ADVANCES IN ENZYMOLOGY

AND RELATED AREAS OF MOLECULAR BIOLOGY

Founded by F. F. NORD

Edited by ALTON MEISTER

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KYNURENINASES: ENZYMOLOGICAL PROPERTIES AND REGULATION MECHANISM

By KENJI SODA and KATSUYUKI TANIZAWA, *Kyoto, Japan*

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I. Introduction

Kynurenine, β-anthraniloyl-α-aminopropionate was first discovered by Matsuoka and Yoshimatsu (1) in the urine of rabbits fed large quantities of tryptophan, and the structure was established by Butenandt et al. (2). Kynurenine is derived from tryptophan through formylkynurenine in ani-

mals, higher plants, and microorganisms (Fig. 1). Kotake and Nakayama (3) observed the conversion of kynurenine into L-alanine and anthranilate by a mammalian liver extract. The enzyme catalyzing the reaction was termed kynureninase by Braunstein et al. (4).

Kynureninase is a key enzyme of the kynurenine–niacin pathway in tryptophan metabolism and catalyzes a unique reaction, the hydrolytic β,γ-cleavage of aryl-substituted γ-keto-α-amino acids.

R = H (kynurenine) or OH (3-hydroxykynurenine)

The enzyme was partially purified from *Pseudomonas fluorescens* (5), *Neurospora crassa* (6,7), rat liver (8), and porcine liver (9), and was characterized enzymologically. Liver kynureninase splits 3-hydroxykynurenine about twice as rapidly as it splits kynurenine (8), while the pseudomonad enzyme hydrolyzes kynurenine five times as rapidly as it hydrolyzes 3-hydroxykynurenine (10). It has been shown that pyridoxal

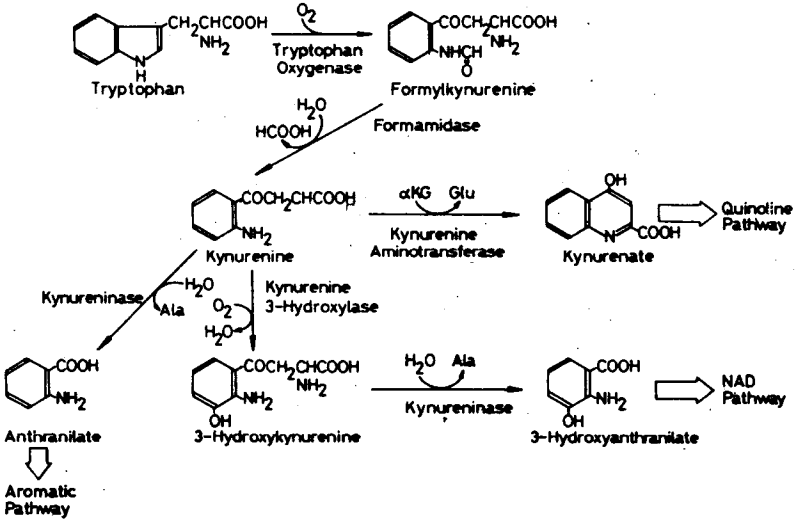

Fig. 1. Metabolic pathways of tryptophan via kynurenine. α-KG, α-ketoglutarate; Ala, L-alanine; Glu, L-glutamate.

5′-phosphate is required as a coenzyme for the enzyme (4,11). Two possible mechanisms were proposed for the enzymatic cleavage of kynurenine: a β-diketone pathway (12) and an α,β-elimination pathway through *o*-aminobenzaldehyde (13).

Recently ample evidence has been obtained for the occurrence of two distinct types of kynureninases, namely kynureninase, which is inducible by tryptophan and is involved in the catabolism of tryptophan, and 3-hydroxykynureninase, which is not inducible and functions primarily in the biosynthesis of NAD from tryptophan (14–16). This review attempts to summarize our current knowledge of these enzymes, with emphasis on the regulatory mechanism. Another two enzymes also are specifically concerned with kynurenine metabolism, namely, kynurenine aminotransferase and kynurenine 3-hydroxylase as shown in Figure 1. Descriptions of these enzymes are beyond the scope of this review.

