KYNURENINASE FROM NEUROSPORA: OCCURRENCE OF TWO ACTIVITIES*

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Summary: The induced enzyme kynureninase from N. crassa has been found to occur in two forms. These forms differ in kinetic properties, and in reaction to the cofactor pyridoxal phosphate. Uninduced cells produce only one form of the enzyme.

The inducible enzyme kynureninase [L-kynurenine-hydrolase, EC 3.7.1.3] (1, 2) was first partially purified and characterized from Neurospora by Jakoby and Bonner (3). Evidence for the presence of kynureninase in Neurospora resulted from studies in which it was found that tryptophan gave rise to kynurenine which was further degraded to anthranilic acid (4, 5). In addition, the ability of tryptophan to serve as a source of nicotinic acid in this organism indicated (6) that two activities could be associated with kynureninase; the conversion of kynurenine to anthranilic acid, an excreted product, and the conversion of 3-OH-kynurenine to 3-OH-anthranilic acid, a nicotinic acid precursor. The results of Jakoby and Bonner (3) indicated that one protein was responsible for both activities since the activities were inseparable throughout a purification of approximately 100 fold. Evidence presented here shows that kynureninase from Neurospora can be separated into two chromatographically distinct proteins each of which retains both activities but has different kinetic properties with respect to kynurenine and 3-OH-kynurenine. They also differ in response to pyridoxal phosphate.

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Further, only one activity is present in non-induced cells whereas both activities are found after induction.

Methods and Materials: Wild-type Neurospora crassa strain 74A was used for preparation of kynureninase. Growth of this organism was described previously (7). Induction of kynureninase was carried out by incubating log phase cells for 3.5 hours after addition of tryptophan (150 mg/ℓ). The cells were harvested by filtration on cheesecloth and crude extracts prepared by grinding the cells in a mortar with sand and potassium phosphate buffer (0.1M, pH 7.8) containing ethylene diamine tetraacetic acid, disodium salt (EDTA, 0.002M).

Crude extract was brought to 50% saturation with $(NH_4)_2 SO_4$ and the resulting precipitate discarded. The supernatant was then brought to 70% saturation in $(NH_4)_2 SO_4$, and the supernatant discarded. The 50-70% precipitate was dissolved in .07M phosphate buffer, pH 7.8 containing 0.002M EDTA (phosphate buffer), and dialyzed against this buffer. After dialysis, the fraction was placed on a 3 x 25 cm diethyl-amino-ethyl cellulose (DEAE) column equilibrated with phosphate buffer, and eluted from the column with this same buffer. Peaks of activity were rechromatographed on a 1 x 30 cm DEAE column.

Kynureninase was assayed fluorometrically as previously described (2). One unit of kynureninase equals that amount of protein required to produce 1 μmole of anthranilic acid per hour at 37°. Protein was estimated by measurement of absorption at 280 nM and 260 nM (8) and by a modification of the Lowry procedure.

Results and Discussion: The elution profile from the first DEAE chromatogram showed two distinct peaks of activity (Fig. 1). The first peak eluting from the column we shall refer to as kynureninase one, (kyn I) the second eluting peak as kynureninase two (kyn II). Kyn I and II were rechromatographed on DEAE, resulting in an approximate doubling in specific activity. No interconversion between the two activities occurred; that is, kyn I from the first DEAE column did not

