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Isocitrate Lyase Activities in *Pinus pinea* Seeds

J. Montoya-Villarroya and M. J. López-Pérez

Department of Biochemistry
Faculty of Pharmacy
Universidad Complutense
Madrid - 3

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Some features of cytoplasmic and glyoxysomal isocitrate lyase obtained from stratified and germinated *P. pinea* seeds, respectively, are described.

Both activities showed a different electrophoretic mobility on cellulose acetate and elution behaviour on Sephadex G-200. Cycloheximide inhibited the increase of both activities during the first days of germination. Chloramphenicol did not show the same effect.

Glyoxysomes are the site of the glyoxylate pathway in fatty seeds (4). When pine seeds germinate, a rapid production of mitochondria and glyoxysomes occurs in the endosperm, were the isocitrate lyase activity is mainly located (9). During stratification only a 15 % of total isocitrate lyase activity could be sedimentated at 15,000 × g. This report describes the behavior of both cytoplasmic and glyoxysomal isocitrate lyase on cellulose acetate electrophoresis and Sephadex G-200 gel filtration.

Materials and Methods

Seeds of *Pinus pinea* were stratified and germinated as previously described (9). Isocitrate lyase activity was determined by DIXON and KORNBERG's method (7) with phosphate buffer at *p*H 7.0. Unless stated otherwise activity was expressed by the increase of optical density at 324 nm per minute of 100 microliters of enzyme.

The cytoplasmic isocitrate lyase —15,000 × g supernatant— was extracted from 30 —days— old stratified seeds (9) and the glyoxysomal enzyme from 5 —days— old germinating seeds —when the enzyme is located in glyoxysomes— grinding during 10 minutes in a morter with the buffer of COOPER and BEEVERS (6), without sucrose, in order to disrupt the organelles. The homogenate was centrifuged at 15,000 × g and the pellet discarded.

Cellulose polyacetate electrophoresis was carried out at 4° C with 1 mA/cm for 90 minutes. After the run, the strips were cut into 15 fractions, which were incubated with 1 ml of 0.06 M phosphate buffer *p*H 7.0, 10 mM Cl_2Mg, 3 mM cystein and 0.07 % phenilhidrazine at

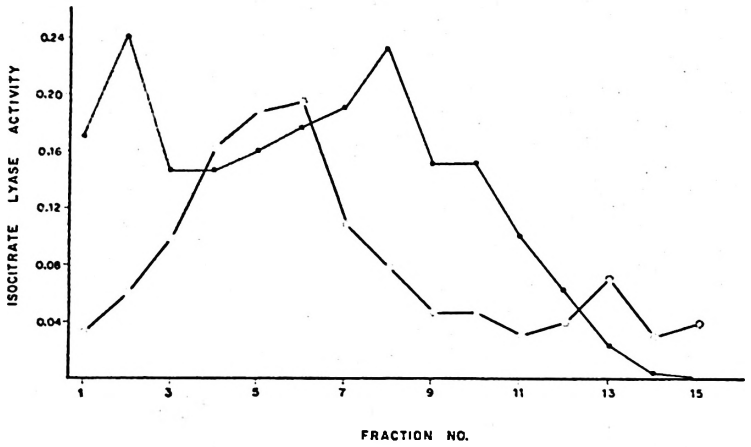

Fig. 1. *Electrophoretic mobility of cytoplasmic and glyoxysomal isocitrate lyase.* Cytoplasmic (●—●), Glyoxysomal (○—○).

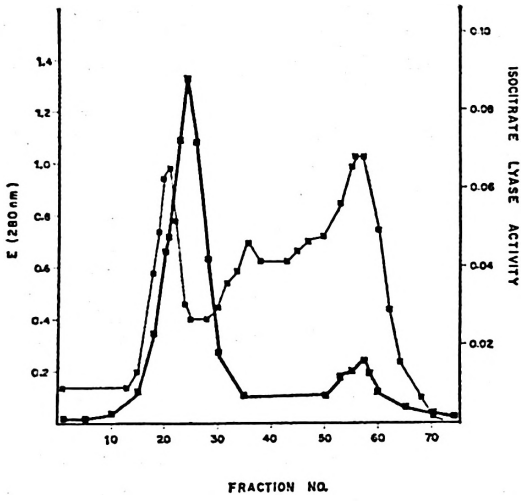

Fig. 2. *Sephadex G-200 gel filtration of glyoxysomal isocitrate lyase.* Protein (■—■), isocitrate lyase (■—■).

35° C during 5 minutes and then, after 10 minutes, 0.1 ml of 25 mM DL-Isocitrate was added, the reaction was stopped by addition of 0.5 ml of 30 % trichloroacetic acid and afterwards it was centrifuged. Enzymatic activity was measured by absorption at 324 nm.

