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Ammonia assimilation in *Vitis vinifera* L.:

III. Glutamate oxaloacetate transaminase from leaf and root tissue

by

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Die Ammonium-Assimilation bei *Vitis vinifera* L.:
III. Glutamat-Oxalacetat-Transaminase aus Blatt- und Wurzelgewebe

Zusammenfassung. — Die Aktivität der Glutamat-Oxalacetat-Transaminase (GOT) in Blatt- und Wurzelgewebe der Rebsorte Chenin blanc (*Vitis vinifera* L.) war auf die lösliche Fraktion beschränkt. *In vitro* betrug die mittlere Enzymaktivität in beiden Organen 4,3 ± 0,8 µmol Oxalacetat je g Frischgewicht und h. Die K_m-Werte von GOT aus Blättern und Wurzeln betrugen (1,9 ± 0,4) · 10^{-4} M für α-Ketoglutarat und (4,7 ± 0,5) · 10^{-3} M für L-Aspartat. Die optimalen Bedingungen der GOT-Aktivität waren *in vitro* ein pH von 7,4—7,8, eine Enzymmenge, die dem Gehalt von 95—130 mg Frischmaterial entsprach, und eine Inkubationstemperatur von 38—39 °C. Weder Blatt- noch Wurzel-GOT reagierten auf zugesetztes Pyridoxal-5'-Phosphat.

Introduction

In metabolic processes that involve interconversion of nitrogen containing molecules, the transfer of amino groups catalyzed by various aminotransferases occupies a central position. Glutamate-oxaloacetate transaminase (L-aspartate : α-ketoglutarate aminotransferase, GOT, EC 2.6.1.1.) catalyzes the reversible interconversions between glutamate, aspartate and their two keto analogues.

WIGHTMAN and FOREST (1978) and GIVAN (1980) have recently reviewed the literature on plant aminotransferases thoroughly. GOT was recognized as the best characterized aminotransferase (GIVAN 1980). Studies on the kinetic properties of this enzyme have been reported for extracts from many annual plants including pea cotyledons (WONG and COSSINS 1966, 1969), *Phaseolus* seedlings (ABBADI and SHANON 1969, FOREST and WIGHTMAN 1973), cauliflower (DAVIS and ELLIS 1961), wheat germ (CRUICKSHANK and ISHERWOOD 1958), oat leaves (REED and HESS 1975) and soybean root nodules (RYAN *et al.* 1972). GOT activity in perennial plants has been detected in *Citrus* leaves (ACHITUR and BAR-AKIRA 1976), apple leaf and bark tissues (SPENCER and TITUS 1972) and pear tissue (ROMANI 1962). However, relatively little work has been done in characterizing GOT in perennial plants.

In two preceding papers characterization and properties of leaf and root glutamate dehydrogenase and glutamine synthetase from grapevine leaf and root tissues were described (ROUBELAKIS-ANGELAKIS and KLIEWER 1983 a,b). In this report several of the properties of GOT from the same tissues are presented.

Materials and methods

GOT was extracted from leaf and root tissue of *Vitis vinifera* L. cv. Chenin blanc. The plant material used, sampling technique and storage conditions were as described earlier (ROUBELAKIS-ANGELAKIS and KLIEWER 1983 a).

Enzyme extraction: 10 g of leaf tissue was homogenized in an Omnimixer 4 times, 15 s each, in 10 volumes (v/w) of ice-cold grinding medium consisting of 100 mM Tris-HCl pH 7.8, 10 mM L-cysteine-HCl and 4 % (w/v) Polyethylene glycol 4000. 10 g of root tissue was pulverized in liquid nitrogen and the fine powder was quantitatively transferred into prechilled Erlenmeyer flask containing 10 volumes (v/w) of ice-cold grinding medium. The mixture was shaken in an ice bath at 160 rpm for 60 min. Thereafter, cell fractionation for both tissues was performed through differential centrifugation according to ROUBELAKIS-ANGELAKIS and KLIEWER (1983 a).

Enzyme assay: GOT activity was determined by following the formation of oxaloacetate. The reaction mixture consisted of 100 mM Tris-HCl pH 7.8, 20 mM L-aspartate, 3 mM α-ketoglutarate, enzyme extract equivalent to about 100 mg fresh tissue and deionized water to a total volume of 3 ml. All substrates were made up in 100 mM Tris-HCl pH 8.0 and neutralized if necessary. The reaction was allowed to proceed for various times up to 60 min in a water bath at 38 °C. The reaction was stopped with 1 ml of 0.1 % (w/v) 2,4-dinitrophenyl hydrazine in 1.35 M HCl (KARMEN *et al.* 1955). After 10 min, 10 ml of 0.4 M NaOH and 10 ml of deionized water were added, the precipitated protein was removed by centrifugation and the absorbance was read at 504 nm. Standards of cis-oxaloacetic acid were run simultaneously with all the components of the reaction mixture, as well as controls in which aspartate was omitted. Enzyme activity was calculated from the initial velocity and expressed as μmol oxaloacetate · g^{-1} of fresh tissue · h^{-1}.