II. Assay

Kynureninase is assayed by spectrophotometric determinations of the disappearance of kynurenine (3,5,17,18) and the formation of alanine (11), or by fluorometric determination of anthranilate produced (6,15,19,20).

A. SPECTROPHOTOMETRIC METHOD

The spectrophotometric method depends on the disappearance of the kynurenine absorption at 360–365 nm (3,5,17,18). This is the simplest and most convenient method, but it is not sensitive enough to be applicable to the assay of the *Neurospora* constitutive and mammalian kynureninases, whose activities are extremely low.

B. FLUOROMETRIC METHOD

The rate of enzymatic formation of anthranilate is followed continuously by measuring the rate of increase in intensity of fluorescence in a cuvette (e.g., excitation, 315 nm and emission, 393 nm) (6,19). Quenching of the fluorescence by kynurenine sometimes interferes with this method. A modified method is based on the separation of anthranilate from the substrate by extraction with ethyl acetate (20). Alternatively, assays are corrected fluorometrically for quenching of the fluorescent product by the substrate (15).

III. Distribution, Purification and General Properties of Kynureninases

A. BACTERIAL KYNURENINASE

Two distinct pathways of tryptophan catabolism were found in pseudomonads that can assimilate L-tryptophan (21). One group of the bacteria metabolizes L-tryptophan through L-kynurenine, anthranilic acid, catechol, and *cis,cis*-muconic acid to β-ketoadipic acid ("aromatic pathway"), and the other group metabolizes exclusively via kynurenic acid ("quinoline pathway") (Fig. 1). Hayaishi and Stanier (5,22) first demonstrated the occurrence of kynureninase in bacteria. The enzyme was purified 7-fold (5) or 100-fold (10) from tryptophan-adapted cells of *Pseudomonas fluorescens*, and the pH optimum and the K_m value for L-kynurenine were determined to be 8.5 and 3.9×10^{-5} M, respectively (5). The bacterial kynureninase was shown to have higher activity and affinity for L-kynurenine than for L-3-hydroxykynurenine, another physiological substrate in the tryptophan–NAD pathway. Recently Shetty and Gaertner (16) also purified the enzyme from *Pseudomonas fluorescens* about 50-fold by DEAE–cellulose column chromatography. The K_m values for L-kynurenine and L-3-hydroxykynurenine were calculated to be 7.14×10^{-5} and 5.00×10^{-4} M, respectively (16). The ratio of the rate of kynurenine hydrolysis to that of L-3-hydroxykynurenine hydrolysis was 4.7 : 1.0 (16). The cells grown in the absence of added tryptophan or kynurenine do not produce kynureninase (16,22). The pyridine moiety of NAD is synthesized from glycerol and L-aspartate in bacteria (23), although it is from L-tryptophan in fungi (24) and higher animals (23). These findings suggest that the bacterial enzyme functions catabolically in the aromatic pathway.

Although most bacterial strains except *Pseudomonas* were reported to lack the inducible enzymes of oxidative tryptophan catabolism (25), Prasad and Srinivasan (26) found that a sporulating culture of *Bacillus cereus* accumulates kynurenine and anthranilic acid. Kynureninase activity reaches a maximum during the early stages of sporulation and then declines. Since the addition of tryptophan to the medium did not significantly alter the enzyme activities of tryptophan catabolism, Prasad and Srinivasan concluded that the induction of tryptophan catabolism occurs concomitantly with the differentiation of the organism after completion of the vegetative growth (26).

The conversion of tryptophan into kynurenine and anthranilate by tryptophan-adapted cells of *Bacillus megaterium* (27,28) also indicates the existence of the inducible kynureninase, but the detailed enzymological study has not yet been performed. *Acinetobacter calcoaceticus* also produces the inducible kynureninase (29).