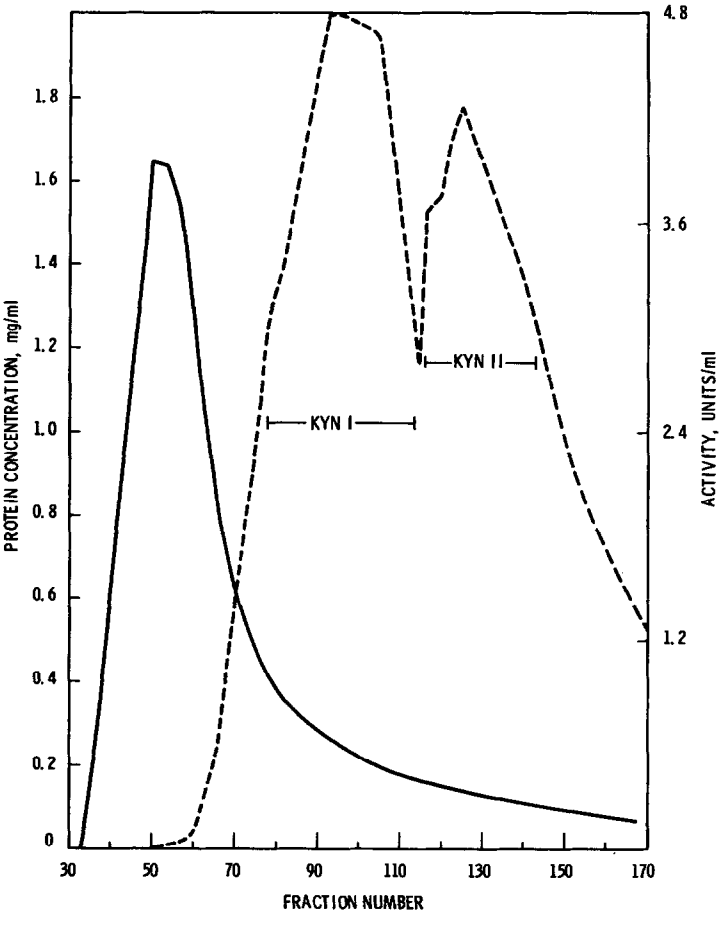

Fig. 1. DEAE chromatography, kynureninase from induced Neurospora (——), protein; (----), kynureninase activity.

give rise to an equilibrium mixture of kyn II and kyn I on the second column. The chromatography described here depends upon a retardation of enzyme activity on the resin and not on a tight adsorption to the DEAE. A typical purification (Table 1) results in a 57.5 fold increase in activity of kyn I with a recovery of 7.7% while kyn II was purified 100 fold with a recovery of 31%. The low yield of kyn I is not due to a smaller amount of this material but rather to a greater degree of contamination of enzyme with bulk protein (Fig. 1 and Fig. 3B).

The column fractions were initially assayed for activity with kynurenine as substrate. Since it had been previously shown that 3-OH-kynurenine could serve as substrate for this enzyme (3), kyn I and kyn

TABLE 1 Purification of Kynureninase

Fraction	Vol.	Units/ml	Units Total	Rec. % Units	Protein mg/ml	Protein Total	S.A.	Fold Purification
Crude ext.	412	2.70	1112	---	11.44	4713	.24	---
50-70 ppt	14.4	42	605	54	46.2	662	.906	3.78
1st DEAE:								
Kyn I	2.3	49.8	114	10.3	7.9	18.2	6.30	26.3
Kyn II	5.0	73.8	370	33.0	8.55	42.7	8.64	36.0
2nd DEAE:								
Kyn I	15	5.7	85.8	7.7	0.413	6.2	13.8	57.5
Kyn II	10	34.3	343.2	31.0	1.43	14.3	24.0	100

II were tested for the ability to utilize the hydroxylated substrate. Table 2 shows Km values obtained at two substrate concentrations for kyn I and kyn II with respect to kynurenine and 3-OH-kynurenine. The Km for kyn II with respect to kynurenine and 3-OH-kynurenine is the same at both substrate concentration ranges but is about 2-fold lower for 3-OH-kynurenine than for kynurenine. The values of Km for kynurenine show an approximate 2-4 fold difference between the two enzymes, with kyn I appearing to have a greater affinity for this substrate. The Km for kyn I with respect to kynurenine at either concentration range is the same. However, with respect to 3-OH-kynurenine the Km for kyn I at the high range is increased by about 5-fold, indicating an apparent decrease in substrate affinity at higher concentrations. This appeared at first sight to resemble a "negative cooperative effect" (9). However, we found that as the affinity decreases, the reaction velocity increases (Fig. 2, Table 2). If this is an example of allosteric alteration it is clear that changes in the binding sites of an enzyme for its substrate need not be accompanied by a commensurate change in the catalytic activity.

Pyridoxal phosphate has been shown to be a cofactor for kynureninase (3). Our results show that kyn I and kyn II differ in response when preincubated for 12 hr with 10^{-5}M pyridoxal phosphate and assayed on kynurenine. Kyn I was inhibited by 10% while kyn II showed an approximate

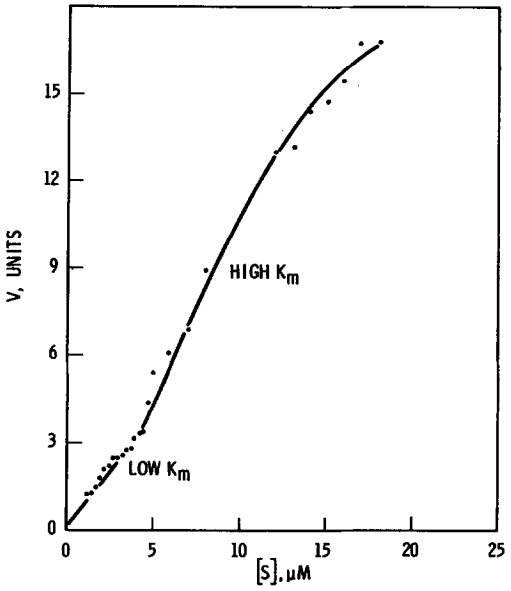

Fig. 2. Kynureninase I, reaction velocity vs concentration of 3-hydroxy-kynurenine.

