JOURNAL OF BACTERIOLOGY, Nov. 1971, p. 902-909
Vol. 108, No. 2
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Evidence for Distinct Kynureninase and Hydroxykynureninase Activities in *Neurospora crassa*

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Received for publication 12 July 1971

Previous studies have indicated that a single enzyme, "kynureninase," catalyzes the reactions of L-kynurenine to anthranilate and L-3-hydroxykynurenine to 3-hydroxyanthranilate in *Neurospora crassa* and in other organisms. The present report describes separate enzymes which catalyze these reactions in *N. crassa*. The first, a *kynureninase*, preferentially catalyzes kynurenine to anthranilate and is induced over 400-fold by tryptophan or a catabolite of tryptophan. The second, a *hydroxykynureninase*, is constitutive or noninducible by tryptophan and preferentially catalyzes L-3-hydroxykynurenine to 3-hydroxyanthranilate. The physiological significance of these enzymes may be inferred from the facts that (i) the noninducible enzyme hydroxykynureninase appears to be the main enzyme present in uninduced cells that is capable of catalyzing L-3-hydroxykynurenine to 3-hydroxyanthranilate for the indispensible synthesis of nicotinamide adenine dinucleotide, and (ii) the inducible enzyme kynureninase is induced by tryptophan to a concentration far in excess of that needed to meet the requirements of the cells for nicotinamide adenine dinucleotide, resulting in the excretion of anthranilate into the medium.

"Kynureninase" (L-kynurenine-hydrolase, EC 3.7.1.3) has been recognized for many years as an enzyme which catalyzes in vitro the conversions of L-kynurenine to anthranilate and of L-3-hydroxykynurenine to 3-hydroxyanthranilate (7). Although it is usually assumed that a physiologically important reaction catalyzed by kynureninase involves L-3-hydroxykynurenine as the substrate, it is known that in *Neurospora crassa* the reaction from L-kynurenine is of importance in the anthranilate-tryptophan cycle (5, 9). Recently, Turner and Drucker (13) gave evidence that two forms of tryptophan-inducible kynureninase exist in *N. crassa*. The two enzymes were partially separated by ion-exchange chromatography and appeared to differ kinetically as well as in the level of induction by tryptophan, but a distinct physiological function for the two enzymes was not made apparent.

We also have observed two peaks of kynureninase activity after ion-exchange chromatography of *N. crassa* extracts. However, in contrast to the enzymes reported by Turner and Drucker, the two enzymes reported here are widely separated on diethylaminoethyl (DEAE) cellulose, one of them is not inducible by tryptophan, and kinetically they differ strikingly in their responses to L-kynurenine and L-3-hydroxykynurenine. Evidence is presented which indicates that the noninducible enzyme may be distinguished as a hydroxykynureninase, whereas the inducible enzyme has catalytic properties consistent with a kynureninase. The possibility that the inducible enzyme described here actually consists of the two enzymes described by Turner and Drucker is discussed.

MATERIALS AND METHODS

Growth of organisms. *N. crassa* was grown in Vogel's minimal medium (14) with and without L-tryptophan. Conidia were washed from 5-ml agar slants with 3 ml of sterile water. One-liter Erlenmeyer flasks containing 500 ml of the appropriate sterilized medium were inoculated with 2.0 ml of spore suspension. The flasks were incubated for 2 days at 30 C in a model G25 incubator-shaker (New Brunswick Scientific Co., Inc.) at 150 rev/min. Each flask was used to inoculate one of five 20-liter nalgene carboys containing 15 liters of medium. Mycelium was harvested in cheese cloth after 2.25 days of growth under rapid forced aeration at 25 C. Under these conditions, yields varied from 200 to 300 g (dry weight).

Preparation of extracts. The mycelium was lyophilized to dryness and ground to a fine powder with a

Wiley mill. Extracts were prepared by suspending 150 g of powdered mycelium in 2,100 ml of 0.05 M potassium phosphate buffer (*p*H 7.0) followed by protamine sulfate and ammonium sulfate fractionation as described previously (4). The protein which precipitated in the 0 to 60% range of saturation with ammonium sulfate was used in subsequent steps.