Xanthomonas pruni is the only anomalous bacterium that possesses the ability to convert tryptophan to NAD (23,30,31). Brown and Wagner (32) showed that the first three enzymes of tryptophan degradation in *Xanthomonas pruni* are induced coordinately by L-tryptophan and that kynureninase is not formed in the absence of L-tryptophan in the medium. The extract of *Xanthomonas pruni* catalyzes the hydrolysis of L-kynurenine 40% more efficiently than it does the hydrolysis of L-3-hydroxykynurenine (32). Gaertner and Shetty (33) reported that kynureninase of *Xanthomonas pruni* shows the same activity on L-kynurenine and L-3-hydroxykynurenine, but the K_m value for L-kynurenine (1.8×10^{-5} *M*) is much smaller than that for L-3-hydroxykynurenine (2.2×10^{-4} *M*).

The detailed physicochemical and enzymological properties of bacterial kynureninase have been shown by Moriguchi et al. (18,34), who purified the enzyme about 130-fold from *Pseudomonas marginalis* and crystallized it. The crystalline enzyme was shown to be homogeneous by the criteria of ultracentrifugation and disk gel electrophoresis (18,34). The molecular weight, estimated by sedimentation equilibrium, is about 100,000 (34). The Sephadex G-150 gel filtration method gave a value of approximately 92,000 (34). The sedimentation coefficient ($S^0_{20,w}$) is 5.87 S. Treatment with sodium dodecyl sulfate causes an irreversible dissociation of the enzyme into subunits having a molecular weight of about 46,000 (35). The amino-terminal amino acid was determined to be methionine by the dansylation method, and nearly 2 moles of methionine were determined per mole of enzyme by Sanger's DNP method (35), suggesting that the enzyme is composed of the two identical subunits.

The absorption spectrum of the purified enzyme has maxima at 280, 337, and 430 nm. No appreciable spectral change is observed on varying the pH between 5.4 and 9.0 (34). The enzyme shows fluorescence upon excitation at 337 and 430 nm, and the emission maxima are observed at 380 and 480 nm, respectively. The characteristic spectrum, and also isolation of ϵ-*N*-pyridoxyllysine from the hydrolysate of borohydride-reduced enzyme (34), show that pyridoxal 5′-phosphate is bound to an ϵ-

amino group of lysine residue of the protein through an aldimine linkage. One mole of pyridoxal 5′-phosphate is bound per mole of enzyme, that is two subunits. As described in Section III.B.1, the fungal inducible enzyme also contains 1 mole of the bound pyridoxal 5′-phosphate for every two identical subunits. Although the mode of binding of both the subunits and the function of the subunit containing no pyridoxal 5′-phosphate are not known at present, kynureninase may have "half of the sites' reactivity" with respect to the cofactor.

The purified enzyme of *Pseudomonas marginalis* has an optimum reactivity at about pH 8.0 and catalyzes most preferentially the hydrolysis of L-kynurenine. L-3-Hydroxykynurenine and *N*′-formyl-L-kynurenine are hydrolyzed with the relative rates of 19 and 15% of that of L-kynurenine, respectively (36). The K_m values were determined to be 3.5×10^{-5} *M* for L-kynurenine, 2.0×10^{-4} *M* for L-3-hydroxykynurenine, 2.2×10^{-3} *M* for *N*′-formyl-L-kynurenine, and 2.3×10^{-7} *M* for pyridoxal 5′-phosphate (34,35).

Kynureninase is strongly inhibited by hydroxylamine and phenylhydrazine and is resolved to the apo-form by treatment with these compounds followed by dialysis (34). The apoenzyme, which lacks absorption peaks at 337 and 430 nm, is also prepared by incubation with L-ornithine or L-alanine (37). Resolution of the enzyme with L-ornithine or L-alanine is interpreted as the result of formation of pyridoxamine 5′-phosphate by transamination as described below (Section IV).