Sephadex G-200 chromatography was carried out at 4° C on a column of 2.5 × 40 cm, eluated with 50 mM KCl, 50 mM Tris *p*H 7.6 with a flow of 10 ml/h, and fractions of 2 ml were collected.

Results and Discussion

K_m is slightly higher in the cytoplasmic (1.10±0.04 mM) than in the glyoxysomal (0.83±0.10 mM) extract. Both preparations were previously chromatographied on Sephadex G-25 and values are average of three determinations.

The electrophoretic mobilities are shown in figure 1. On cellulose polyacetate strips, the cytoplasmic activity has a relatively higher mobility than the glyoxysomal one. Similar results were found with soluble and glyoxysomal malate dehydrogenase by BREIDENBACH (3).

Sephadex G-200 gel filtration has proved very useful for partial separation. The glyoxysomal activity (fig. 2) shows mostly a faster eluating peak with a low $K_{av} = 0.05$, corresponding for globular proteins, to an apparent molecular weight of about 500,000. The cytoplasmic protein (fig. 3) shows a K_{av} of 0.7 with an apparent molecular weight of 25.000, according to

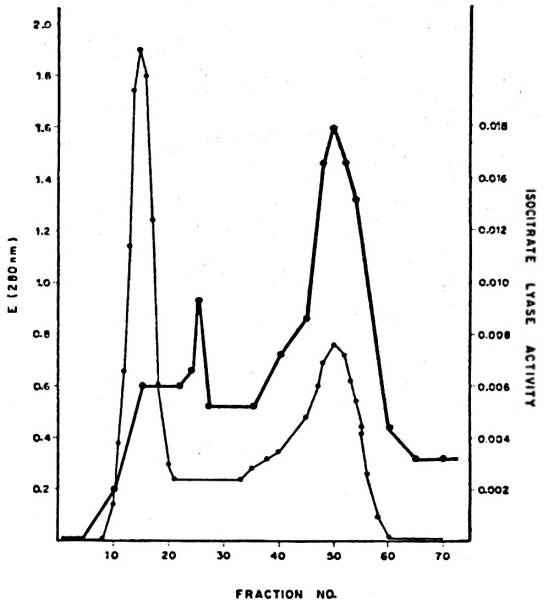

Fig. 3. *Sephadex G-200 gel filtration of cytoplasmic isocitrate lyase.*
Protein (●—●), isocitrate lyase (●▬●).

Table I. *Ultracentrifugation of glyoxysomal isocitrate lyase.*
Ultracentrifugation was carried out at 100,00×g for one hour at 2° C.

Fraction	Volume	Enzymatic activity	% of total activity
Upper lipidic layer	1.4	0.60	27
Supernatant	2.8	0.70	66
Pellet	0.7	0.29	7

Table II. *Inhibition of Isocitrate lyase activities by cloramphenicol and cycloheximide.* Enzymatic activity is expressed by the increase of optical density per minute per 25 microliters of enzyme.

Fraction	Control	Chloramphenicol	Cycloheximide
15,000×g supernatant activity	0.42	0.39	0.27
15,000×g pellet activity	0.27	0.25	0.13

ANDREWS (1), contaminated with the glyoxysomal fraction.

It can be concluded from these data that the glyoxysomal isocitrate lyase is indeed different in some properties from the soluble enzyme. This could be explained considering that the glyoxysomal enzyme forms some kind of complex or aggregate although it is not bound to any membrane particle as shown by the fact that almost no isocitrate lyase activity is pelleted al 100,000 × g in the glyoxysomal preparation (table I). Similar results have been shown with malate dehydrogenase (8) and citrate synthetase (2) isoenzymes, found in the soluble, glyoxysomal and mitochondrial fractions of corn scutella and castor bean endosperm respectively.

Treatment with 0.5 mM cycloheximide from the end of the stratification till the radicle appears, inhibits 60 % the production of the 15,000 × g sedimentating isocitrate lyase (9) and a 30 % the soluble enzyme (table II). A much lesser effect is found with 0.5 mM chloramphenicol. This suggests that both enzymes have the same subcellular origin, synthesized by cycloheximide-sensitive cytoplasmic ribosomes and not by the chloramphemicol sensitive protein biosynthetic system reported by CHING (5) in glyoxysomes.

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Resumen

Se describen algunas características de la isocitrato liasa citoplásmica y glioxisómica obtenidas de semillas estratificadas y germinadas de *Pinus pinea*, respectivamente.

Ambas muestran una diferente movilidad electroforética y aparecen en formas proteicas de diferentes pesos moleculares, según se comprueba por gel filtración en Sephadex G-200.

La cicloheximida inhibe el incremento de ambas actividades durante los primeros días de la germinación de la semilla, si bien el cloranfenicol ejerce una acción poco significativa.

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