of darkness	0.08 a	0.11 a	0.05 a
3 d of low light**	0.11 a	0.19 b	0.04 a
7 d of low light	0.07 a	0.06 a	0.22 b
Sediment-free plants air bubbled			
24 h, light	0.25 b	0.20 b	0.16 b
24 h, dark	0.34 b	0.39 b	0.13 a
nitrogen bubbled			
24 h, light	0.65 c	0.61 c	0.32 b
24 h, dark	1.00 c	5.07 d	8.20 d
48 h, dark	1.07 c	6.09 d	0.36 b

* Saturation light intensity > 100 μE m^{-2}s^{-1}

** Low-light conditions werre 25 μE m^{-2}s^{-1} for the 16-h light period each day (16 hl:8 hD)

Table 2. *Zostera marina.* Malate and α-keto acid content (mean ± 1 SD) of roots following aerobic and anoxic treatments. Whole plants washed free of sediments were used. Aerobic treatment: 24 h, air bubbled, saturating light. Anoxic treatment: 24 h, N_2 bubbled, total dark. Different letters (a–e) indicate significantly different mean concentrations ($p < 0.05$)

Organic acid (nmol g fresh wt^{-1})	Plant treatment	
	Aerobic	Anaerobic
Malate	3 470.0 (±1,980.0) a	3 100.0 (±430.0) a**
Pyruvate	7.72 (±9.29) b*	58.18 (±5.52) c*
Glyoxylate	60.28 (±12.54) d	72.83 (±13.57) d
α-ketoglutarate	192.12 (±9.29) e*	158.37 (±38.38) e

* Sample number for these analyses was $N = 2$; all others $N = 3$
** Sample number for this analysis was $N = 4$

not shown). Thus the pool size of malate certainly does not increase during anoxia, and may very well decrease.

The levels of the α-keto acid pryuvate increased about 8-fold when roots were kept under anaerobic conditions for 24 h compared with an equivalent aerobic treatment (Table 2). No significant changes in response to anoxia were observed in the levels of α-ketoglutarate or glyoxylate (Table 2).

Discussion

Eelgrass (*Zostera marina*) is a marine angiosperm that inhabits shallow-subtidal sediments along many temperate coastlines. In most eelgrass populations the roots extend down into anoxic sediments that are characterized by high organic content and high rates of ammonium regeneration (Fenchel, 1977; Iizumi *et al.*, 1982). While such rhizosphere conditions are detrimental to growth for most terrestrial plants, eelgrass populations are among the most productive plant communities in the world (Phillips and McRoy, 1980). The present study sought an explanation for this apparent paradox.

When terrestrial plants experience chronic flooding, as is common in swamp and wetland species, or episodic flooding, as is often observed in crop plants, the accumulation of ethanol from anaerobic root metabolism may be observed (John and Greenway, 1976; Smith and ap Rees, 1979a, b; Joly and Crawford, 1982; Rumpho and Kennedy, 1983). In contrast, some other species appear to divert carbon away from ethanol to a variety of products, such as the oganic acids malate or shikimate (Davies, 1980; Joly and Crawford, 1982) or amino acids (Dubinina, 1961; Fulton *et al.*, 1964; Streeter and Thompson, 1972a; Smith and ap Rees, 1979a).

Spartina alterniflora, the dominant cordgrass of North Atlantic salt marshes, responds to flooding in a manner similar to terrestrial plants. Tidal inundation in daylight creates hypoxia in shoots and roots, while submersion at night causes anoxia (Gleason and Zieman, 1981). Under these conditions, the roots accumulate malate (Mendelssohn *et al.*, 1981). When the marsh soils become highly reduced, the roots increase their alcoholic fermentation (Mendelssohn *et al.*, 1981). In eelgrass roots, however, ethanol does not accumulate in plants still rooted in sediments, and the pool size of malate does not increase and may very well decrease slowly over extended periods of anoxia.

In response to anoxia in eelgrass roots, the levels of specific amino acids change very rapidly; alanine and GABA accumulate, and glutamate and glutamine decline within 2 to 4 h. In contrast, when the roots become aerobic, GABA declines rapidly, glutamate and glutamine accumulate, and alanine declines gradually but markedly. Low amounts of ethanol are still present under aerobiosis, and malate levels remain relatively constant. The α-keto acid pyruvate increases 8-fold under anoxia, a response documented in other species (Dubinina, 1961). It is unlikely that pyruvate accumulates significantly during anoxia until after the levels of alanine plateau (2 to 4 h), as pyruvate provides the carbon skeletons for the synthesis of this amino acid.