Column chromatography. The pellet obtained after ammonium sulfate precipitation and centrifugation was dissolved in 0.05 M potassium phosphate (*p*H 7.0) to a final volume of 80 to 120 ml. One half of this extract was desalted on a column (4 by 90 cm) of Sephadex G-25 with 0.05 M potassium phosphate (*p*H 7.0) containing 2 $\times$ 10^{-4} M dithiothreitol and 10^{-4} M ethylenediaminetetraacetic acid. All buffers used after this point contained these sulfhydryl protective agents. The excluded fraction was collected, and the total volume of approximately 200 ml was placed on a column (2 by 80 cm) of DEAE cellulose. Chromatography with a 1,500-ml linear gradient of 0.01 to 0.15 M potassium phosphate buffer (*p*H 6.5), followed by elution with 500 ml of 0.15 M phosphate buffer, was carried out as described previously (3, 4). Fractions (9.0 ml) were collected every 12 min.

Assays. The catalyses of L-kynurenine to anthranilate (kynureninase activity) and of L-3-hydroxykynurenine to 3-hydroxyanthranilate (hydroxykynureninase activity) were measured fluorometrically, essentially as described by Jakoby and Bonner (8). The excitation and emission wavelengths of the Aminco-Bowman spectrophotofluorometer were 315 and 400 nm, respectively. The temperature of the reagents and the cuvette chamber was regulated at 25 C with a Lauda circulating water bath. The increase in fluorescence due to anthranilate or 3-hydroxyanthranilate was followed continuously or at intervals with a model Eu20B Heath recorder equipped with a multispeed chart drive. The assay routinely used for measuring kynureninase and hydroxykynureninase activities contained 0.1 M potassium phosphate (*p*H 7.0), 4 $\times$ 10^{-6} M pyridoxal phosphate, 2 $\times$ 10^{-3} M $MgSO_4$, 4 $\times$ 10^{-4} M L-kynurenine or L-3-hydroxykynurenine, and enzyme. In other assays involving these activities, the reagents were varied as indicated for specific experiments. A unit of activity is defined as 1.0 μmole of anthranilate or 3-hydroxyanthranilate formed per min at 25 C. Fluorescence was converted to micromoles of anthranilate or 3-hydroxyanthranilate with the use of the appropriate buffered standards. Assays were corrected for quenching of the fluorescent product by the respective substrate. Standard anthranilate and 3-hydroxyanthranilate solutions (10^{-6} M) were prepared in 0.1 M potassium phosphate buffer, *p*H 7.0. Quenching was determined and corrected as the per cent decrease in fluorescence.

Anthranilate-5-phosphoribosylpyrophosphate phosphoribosyltransferase (PR transferase) was assayed by converting the product of the reaction, *N*-(5'-phosphoribosyl) anthranilate, to indole-3-glycerol 5-phosphate with an excess of a multienzyme complex containing the necessary coupling enzymes, *N*-(5'-phosphoribosyl) anthranilate isomerase and indole-3-glycerol 5-phosphate synthase (3). The formation of indole-3-glycerol 5-phosphate was followed continuously at 280 nm in the Gilford model 2400 recording spectrophotometer. The coupling enzymes were obtained free of PR transferase essentially as described in the two preceding sections by DEAE cellulose chromatography of an entire 1.65 to 2.0 M ammonium sulfate fraction (40 to 50 cut) derived from 150 g (dry weight) of wild-type *N. crassa*. Such DEAE cellulose preparations have been found to be a convenient source of these coupling enzymes, since they may be stored at −25 C for more than a year without significant loss of activity. The assay contained 0.1 M potassium phosphate (*p*H 7.0), 3.5 $\times$ 10^{-4} M 5-phosphoribosylpyrophosphate, 6 $\times$ 10^{-4} M anthranilate, 0.02 ml of coupling enzyme (DEAE cellulose peak, undiluted fraction no. 120; *see* Fig. 1 for example), 5 $\times$ 10^{-3} M $MgSO_4$, and 0.02 ml of diluted or undiluted enzyme sample in a final volume of 0.2 ml. Coupling enzymes were determined to be nonlimiting if a fivefold dilution of coupling enzymes produced no significant change in rate for the most active enzyme sample. The temperature was regulated at 25 C with a Haake circulating water bath equipped with a cooling coil. A unit of activity is defined as 1.0 μmole of indole-3-glycerol 5-phosphate formed per min at 25 C. The molar extinction coefficient used for indole-3-glycerol 5-phosphate at 280 nm was 5,440. The quartz microcuvettes used in this assay had a 1-cm path length with a capacity of 0.5 ml. All other assays were performed as described previously (4).