Results and discussion

GOT activity was found in extracts from grapevine leaf and root tissues, and was dependent on the presence of both reactants, L-aspartate and α-ketoglutarate. Mean enzyme activity of both tissues was 4.3 ± 0.8 μmol oxaloacetate formed per g of fresh tissue per hour.

GOT activity in the leaf and root extracts was present in the 25,000 *g* supernatant fraction. In most plant aminotransferases the bulk of activity has been associated with the soluble cellular fraction. However, in some plants a small proportion of total activity is apparently associated with the particulate fractions (GIVAN 1980). In pea cotyledons (WONG and COSSINS 1966, 1969), and bushbean seedling roots, shoots and cotyledons (FOREST and WIGHTMAN 1973) activity of GOT in the soluble fraction exceeded 90 % of the total. In oat leaves (REED and HESS 1975) soluble GOT accounted for 88 % and in pear tissue (ROMANI 1962) 73 % of the total activity. In soybean root nodules (RYAN *et al.* 1972) and in rice roots (KANAMORI and MATSUMOTO 1974) cytosolic and mitochondrial GOT activities were reported, whereas in tobacco cultured cells (WASHITANI and SATO 1978) GOT activity was associated with proplastids, and with chloroplasts in *Vicia faba* (KIRK and LEECH 1972) and maize (SCANDALIOS *et al.* 1975).

GOT from grapevine tissues was considerably more stable than glutamate dehydrogenase from the same tissues. After incubation at 4 °C for 90 h, GOT activity decreased by only 5—7 % whereas glutamate dehydrogenase activity was mostly lost whithin 72 h (ROUBELAKIS-ANGELAKIS and KLIEWER 1983 a).

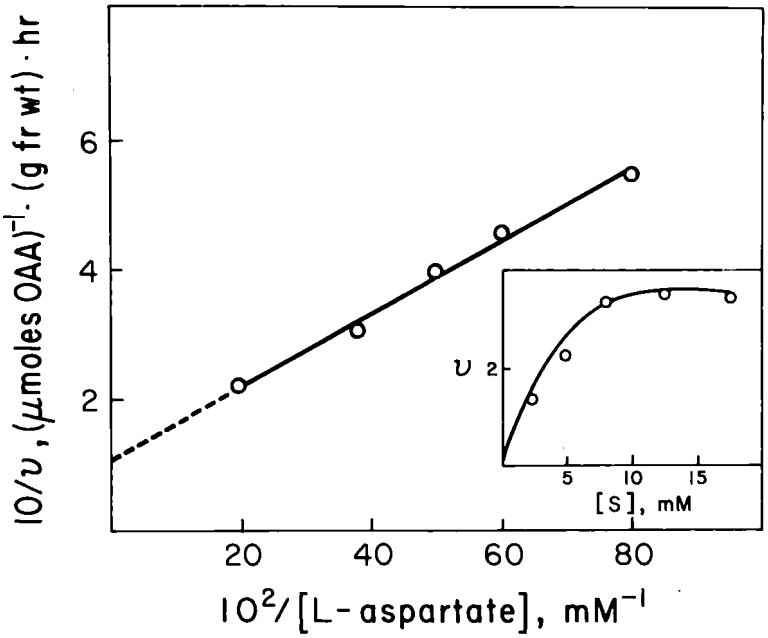

Fig. 1: Dependence of glutamate-oxaloacetate transaminase activity on L-aspartate concentration. Lineweaver-Burk and Michaelis-Menten (inset) plots. Other reaction components and conditions were as described in the text.

Abhängigkeit der Glutamat-Oxalacetat-Transaminase-Aktivität von der L-Aspartat-Konzentration. Enzymkinetik nach Lineweaver-Burk und Michaelis-Menten (Einsatz). Übrige Reaktionskomponenten und -bedingungen s. Text.

Leaf and root GOT *in vitro* activities were similarly affected by the concentration of α-ketoglutarate in the reaction mixture. The enzyme from both tissues exhibited normal Michaelis-Menten kinetics with increasing concentrations of the substrate (Fig. 1, inset). The K_m value for α-ketoglutarate calculated from the double reciprocal plot (Fig. 1) was $(1.9 \pm 0.4) \cdot 10^{-4}$ M and did not differ significantly between leaf and root enzyme. This value is slightly lower than that reported for other plant GOTs. K_m values for α-ketoglutarate in plant GOTs have ranged from $2.2 \cdot 10^{-4}$ to $6.3 \cdot 10^{-4}$ M (GIVAN 1980).