B. FUNGAL KYNURENINASE

1. Inducible Enzyme

Earlier observations that mycelial pads of *Neurospora crassa* convert tryptophan to kynurenine and anthranilic acid (38) and that kynurenine is also oxidized to 3-hydroxyanthranilic acid through 3-hydroxykynurenine (39) led to the initial studies on fungal kynureninase. Jakoby and Bonner (6) showed that kynureninase activity of *Neurospora crassa* was increased about 600-fold by the addition of L-tryptophan to the growth medium and that the enzyme purified 70- to 100-fold catalyzed the conversion of L-kynurenine, L-3-hydroxykynurenine, and *N*′-formyl-L-kynurenine into alanine and anthranilic acid, 3-hydroxyanthranilic acid, and formylanthranilic acid, respectively. D-Kynurenine and N^{α}-acetyl-L-kynurenine were inactive as substrates (6). Jakoby and Bonner concluded that a single enzyme is responsible for both the biodegradative

aromatic pathway of tryptophan metabolism and the biosynthetic NAD pathway, since the ratio of the activities for kynurenine and 3-hydroxykynurenine was constant (about 1.74) throughout the course of purification (6). The K_m values were 6×10^{-6} *M* for L-kynurenine and 3×10^{-6} *M* for L-3-hydroxykynurenine, and the concentration of pyridoxal 5′-phosphate for half maximal activation of the enzyme was 6×10^{-7} *M*. Magnesium ions activated the enzyme from 15 to 45%. Although Saran (40) also reported that Mn^{2+} and Ca^{2+} ions activated the enzyme, such activations have not been verified by recent works (15,36).

The mechanism of kynureninase induction (19,41) and the role of the enzyme in biosynthesis and degradation of tryptophan (42,43) in *Neurospora crassa* also have been investigated in some detail. We recently purified the *Neurospora crassa* kynureninase to homogeneity by several steps, including affinity chromatography with kynurenine-bound Sepharose 4B, and crystallized it (36). The enzyme ($S_{20,w}$ = 6.55 S) has a molecular weight of about 105,000 and is composed of two identical subunits (M_r 50,000) with valine as the amino-terminal amino acid residue (44). The enzyme exhibits absorption maxima at 280 and 430 nm and emits fluorescent light with the maxima at 338 and 495 nm, respectively, upon excitation at the wavelength of the absorption maxima. As stated above the fungal kynureninase also contains 1 mole of pyridoxal 5′-phosphate per mole of enzyme. It is released from the enzyme by the treatment with hydroxylamine or by transamination of the coenzyme moiety with L-ornithine (44) (see Section IV).

Although the bacterial kynureninase catalyzes the hydrolysis of L-kynurenine, almost exclusively, the *Neurospora crassa* enzyme acts on both L-kynurenine and L-3-hydroxykynurenine (36). L-3-Hydroxykynurenine is the best substrate for the fungal enzyme with a V_{max} value of 5.6 μmole/min mg of protein, and the value for L-kynurenine is 2.7 μmole/min mg of protein (44). The K_m values were calculated to be 1.8×10^{-6} *M* for L-kynurenine, 3.5×10^{-5} *M* for L-3-hydroxykynurenine, and 1.4×10^{-7} *M* for pyridoxal 5′-phosphate (36).

The inducible kynureninase of *Neurospora crassa* is strongly inhibited by thiol reagents such as $HgCl_2$ and *p*-chloromercuribenzoic acid (36), as shown for the bacterial enzyme (34). Saran (40) studied the inhibition of *Neurospora crassa* kynureninase by *p*-chloromercuribenzoic acid to determine the presence of functional thiol groups in kynureninase. The direct involvement of thiol groups in the kynureninase reaction, however, has not yet been proved.