Materials. L-Kynurenine sulfate, L-3-hydroxykynurenine, and 3-hydroxyanthranilate were obtained from Calbiochem; pyridoxal-5'-phosphate was from Sigma Chemical Co. Type 20 DEAE cellulose was obtained as a 10-lb. (4.5 kg) batch (no. 2277) from Brown Paper Co.

RESULTS

Two peaks of kynureninase activity are observed when extracts from wild-type *N. crassa* are chromatographed on DEAE cellulose. It can be seen in Fig. 1A that from cells grown on minimal medium the two peaks catalyzing L-kynurenine to anthranilate are widely separated and that the second peak (fractions 180 to 200) contains about 10 times the activity of the first (fractions 110 to 130). However, as shown in Fig. 1B, when wild-type cells are grown with 400 μg of L-tryptophan added to the medium per ml, the first peak increases in activity approximately 450-fold, whereas the second peak remains essentially unchanged, varying less than 13% in two such experiments (*see* also Fig. 6). Hereafter, for clarity and as justified by these findings, the enzymes contained in the first and second peaks will be referred to as "inducible" and "noninducible," respectively. Recovery of kynureninase activity from crude extracts after DEAE cellulose chromatography was 53% in the uninduced case and 73% in the induced.

Our initial interest in the noninducible enzyme came from an observation of its close association on DEAE cellulose with one of the enzymes of the tryptophan pathway, PR transferase. As can be seen in Fig. 1 (*see* also Fig. 6), PR transferase and the noninducible activity migrate at