GOT from grapevine leaves and roots also showed Michaelis-Menten response to varying concentrations of L-aspartate (Fig. 2, inset). The K_m values did not differ significantly between leaf and root enzyme and averaged $(4.7 \pm 0.5) \cdot 10^{-3}$ M which falls within the range reported for other plant GOTs (GIVAN 1980). In general, GOT affinity to L-aspartate is lower than its affinity to α-ketoglutarate, although K_m values may depend on the ionic strength of the buffer used (GIVAN 1980).

Pyridoxal-5′ phosphate (PLP) has been suggested to be essential for transamination activity. However, neither grapevine leaf nor root GOT responded to exogenous PLP. There is conflicting literature on the response of GOT to PLP. For example, GOT from oat leaf (REED and HESS 1975), tobacco (WASHITANI and SATO 1978) and spinach leaves (HUANG *et al.* 1976) showed no response to exogenous PLP, whereas activity from pea cotyledons (WONG and COSSINS 1966) showed an absolute dependence on exogenous PLP; from wheat germ (CRUICKSHANK and ISHERWOOD 1958) increased 50—80 % and from pear tissue (ROMANI 1962) increased 7.5 % upon PLP addition to the reaction mix-

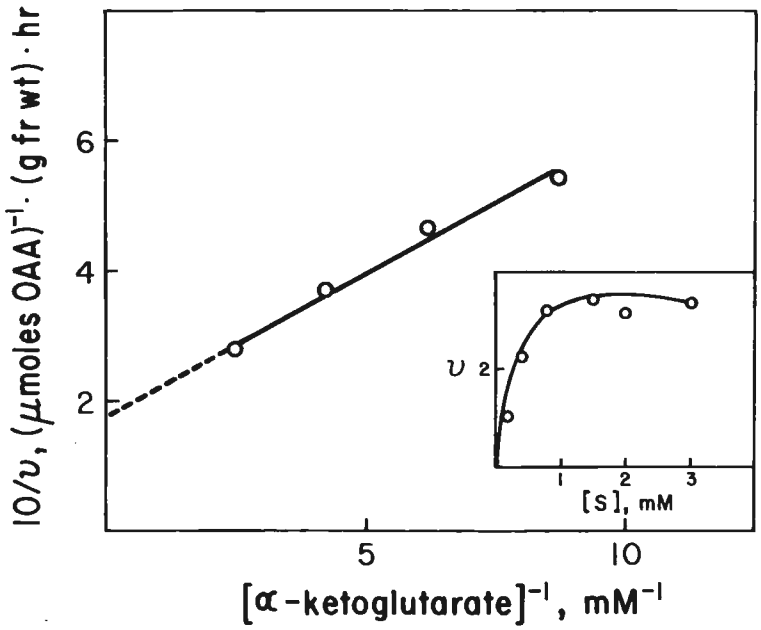

Fig. 2: Dependence of glutamate-oxaloacetate transaminase activity on α-ketoglutarate concentration. Lineweaver-Burk and Michaelis-Menten (inset) plots. Other reaction components and conditions were as described in the text.

Abhängigkeit der Glutamat-Oxalacetat-Transaminase-Aktivität von der α-Ketoglutarat-Konzentration. Enzymkinetik nach Lineweaver-Burk und Michaelis-Menten (Einsatz). Übrige Reaktionskomponenten und -bedingungen s. Text.

ture. PLP is believed to be tightly linked to the plant apoenzyme and its removal does not ordinarily occur, even during very drastic purification procedures (GIVAN 1980). In addition, *in vitro* response of plant GOT to exogenous PLP may depend on the nutritional status of the plant with respect to phosphorus. ACHITAR and BAR-AKIRA (1976) found that GOT activity from phosphate deficient *Citrus* trees showed PLP dependence, whereas this enzyme from trees grown under sufficient phosphate supply showed no response to exogenous PLP.

Optimum conditions for *in vitro* GOT activity from grapevine leaf and root tissues were pH 7.4—7.8, amount of enzyme extract equivalent to 95—130 mg of fresh tissue and incubation temperature of 38—39 °C.

Summary

Glutamate-oxaloacetate transaminase (GOT) activities in *Vitis vinifera* L. cv. Chenin blanc leaf and root tissues were associated only with the soluble fraction. Mean *in vitro* enzyme activity of both tissues was 4.3 ± 0.8 μmol oxaloacetate formed per g fresh tissue per hour. K_m values of GOT from leaves and roots were (1.9 ± 0.4) · 10^{-4} M for α-ketoglutarate and (4.7 ± 0.5) · 10^{-3} M for L-aspartate. Optimum *in vitro* conditions for GOT activity were pH 7.4—7.8, amount of enzyme equivalent to 95—130 mg fresh tissue and incubation temperature 38—39 °C. Neither leaf nor root GOT responded to exogenous pyridoxal-5′ phosphate.

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