2. *Constitutive Enzyme*

It was long believed that a single inducible kynureninase functions in the pathways of both tryptophan degradation and NAD biosynthesis in *Neurospora crassa:* the enzyme acts on both L-kynurenine and L-3-hydroxykynurenine (6). Gaertner et al. (15) have demonstrated the occurrence of two types of kynureninases in *Neurospora crassa,* which are separated by DEAE–cellulose chromatography. A kynureninase, preferentially catalyzing hydrolysis of kynurenine (K_m is 6.7×10^{-5} *M* for L-kynurenine and 2.5×10^{-4} *M* for L-3-hydroxykynurenine) is induced over 400-fold by tryptophan, whereas the other kynureninase, termed 3-hydroxykynureninase is a constitutive enzyme and predominantly catalyzes hydrolysis of L-3-hydroxykynurenine (K_m is 2.5×10^{-4} *M* for L-kynurenine and 5×10^{-6} *M* for L-3-hydroxykynurenine) (15). Turner and Drucker (14) also showed that the tryptophan-induced mycelium of *Neurospora crassa* have two distinct kynureninases (kynureninases I and II). The enzymes differ in their kinetic properties and in their behaviors with pyridoxal 5′-phosphate (14). Schlitt et al. (20) described the different responses of the two kynureninases to mutagenic treatment and confirmed the existence of constitutive kynureninase.

We also observed (44) the very low but definite activity of constitutive kynureninase in *Neurospora crassa* grown in the defined minimal medium of Vogel (45). This constitutive enzyme is eluted from DEAE–cellulose with 0.15–0.2 *M* potassium phosphate buffer (pH 7.2), while the inducible kynureninase, which is inducibly formed by L-tryptophan, *N*′-formyl-L-kynurenine, or L-kynurenine, is eluted with 0.065–0.1 *M* buffer (44). The immunological relation between the constitutive enzyme and the inducible one was investigated by an Ouchterlony double-diffusion analysis. As shown in Figure 2*A*, no precipitin band formed between the anti-inducible kynureninase antiserum and the constitutive enzyme under the conditions of varying the ratio of the amount of the enzyme to that of the antiserum (44). The constitutive enzyme was neither inhibited nor precipitated when titrated with the anti-inducible kynureninase antiserum, showing that the kynureninases are distinct from each other. Therefore, the constitutive kynureninases can be specifically assayed in the presence of the inducible enzyme by precipitation of the inducible one with the antiserum. This specific assay method allowed us to show that the constitutive enzyme is formed almost independently of the presence of L-tryptophan in the growth medium and

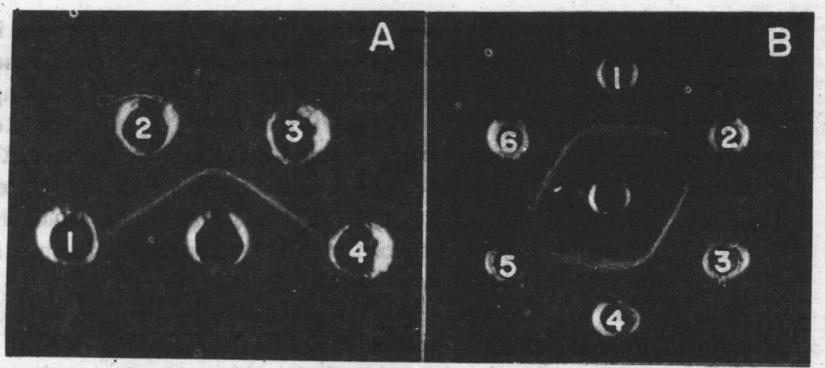

Fig. 2. Ouchterlony double-diffusion analyses of fungal kynureninases (44). (*A*) Center well, antiserum against inducible kynureninase of *Neurospora crassa* (IFO 6068): well *1*, cell extract of *Neurospora crassa* (IFO 6068) grown in the absence of tryptophan; well *2*, cell extract of *Neurospora crassa* (IFO 6068) grown in the presence of 0.1% tryptophan; well *3*, crystalline inducible kynureninase of *Neurospora crassa* (IFO 6068); well *4*, purified constitutive kynureninase of *Neurospora crassa* (IFO 6068). (*B*) Center well, antiserum against inducible kynureninase of *Neurospora crassa* (IFO 6068); wells *1* and *4*, inducible kynureninase of *Neurospora crassa* (IFO 6068); wells *2*, *3*, *5*, and *6*, tryptophan-induced cell extracts of *Neurospora sitophila* (ICR 3551), *Neurospora sitophila* (IFO 6070), *Neurospora tetrasperma* (IFO 8650), and *Neurospora crassa* (IFO 6979), respectively.