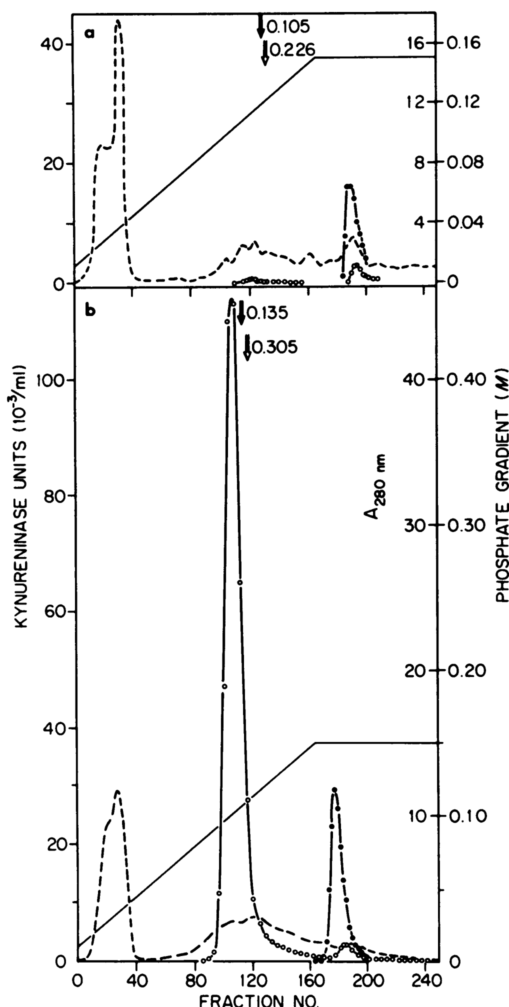

FIG. 1. *DEAE cellulose chromatography of extracts from wild-type N. crassa 74A. Cells were grown in minimal medium (a) and in medium with 400 μg of L-tryptophan per ml (b). In cultures with tryptophan, cells were grown for the first day on 40 μg of L-tryptophan per ml and for the remaining period of 1.25 days on 400 μg/ml. This regimen was followed to maximize induction and to allow comparison of wild type with tryptophan auxotrophs. Symbols: A_{280} (broken line), kynureninase activity in international units (O——O), PR transferase in arbitrary units (●——●), potassium phosphate concentration (solid line). The peak positions of anthranilate synthase (↓) and dehydroshikimate reductase (⇩) and their peak activities in international units per milliliter are recorded for reference.*

the same position in the ion-exchange column. This coincidence seemed particularly significant, since a relatively high salt concentration is needed to elute PR transferase from DEAE cellulose compared to other enzymes of the aromatic-tryptophan pathway (4). We first considered that the coincidence of the two enzymes on the column was due to their physical association in the form of an aggregate or multienzyme complex. We exclude this possibility since (i) these activities in various other fractionation procedures do not coincide exactly (Vitto and Gaertner, *unpublished observations*) and (ii) a mutant lacking PR transferase is unaltered with respect to the noninducible activity (Fig. 2).

The inducible and noninducible enzymes from *N. crassa* were compared for their ability to catalyze the reactions from L-kynurenine and L-3-hydroxykynurenine. As can be seen in Fig. 3 and 4, the inducible enzyme catalyzes the L-kynurenine reaction more favorably than the L-3-hydroxykynurenine reaction, with K_m values of 6.7×10^{-5} M and 2.5×10^{-4} M, respectively. In sharp contrast to this result, the noninducible enzyme catalyzes the L-3-hydroxykynurenine reaction more favorably at low concentrations of substrate, with K_m values for L-kynurenine and L-3-hydroxykynurenine of 2.5×10^{-4} M and 5×10^{-6} M, respectively. An additional significant difference which may be seen in these data is that the maximum velocity (V_{max}) of the inducible enzyme is the same for L-kynurenine and L-3-hydroxykynurenine, but V_{max} for the noninducible enzyme is several times greater for L-kynurenine. Thus, at high concentrations of substrate the noninducible enzyme catalyzes the reaction from L-kynurenine more favorably, whereas at low substrate concentrations it is highly active

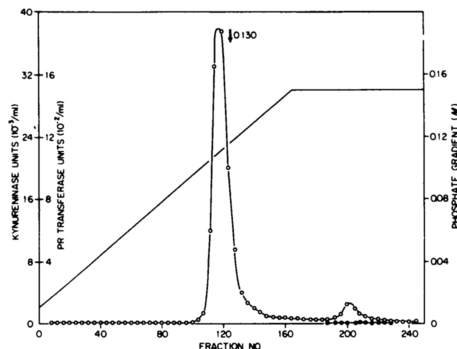

FIG. 2. *DEAE cellulose chromatography of an extract from a tryptophan auxotroph, PR transferase mutant, tryp-4-3 (2). Cells were grown in minimal medium plus 40 μg of indole per ml. Other conditions were as described in Fig. 1. Symbols: kynureninase activity (O—O), PR transferase (●—●), potassium phosphate concentration (solid line), and anthranilate synthetase with peak activity in international units per milliliter. (↓) Dehydroshikimate reductase was not measured. PR transferase activity was not detectable in this strain compared to an average value in international units per milliliter of 0.08 in wild type.*