We present the following metabolic model to account for the changes in metabolites observed here (Fig. 4). When eelgrass shoot photosynthesis stops at night or is low under conditions of heavy overcast or high water turbidity, transport of oxygen to the rhizomes and roots is

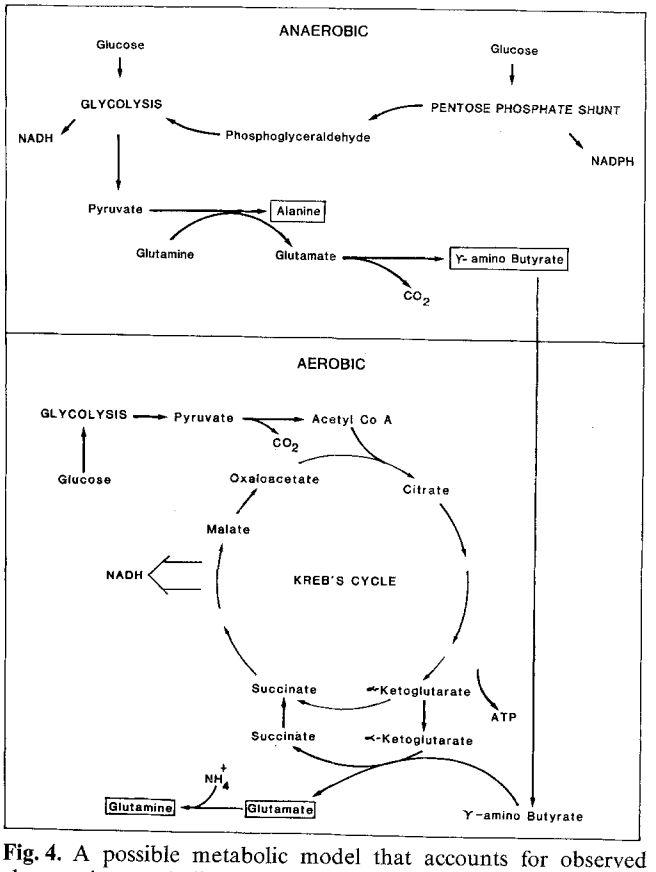

Fig. 4. A possible metabolic model that accounts for observed changes in metabolite levels in eelgrass roots during anaerobic and aerobic treatments. Small boxes enclose those substances that accumulate (alanine and γ-amino butyrate under anerobiosis vs glutamate and glutamine under aerobiosis)

minimal or ceases; consequently root aerobic respiration consumes available oxygen, leading to hypoxia and/or anoxia within a very short time (Smith *et al.*, 1984). Kreb's cycle activity is extremely limited or ceases, and metabolism of glucose proceeds via glycolysis and/or the pentose phosphate shunt. The eventual product of these pathways is pyruvate, which is converted to ethanol in roots of some other species under anoxia. However, in *Zostera marina* pyruvate can be transaminated by glutamate or perhaps glutamine to alanine. Alanine has been observed to accumulate in anaerobic tissues of some plants (Dubinina, 1961; Streeter and Thompson, 1972a; Smith and ap Rees, 1979a). The glutamate pool, which is augmented by the deamination of glutamine, can be converted via a decarboxylation reaction to GABA. GABA has been found to accumulate in some plants subjected to extended anoxia (Dubinina, 1961; Fulton *et al.*, 1964; Streeter and Thompson, 1972a, b). Fulton *et al.* (1964) determined that the increased levels of GABA were not harmful. Thus, when *Z. marina* roots are under anaerobic conditions, the amino acids alanine and GABA should accumulate at the expense of glutamate and glutamine. Pyruvate from glycolysis can be converted to alanine rather than ethanol; therefore, little or no ethanol accumulation should be observed in eelgrass roots.

