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PURIFICATION AND PROPERTIES OF ALLIIN LYASE FROM THE FUNGUS *PENICILLIUM CORYMBIFERUM*

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SUMMARY

Penicillium corymbiferum contains alliin lyase (cysteine sulfoxide lyase; alliin alkyl sulfenate-lyase, EC 4.4.1.4). This appears to be the first report of such an enzyme being isolated from a fungus. A 45-fold purification in two steps was effected by taking a 40–65% $(NH_4)_2SO_4$ cut from a mycelial homogenate and chromatographing it, after dialysis, on Sephadex G-200. The resulting preparation required pyridoxal phosphate, had an apparent K_m using alliin (*S*-allyl-L-cysteine sulfoxide) as a substrate of 5.8 mM, did not utilize *S*-alkylcysteines, and had a pH optimum of 6.5. These properties are similar to those of alliin lyase from garlic. It differed from this higher plant enzyme in that it had relatively little activity at pH 8.0, and was not stimulated by divalent ions or EDTA.

S-Alkyl-substituted cysteine sulfoxides occur as major constituents of the free amino acid pools of selected higher plants, principally species of Cruciferae and Liliaceae. Such plants also contain enzymes that degrade these compounds to the corresponding thiosulfinates, pyruvate and ammonia when the tissue is mechanically injured[1–3]. In at least one case it has been suggested that the resulting thiosulfinate may protect the plant against potential pathogens that enter through wounds[4].

Recently we have found that some fungi in this category, namely Penicillium spp., contain enzymes that act on *S*-alkylcysteine sulfoxides in the same manner as do the enzymes from higher plants. This appears to be the first report of their occurrence in fungi. Interestingly, such enzyme activity appears to be restricted to those fungi that attack members of the Cruciferae and Liliaceae. This paper reports the purification and some properties of one of these enzymes, (an alliin lyase cysteine sulfoxide lyase; alliin alkylsulfenate-lyase, EC 4.4.1.4) from *P. corymbiferum* Westling which causes bulb decay of garlic.

Enzyme activity was determined by using a 2,4-dinitrophenylhydrazine assay for pyruvic acid after differential extraction of pyruvate 2,4-dinitrophenylhydrazone (see ref. 5, Method B). The reaction mixture contained 50 mM alliin (*S*-allyl-L-cysteinesulfoxide), 20 μM pyridoxal 5'-phosphate, 0.05 M sodium phosphate buffer,

pH 6.5, containing 10% (v/v) glycerol, and enzyme in a total volume of 1 ml. A unit of enzyme activity is defined as the amount of enzyme that would catalyze the formation of 1 μmole of pyruvate per min at 24°. Protein concentration was determined by using a modification of Lowry's method[6].

Flasks containing a sucrose–mineral salts medium were inoculated with a conidiospore suspension of *P. corymbiferum*. Still cultures were then incubated for 6 days at 22°. After the mycelial mats were washed in cold 0.05 M phosphate buffer, pH 6.5, containing 10% glycerol, they were suspended in the same buffer (1:1, w/v) at 3° and ground in a Duall tissue homogenizer. The homogenate was centrifuged (12 000 × *g*, 20 min), then filtered through cheesecloth. The supernatant was treated with solid $(NH_4)_2SO_4$ without further pH control to give 45% saturation. After the suspension was stirred for an additional 30 min at 3°, it was centrifuged (20 000 × *g*, 30 min), and the supernatant treated with additional $(NH_4)_2SO_4$ to give a final concentration of 65%. After centrifugation (20 000 × *g*, 30 min), the pellet was dissolved in 0.05 M sodium phosphate buffer, pH 6.5, containing 10% glycerol and dialyzed for 5 h at 3° against the same buffer.

TABLE I

PURIFICATION OF ALLIIN LYASE FROM *P. corymbiferum*

Fraction	*Total vol. (ml)*	*Protein (mg/ml)*	*Total enzyme units*	*Specific activity (units/mg)*	*Recovery (%)*
(1) Crude extract	30	2.2	1.7	0.026	—
(2) 40–65% satd. $(NH_4)_2SO_4$	3.5	5.0	1.1	0.063	65
(3) Sephadex G-200	4.0	0.15	0.7	1.17	41

This preparation was applied to a column of Sephadex G-200 (1.5 cm × 32 cm) equilibrated with 0.05 M phosphate buffer, pH 6.5, containing 10% glycerol. Development was performed at 3° with the same buffer. 2-ml fractions were collected, and those containing the highest specific activity were pooled and used for studies on the enzyme's properties. These two purification steps effected a 45-fold purification with a 41% yield (Table I).

The resulting preparation would stoichimetrically convert alliin to pyruvate, ammonia and a substance having the same characteristic absorption spectra as allicin (*S*-oxodiallyl disulfide) after reaction with *N*-ethyl maleimide. A comparison of the 2,4-dinitrophenylhydrazone derivative obtained from the reaction with authentic pyruvate 2,4-dinitrophenylhydrazone showed that both had the same infrared absorption spectra, melting points (213°) and chromatographic mobilities on Whatman 1 filter paper in three solvent systems (*n*-butanol–ethanol–0.5 M NH_4OH, (7:1:2, by vol.); *n*-butanol–1.0 M $NaHCO_3$ (1:2, by vol.); *n*-butanol–ethanol–water, (5:1:4, by vol.).

Most of the properties of the partially purified fungal alliin lyase were quite similar to those reported for the same enzyme from garlic[2]. For example: (a) its K_m, using alliin as a substrate, was 5.8 mM; (b) pyridoxal phosphate was an absolute requirement for activity; (c) glycerol significantly increased enzyme stability; and

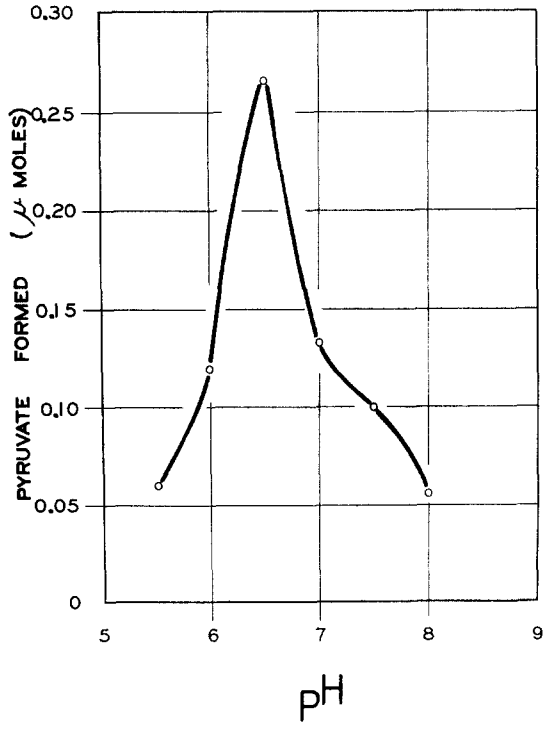

Fig. 1. Effect of pH using 0.05 M sodium phosphate buffers on the reaction rate.

(d) the enzyme did not utilize *S*-methyl- or *S*-ethyl-L-cysteines. However, although the fungal enzyme's pH optimum for activity was 6.5, the same as that for the enzyme from garlic, the activity curve of the former was much narrower in that relatively little activity could be demonstrated at pH 8.0 (Fig. 1). Other differences were that the activity of the fungal enzyme was not stimulated by divalent ions (Mg^{2+}, Mn^{2+} or Fe^{2+} at 5 mM) or by EDTA (10 mM).

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