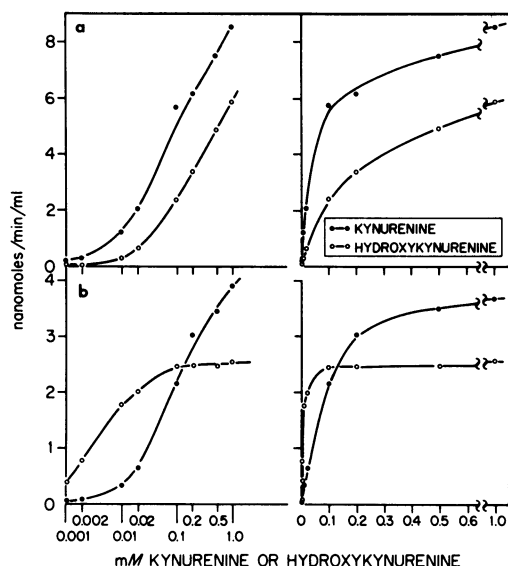

FIG. 3. *Dependency of the rate of the reaction catalyzed by the inducible and noninducible enzymes on the concentration of the substrates L-kynurenine and L-3-hydroxykynurenine. Reactions were carried out as described in Materials and Methods, except that the substrate concentration was varied as indicated. Data are presented in semilogarithmic and linear form for comparison of inducible enzyme (a) with noninducible enzyme (b). Symbols: L-kynurenine (●—●), and L-3-hydroxykynurenine (O—O).*

with L-3-hydroxykynurenine and almost totally inactive with L-kynurenine.

We have also found the inducible and noninducible enzymes to differ in their response to pyridoxal phosphate and $MgSO_4$. As shown in Table 1, the activity of the inducible enzyme is reduced 46 to 80% in 10 min in the absence of pyridoxal phosphate and is inhibited up to 47% by $MgSO_4$. On the other hand, the noninducible enzyme is nearly inactive in the absence of added pyridoxal phosphate and is insignificantly affected by $MgSO_4$. $MgSO_4$ is usually included in assays for kynureninase (7, 13). However, it seems not to be of value here and appears to be inhibitory for the inducible activity. We have included it in our assays for the sake of continuity within this study, but for future work it may be advisable to leave it out.

The above assays have been corrected for quenching of fluorescence of anthranilate and 3-hydroxyanthranilate by L-kynurenine and L-3-hydroxykynurenine. The quenching is more severe for the nonhydroxylated system (Fig. 5). As pointed out recently by Smith and Dietrich (4), the substrate inhibition of *N. crassa* kynureninase reported by Jakoby and Bonner (8) was possibly due to a fluorescence-quenching arti-

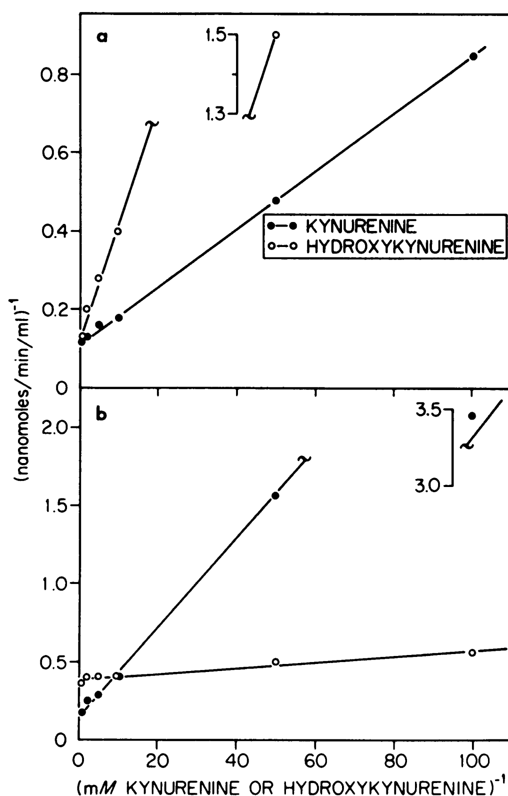

FIG. 4. *Lineweaver-Burk plots of the kynureninase and hydroxykynureninase reactions catalyzed by the inducible and noninducible enzymes. Data shown in Fig. 3 are presented in double reciprocal form as an additional means for comparison of the inducible enzyme (a) with the noninducible enzyme (b). Symbols: L-kynurenine (●——●), and L-3-hydroxykynurenine (O——O).*

fact. As with the findings of Smith and Dietrich on chick liver kynureninase, we also found no substrate inhibition for the *Neurospora* enzyme, provided the quenching of anthranilate fluorescence by L-kynurenine and the quenching of 3-hydroxyanthranilate fluorescence by L-3-hydroxykynurenine were taken into account.

