Two-step hydrolysis of amygdalin in molds

Rivista di biologia-biology forum

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Two-Step Hydrolysis of Amygdalin in Molds

Abstract. Mucor circinelloides *LU M40 and* Penicillium aurantiogriseum *P 35, characterized by extracellular ß-glucosidase activity on cyanogenic glycosides, hydrolyse amygdalin by a two-step reaction mechanism being the first step of hydrolysis, from amygdalin to prunasin, very rapid (15 min) and the second one, from prunasin to mandelonitrile, much slower (120 min).*

Dear Editor,

Beta-glycosidases of plant origin are known which catalyze the breakdown of cyanogenic ß-bis-glycosides (disaccharide glycosides) by means of ß-bis-glycosidases responsible for a simultaneous mechanism of hydrolysis (Fig. 1a), while in other plants the hydrolysis is carried out by the concerted action of two enzymes, with a typical sequential mechanism (Fig. 1b) (Haisman & Knight [1967]; Kasai et al. [1981], Kuroki et al. [1984]; Fan & Conn [1985]; Guo et al. [1995]). To the best of our knowledge, however, nothing has been reported concerning the hydrolysis of cyanogenic ß-bis-glycosides by microorganisms/microbial enzymes.

The present note deals with the mechanism of hydrolysis of the bis-beta-glucoside (gentiobioside) amygdalin by the ß-glycosidase activity of two fungal organisms, *Mucor circinelloides* LU M40 and *Penicillium aurantiogriseum* P 35, previously selected for their ability to

Rivista di Biologia / Biology Forum 89 (1996), pp. 493-496.

degrade amygdalin and/or linamarin (Brimer et al. [1993]; Brimer et al. [1994]).

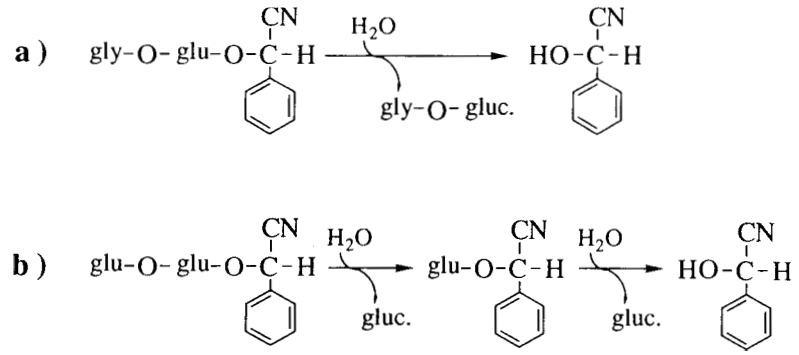

Fig. 1 – Cleavage of cyanogenic glycosides: 1a. Cleavage by a ß-bis-glycosidase (e.g. cleavage of vicianin in *Davallia* species); 1b. Cleavage of amygdalin by the concerted action of two hydrolytic enzymes. Glu = glucose moiety; gluc = glucose; gly = a monosaccharide moiety.

Isolates were grown as previously reported (Brimer et al. [1994]) and the culture supernatants used as enzyme solutions for TLC analysis. One ml of culture broth (ca. 32 mU of enzyme activity) was incubated with 1 ml of a solution of 2 mM amygdalin in 6.6 mM phosphate buffer, pH 6.0, at 50°C. Samples of 50 µl were taken at different incubation times (5, 15, 30, 60, 90, 120 and 240 min) and the reaction stopped by the addition of 20 µl of 0.4 N H_2SO_4. Analyses were performed by thin layer chromatography (TLC) by applying 20 µl of sample on aluminium sheets (20 x 20 cm) silica gel 60 F_{254} (Merk, 64271 Darmstadt, Germany); the adsorbent layer thickness was 0.2 mm. Standards (4 µmol) were amygdalin, prunasin (D-mandelonitrile ß-D-glucoside) and cyanohydrin (DL-mandelonitrile). The solvent system used consisted of ethyl acetate, acetone, chloroform, methanol and water in a ratio of 5 : 3.75 : 1.5 : 1.25 : 1. The development of the spots' reaction products was carried out by the procedure of Brimer et al. [1983].

TLC analysis of the reaction mixtures (culture supernatants plus amygdalin) showed that prunasin was formed during the degradation

of the ß-bis-glycoside irrespective of the enzyme source, *P. aurantiogriseum* or *M. circinelloides.* After 15 min of incubation, in fact, prunasin was already clearly detectable in the reaction mixture (Table 1). On the contrary, a much longer incubation time was necessary for the second step of hydrolysis, from prunasin to cyanohydrin. The latter compound, in fact, appeared only after 120 min of incubation (Table 1).

Table 1 – R_f values of amygdalin degradation products, and relative standards, by TLC analysis, as obtained after different incubation times (15, 60 and 120 min) with culture broth supernatants from *Mucor circinelloides* LU M40 and *Penicillium aurantiogriseum* P 35.

	R_f values[a]		
	Amygdalin	Prunasin	Cyanohydrin
Standards	0.30±0.02	0.59±0.01	0.90±0.03
M. circinelloides LU M40			
15 min	0.28±0.01 (++)[b]	0.59±0.01 (+)	ND[c]
60 min	0.28±0.01 (+)	0.59±0.00 (++)	ND
120 min	0.29±0.01 (+/-)	0.59±0.01 (+)	0.89±0.00 (+)
P. aurantiogriseum P35			
15 min	0.29+0.00 (++)	0.58±0.00 (+)	ND
60 min	0.29±0.01 (+)	0.58±0.01 (++)	ND
120 min	0.29±0.01(+/-)	0.57±0.01(+/-)	0.90±0.01 (+)

[a] Values represent means of two or more repetitions ± standard deviation.

[b] In parenthesis, color intensity of spots: +/-, very weak; + and ++, increasing intensity.

[c] ND, not detectable (spot not present).

These results clearly prove that the two fungal organisms herein studied produce enzymes that hydrolyze the disaccharide glycoside amygdalin by a two-step sequential mechanism; further investigations, however, are still needed to show whether the present findings are

representative of all fungi.

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Riassunto. *Mucor circinelloides* LU M40 e *Penicillium aurantiogriseum* P 35 sono microrganismi fungini filamentosi caratterizzati da attività ß-glucosidasica in grado di degradare glicosidi cianogenici di origine vegetale. Tra questi, l'amigdalina, ß-bis-glicoside cianogenico (gentiobioside), viene idrolizzata attraverso un meccanismo a due *step* con il primo *step,* da amigdalina a prunasina, molto rapido (ca. 15 min) ed il secondo, da prunasina a mandelonitrile, molto più lento (ca. 120 min). Ulteriori ricerche saranno necessarie per stabilire se le presenti osservazioni si possano estendere a tutti i funghi.