that the inducible enzyme activity is increased over 500-fold by addition of 0.04% L-tryptophan (Fig. 3) (44).

The constitutive enzyme has been purified approximately 650-fold, though the specific activity was one-tenth that of the crystalline inducible enzyme, and was shown to be free of the inducible enzyme activity upon analytical disk gel electrophoresis and isoelectric electrophoresis (44). The molecular weight (about 110,000 by the Sephadex G-200 gel filtration method) and the optimum pH (pH 8.5) of the constitutive enzyme are similar to those of the inducible one. However, they differ in substrate specificity: the ratio of the reactivity of L-3-hydroxykynurenine to that of L-kynurenine is 3.16 for the constitutive enzyme and 1.92 for the inducible one, and K_m values of the constitutive enzyme are 3.4×10^{-5} *M* for L-kynurenine and 3.7×10^{-6} *M* for L-3-hydroxykynurenine. On the basis of the kinetic characteristics of the constitutive enzyme, the enzyme was also designated hydroxykynureninase by Gaertner et al. (15) and is thought to function biosynthetically in the tryptophan–NAD pathway.

To substantiate the hypothesis concerning with the differences in physiological functions between inducible and constitutive kynureninases (see Section VI), Shetty and Gaertner (16) studied the enzyme from fungi other than *Neurospora crassa*. *Rhizopus stolonifer*, a phycomycetes fungus, was found to contain only a constitutive enzyme, whose K_m values for L-3-hydroxykynurenine and L-kynurenine are 6.67×10^{-6} and 2.5×10^{-4} *M*, respectively. *Aspergillus niger* and *Penicillium roqueforti* belonging to fungi imperfecti produce both the inducible kynureninase (K_m for L-3-hydroxykynurenine and L-kynurenine is 5.9×10^{-5} to 14.3×10^{-5} *M*) and the constitutive enzyme ($K_m = 4 \times 10^{-6}$ *M* for L-3-hydroxykynurenine and $K_m \doteq 10^{-4}$ *M* for L-kynurenine).

NAD is synthesized in fungi through the aerobic tryptophan pathway. In all the strains tested the constitutive kynureninase activity has been found (Table I). Three species of *Mucor* produce relatively high enzyme activity. Besides *Neurospora*, *Aspergilus*, and *Penicillium* fungi, which are known to produce the inducible enzyme as described above, strains of *Fusarium* and *Gibberella* also possess two types of kynureninases. The ratios of the inducible enzyme activity to the constitutive one (*I*/*C*) also are given in Table I. All the strains of *Mucor* and *Rhizopus* tested show small values, indicating that little inducible kynureninase is produced in them. Although *Neurospora crassa* has been known to produce kynureninase inducibly with L-tryptophan since the early study (6), and

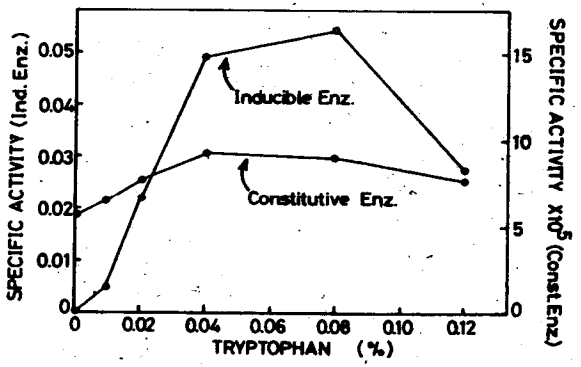

Fig. 3. Effect of tryptophan added in the medium on kynureninase activities (44). The organism was grown in the minimal medium containing indicated concentrations of L-tryptophan. The constitutive enzyme activity was assayed after precipitation of the inducible one with the antiserum.