The noninducible activity was observed originally with a niacin-tryptophan auxotroph which we have been attempting to characterize. By analysis of complementation patterns and nutritional requirements (Gaertner and Bradford, *unpublished data*), we have shown that our isolate, nt 295, is a noncomplementing allele of nt C86, a niacin-tryptophan auxotroph that was isolated over 20 yr ago (10). Thus far it has not been possible to determine a biochemical basis for these perplexing auxotrophs (12). The nt 295 mutant (Fig. 6) appears to be normal with respect to the two kynureninase activities, except

TABLE 1. *Requirement of the inducible and noninducible enzymes for pyridoxal phosphate and magnesium sulfate*[a]

Reaction mixture	Inducible enzyme				Noninducible enzyme			
	Initial rate	Per cent	Rate in 10 min	Per cent	Initial rate	Per cent	Rate in 10 min	Per cent
Kynurenine complete	54	100	25	100	34	100	33	100
Kynurenine complete-B_6	41	76	12	48	1.4	4	1.1	3
Kynurenine complete-$MgSO_4$	70	130	45	180	38	112	38	115
Kynurenine complete-B_6, $MgSO_4$	47	87	17	68	1.6	5	1.0	3
Hydroxykynurenine complete	49	100	18	100	28	100	25	100
Hydroxykynurenine complete-B_6	30	63	8	44	2.4	9	1.5	6
Hydroxykynurenine complete-$MgSO_4$	44	90	34	190	31	111	25	100
Hydroxykynurenine complete-B_6, $MgSO_4$	31	63	7	39	2.6	9	1.4	6

[a] Pyridoxal phosphate (B_6) and $MgSO_4$ were deleted from the complete reaction mixtures as indicated. Rates are in international units $\times$ 10^{-4} per ml at 25 C and are recorded as the average initial rate obtained in the first 2 min of reaction and the average rate after 10 min.

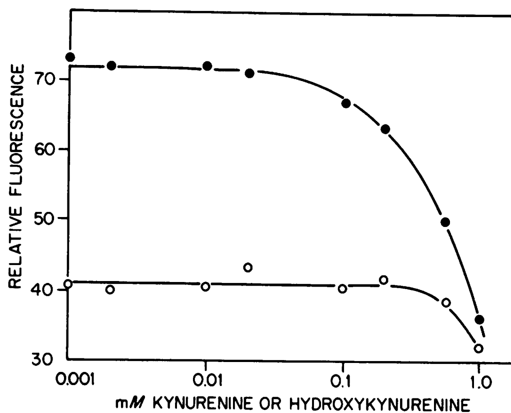

FIG. 5. *Quenching of fluorescence by L-kynurenine and by L-3-hydroxykynurenine. The indicated concentrations of L-kynurenine (●—●) and L-3-hydroxykynurenine (O—O) were added to 10^{-6} M standard solutions of anthranilate and L-3-hydroxyanthranilate, respectively. Fluorescence at an excitation of 315 nm and emission at 400 nm (pH 7.0, 25 C) was recorded.*

for two- to threefold less activity in the first peak, as compared to wild type (Fig. 1). As in wild type, the second peak, which is noninducible, is essentially the same in the presence and in the absence of tryptophan, whereas the activity of the first peak increases approximately 400-fold in the presence of tryptophan. As is discussed below, the relatively lower level of activity in the first peak may reflect the biochemical basis of the auxotrophy.