We feel that the low levels of ethanol found in plants maintained in their sediment cores reflect the typical response of the roots. Penhale and Wetzel (1983) also found low levels of ethanol in field-collected *Zostera marina*. In contrast, when whole plants are subjected to anoxia, significant amounts of ethanol accumulate. The increased levels of ethanol in the roots may indicate (1) that whole-plant anaerobiosis is an extreme stress that results in abnormal production of ethanol in the roots or (2) that the shoots and rhizomes perform fermentation of glucose and produce ethanol, some of which diffuses down into the roots. Thus, different tissues of eelgrass exhibit very different responses to anoxia (see Table 1). Roots, which are diurnally anoxic, probably divert the majority of their pyruvate to alanine rather than ethanol, while shoots, which are always in oxygenated water, and rhizomes, which extend laterally in the oxidized upper few centimeters of sediments, respond to anoxia as many terrestrial plants do, i.e. by producing and accumulating ethanol.

A decline in the pool size of malate under anaerobic conditions (see Tables 2) in eelgrass, if it occurs, may be due to the conversion of malate to oxaloacetate in a reaction that yields reductant. The oxaloacetate may subsequently be used as a substrate for gluconeogenesis. It is also possible that malate may be transported to the shoots; Dubinina (1961) observed an increase in organic acid content in root sap of plants under anoxia.

Upon the resumption of saturated photosynthesis in daylight, eelgrass roots become oxygenated within minutes (see Smith *et al.*, 1984). After this return to aerobic conditions, anaerobically accumulated alanine is probably consumed through protein synthesis in the roots; protein synthesis is suppressed during anaerobiosis, but resumes quickly in aerobic conditions in rice roots (Bertani *et al.*, 1981). However, some alanine may be transported to the shoot meristem to support the protein synthesis required for rapid shoot growth. Once aerobiosis is reestablished, the anaerobically accumulated GABA can be readily processed in roots in a transamination reaction with α-ketoglutarate (2-oxoglutarate) to yield succinate and glutamate (Fig. 4). According to this scheme, the entry of a GABA carbon skeleton into the Kreb's cycle precisely matches the loss of each α-ketoglutarate, and prevents any interruption in Kreb's-cycle ATP production and NADH production, which drives electron transport.

Glutamate accumulates under aerobic conditions, but achieves a level that is surpassed by glutamine within a few hours. This is most likely due to the activity of the enzyme glutamine synthetase (GS), which combines free NH_4^+ with glutamate to yield glutamine. Glutamine synthetase is often the most important enzyme for initial NH_4^+ assimilation in roots (Miflin and Lea, 1977).

Based on this model, NH_4^+ uptake by roots will occur primarily during daylight hours for two reasons: first, the enzyme GS requires ATP in order to form glutamine, and under anoxic conditions the levels of ATP in roots is likely to be very low (Saglio *et al.*, 1980); secondly, the pool size of glutamate, the requisite substrate for GS, declines markedly within a few hours after the onset of root anoxia. We take exception to the conclusion of Iizumi and Hattori (1982) that there is no diurnal variation in NH_4^+ uptake by *Zostera marina* roots. The incubations they used for ^{15}N-NH_4^+ uptake in day versus night exposed roots to label for only 3 h, and the roots and rhizomes of only three plants were placed in 12-l boxes filled with filtered sea water. We suggest that the large volume of aerobic water and the short incubation time effectively created an oxidized condition for the eelgrass roots, and the NH_4^+ uptake observed at night (82% of the daylight uptake) is artifactually high. In their measurements of translocation of ^{15}N-labelled compounds, the roots and rhizomes were either kept in the intact sediment cores or placed in only 500 ml of sea water. Thus, their observation of photosynthesis-enhanced nitrogen translocation from roots to leaves may well be due to increased NH_4^+ assimilation by oxygenated roots.

In contrast to flooded terrestrial and salt marsh plants, in which the period of root anoxia usually depends upon the duration of flooding, *Zostera marina* roots experience a diurnal fluctuation from aerobic to anoxic conditions that is dependent upon shoot photosynthesis. The present research demonstrates that: (1) the metabolic response of roots to anoxia is very rapid, with major changes in metabolite pools observed within 1 to 4 h; (2) the levels of root tissue ethanol remain low, and (3) there is no accumulation of malate under anoxia. Further, it can be inferred from the data presented that: (1) the amino acids that accumulate under anoxic conditions (alanine and GABA) conserve carbon skeletons and assimilated nitrogen and are not toxic to the plant; and (2) the products that accumulate under anaerobic conditions permit a

rapid return to aerobic respiration and ammonium assimilation upon the resumption of shoot photosynthesis. These apparently adaptive metabolic features must contribute to the ability of eelgrass to exploit successfully marine coastal sedimentary habitats that have excluded nearly all other vascular plants.

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