TABLE I
Distribution of Constitutive and Inducible Kynureninases of Fungi (44)

Strain	Specific activity (units × 10^3/mg of protein)		
	Constitutive enzyme (C)[a]	Inducible enzyme (I)[b]	I/C
Aspergillus niger ICR 3303	0.068	5.61	83
Aspergillus niger IFO 4318	0.132	18.3	139
Aspergillus oryzae IFO 4181	0.248	48.8	197
Aspergillus wentii IFO 4107	0.162	4.31	27
Fusarium oxysporum IFO 5942	0.112	17.5	156
Gibberella fujikuroi IFO 6356	0.146	12.5	86
Mucor ambiguus IFO 6742	0.509	0.109	0
Mucor javanicus IFO 4569	1.22	0[c]	0
Mucor fragilis IFO 6449	0.771	0.42	1
Neurospora crassa IFO 6067	0.140	0.372	3
Neurospora crassa IFO 6068	0.286	42.3	148
Neurospora crassa IFO 6660	0.059	0.321	5
Neurospora crassa IFO 6979	0.134	26.6	199
Neurospora sitophila ICR 3551	0.669	102	152
Neurospora sitophila IFO 6070	0.596	32.1	54
Neurospora tetrasperma IFO 8650	0.160	39.4	246
Penicillium notatum IFO 4640	0.803	57.4	71
Penicillium purpurogenus ICR 3402	0.256	56.4	220
Penicillium urticae IFO 7011	0.084	16.0	190
Rhizopus javanicus IFO 5442	0.223	0.020	0
Rhizopus oryzae IFO 4706	0.491	0[c]	0

[a] Activity of extracts of the fungal mycelia grown in Vogel minimal medium (45) containing 0.03% yeast extract and 2% sucrose.

[b] The activity of mycelia grown in the medium supplemented with 0.1% *L*-tryptophan minus the constitutive enzyme activity.

[c] 0 indicates that the enzyme activities of tryptophan-induced mycelia were lower than those of noninduced mycelia.

this is the case for *Neurospora crassa* IFO 6068 and 6979, two other strains tested (IFO 6067 and 6660) have only low inducibility. The enzymes from the mycelia of both the strains grown in the presence of tryptophan do not react with the antiserum of the *Neurospora crassa* (IFO 6068) inducible enzyme, which is identical immunologically with the inducible enzymes from *Neurospora crassa* IFO 6979, *Neurospora sitophila* IFO 6070, *Neurospora sitophila* ICR 3551, and *Neurospora*

tetrasperma IFO 8650 (Fig. 2*B*). Thus the *Neurospora crassa* strains of IFO 6067 and 6660 produce only the constitutive-type enzyme.

Guerdoux (46) showed that *Coprinus radiatus,* a strain of basidiomycetes, produces the inducible kynureninase, but detailed studies have not been published.

C. YEAST KYNURENINASE

The nicotinic acid moiety of NAD originates from L-tryptophan in a yeast, *Saccharomyces cerevisiae,* under aerobic conditions as found in fungal and mammalian systems, but from aspartate and glutamate under anaerobic conditions through a pathway similar to that proposed for several bacteria,(24). Quinolinic acid is the common intermediate in both biosynthetic pathways of NAD in yeasts. Cells of *Saccharomyces cerevisiae* grown in the L-tryptophan medium slowly excrete 3-hydroxyanthranilate but do not anthranilate and do not rapidly deplete L-tryptophan in the medium (47), suggesting that the yeast lacks catabolically functioning kynureninase. Moriguchi et al. (34) also reported that no appreciable activity of kynureninase was observed in the following strains of yeast when they were assayed spectrophotometrically; *Saccharomyces cerevisiae, Saccharomyces fragilis, Saccharomyces marxianus, Schizosaccharomyces liquefaciens, Schwanniomyces occidentalis, Candida utilis, Cryptococcus albidus, Debaryomyces globosus, Debaryomyces hansenii, Endomyces hordei, Hansenula wingei, Hansenula anomala, Hansenula beijerinckii, Hansenula jadinii, Hansenula matritensis,* and *Pichia polymorpha.*