TABLE 2 Kinetic constants for Kyn I and Kyn II

	Km Values, mole/l			
	Substrate Concentration			
	1-5 μM		5-20 μM	
Substrate:	Kyn I	Kyn II	Kyn I	Kyn II
3-OH-kynurenine	1.6×10^{-5}	2.6×10^{-5}	9.5×10^{-5}	2×10^{-5}
Kynurenine	1.5×10^{-5}	6.4×10^{-5}	2.3×10^{-5}	4×10^{-5}
	V_{max} values, μmoles/hr			
3-OH-kynurenine	.016	.031	.109	.045
kynurenine	.013	.008	.023	.040

2-fold increase in activity. A symptom of pyridoxal phosphate deficiency in mammals is the decreased ability to degrade tryptophan characterized by an increased excretion of kynurenine, OH-kynurenine, xanthurenic acid (10, 11) and other metabolites of tryptophan. This differential response of

kyn I and kyn II to pyridoxal phosphate may be a reflection of the regulatory system in Neurospora which promotes channelling of excess tryptophan to anthranilic acid rather than to nicotinic acid.

During induction of kynureninase in Neurospora the specific activity in crude cell extracts is increased by about 100 fold. Figure 1 shows the DEAE elution of activity from induced cells in which the ratio of activities, kyn I/kyn II, in the peak tubes is 1.2/1.0. Figure 3 (top panel) shows the profile of kynureninase from non-induced cells and Fig. 3 (bottom panel) shows purified kyn I on an identical DEAE column. Noninduced cells have kyn I at very low levels with no detectable kyn II. This suggests that induction results in the de novo production of kyn II and increased synthesis of kyn I. Induction of these two activities is therefore similar to a promoter response (kyn I) and an operator response (kyn II) when expressed in terms of the bacterial induction system.

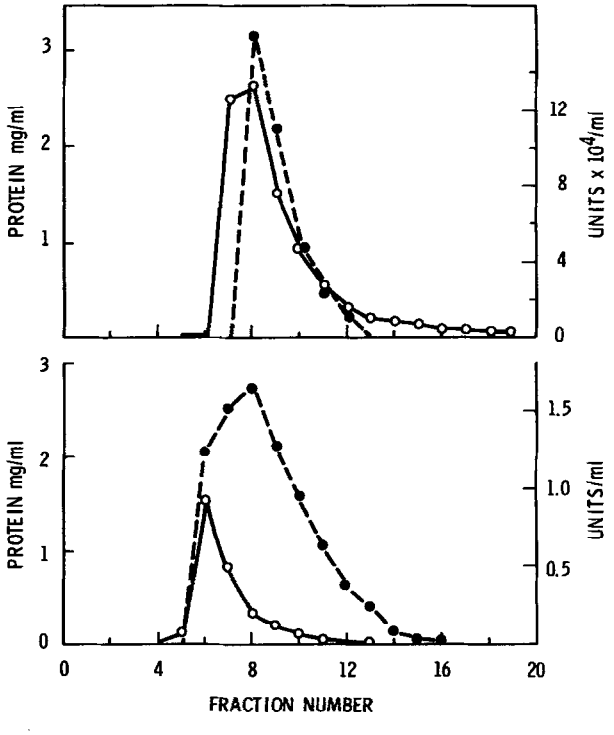

Fig. 3. DEAE chromatography, kynureninase. Top panel, uninduced wild-type, dialyzed 50-70% $(NH_4)_2SO_4$ precipitate (10 fold purified). Bottom panel, purified kyn I from induced wild-type (26 fold purified). (O—O), protein; (●—●), kynureninase activity.

In some respects, the kynureninase system might be compared to the mammalian lactic dehydrogenase system (12) in that two enzymes of different kinetic properties are involved in the degradation of a single substrate. The yeast cytochrome c system (13) may also be similar, as differential rates of synthesis of two forms of cytochrome c seem to play a role in respiratory control.

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