Shetty and Gaertner (47) demonstrated a constitutive kynureninase from *Saccharomyces cerevisiae* and purified it about 75-fold. Kinetic analysis (K_m = 6.7 × 10^{-6} *M* for L-3-hydroxykynurenine and K_m = 5.4 × 10^{-4} *M* for L-kynurenine) shows that the enzyme functions biosynthetically as the constitutive enzyme from *Neurospora crassa.*

Recently the kynureninase of *Saccharomyces cerevisiae* was purifed to near homogeneity (48). The enzyme shows characteristics of 3-hydroxykynureninase; K_m for L-3-hydroxykynurenine and L-kynurenine, 3 × 10^{-6} and 8 × 10^{-5} *M*, respectively; V_{max} (L-3-hydroxykynurenine)/V_{max}(L-kynurenine) = 6. The isoelectric point and the molecular weight were determined to be pH 4.3 and 67,000, respectively.

D. MAMMALIAN KYNURENINASE

Kynureninase of mammalian liver was demonstrated by Kotake and Nakayama (3) and was purified partially and characterized by Wiss

(11,49). Braunstein et al. (4) showed that the kynureninase activity is decreased by pyridoxine deficiency and that the enzyme requires pyridoxal 5′-phosphate as a coenzyme. Several other workers also studied the effect of pyridoxine deficiency on tryptophan metabolism, particularly kynureninase activity (8,50,51). The liver kynureninase activity is not affected by intraperitoneal administration of kynurenine and tryptophan (8), although tryptophan oxygenase is formed inducibly by adrenal cortical hormones or L-tryptophan (52–54).

All livers of mammals tested, for example dog, mouse, guinea pig, beef, and human, contain relatively high kynureninase activity, while the activity of kidneys is low (17), and those of spleen, lung, brain, heart, and muscle are negligibly small (55). The livers of other vertebrates, that is, birds, reptiles, amphibia, and fishes, also show kynureninase activity (33).

Recently we purified the enzyme to homogeneity from hog liver to elucidate the enzymological properties, which are presented with the properties of some other mammalian kynureninase preparations in Table II. Although some preparations have been described to be homogeneous or nearly homogeneous, their activities are much lower than that of our homogeneous preparation. The liver enzymes can be regarded as 3-hydroxykynureninase on the basis of a much higher reactivity of L-3-hydroxykynurenine than of L-kynurenine. The enzymes hydrolyze L-3-hydroxykynurenine 2 to 10 times as rapidly as L-kynurenine, and the affinity for L-3-hydroxykynurenine is about 100 times as high as that of L-kynurenine.

IV. Regulation Mechanism

In the initial studies (6,7,62) on the kynureninase of *Neurospora crassa* it was found that this enzyme is markedly inhibited by various amino acids and amines (dihydroxyphenylalanine, lysine, ornithine, cadaverine, and putrescine are highly inhibitory) and that pyridoxal 5′-phosphate probably counteracts the inhibition. Although it was suggested that the inhibition was due to the unavailability of the coenzyme as a result of formation of a Schiff base between the coenzyme and amino acids or amines added, the differences of the amino acids and amines in inhibitory effects were not explained, and no experimental data were obtained to support the proposed mechanism. Meister (63) suggested from the standpoint of reaction mechanism that the inhibition of kynureninase of *Neurospora* may be analogous to that of aspartate β-