DISCUSSION

The present investigation was initiated to examine a possible physical association of PR transferase and kynureninase activities in *N. crassa*. PR transferase catalyzes the condensation of 5-phosphoribosylpyrophosphate and anthranilate to form PR anthranilate, the second step in the biosynthesis of L-tryptophan from chorismate. The enzyme that has generally been designated "kynureninase" catalyzes in vitro the conversion of L-3-hydroxykynurenine to 3-hydroxyanthranilate, the fourth step in the biosynthesis of nicotinamide adenine dinucleotide (NAD) from L-tryptophan (Fig. 7). It is also known that kynureninase catalyzes the conversion of L-kynurenine to anthranilate, and it is this reaction for which the enzyme is named (7). Through this reaction, kynurenine may be diverted from step 3 in the tryptophan-to-NAD pathway (Fig. 7) and excreted as anthranilate or cycled back to tryptophan by means of the consecutive kynureninase and PR transferase reactions (9). Hence, a possible basis for an association on DEAE cellulose of PR transferase and a previously unrecognized second peak of kynureninase was that these were metabolically consecutive enzymes associated in a multienzyme complex. However, subsequent experiments did not support this hypothesis. Moreover, response to growth conditions and catalytic specificity were not consistent with the idea that the activity associated with PR transferase on DEAE cellulose was in fact the metabolically consecutive enzyme, kynureninase, of the tryptophan-anthranilate cycle. On the contrary, the activity in question had properties consistent with hydroxykynureninase, an enzyme sequentially removed by eight steps from PR transferase in the biosynthesis of tryptophan and NAD. We conclude, therefore, that the apparent association of these two enzymes is indicative only that they have approximately the same size and charge.

Of greater significance is our finding that the enzyme associated with PR transferase on

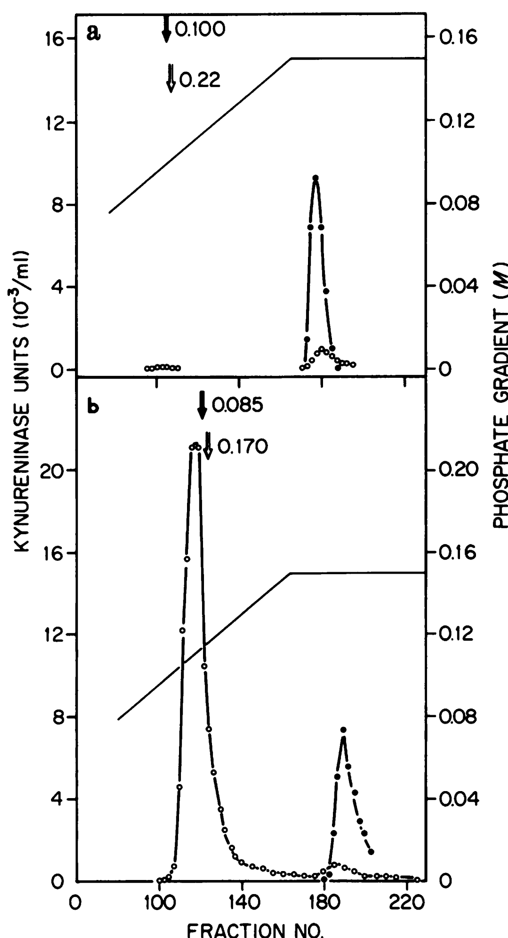

FIG. 6. *DEAE cellulose chromatography of extracts from a tryptophan-niacin auxotroph, nt 295A. Cells were grown in minimal medium plus 4 μg of niacin per ml (a) and in medium with 400 μg of L-tryptophan per ml (b). Other conditions were as described in Fig. 1. Symbols: kynureninase activity in international units* (O—O), *PR transferase in arbitrary units* (●—●), *potassium phosphate concentration (solid line), anthranilate synthase* (⬇), *and dehydroshikimate reductase* (⇩); *with peak activities in international units per milliliter.*

DEAE cellulose is not inducible by tryptophan and therefore is unlike other microbial kynureninases previously described (1, 6, 12). This constitutive behavior is in sharp contrast to the behavior of the more familiar, inducible kynureninase of *N. crassa*, the level of which we and others have found to vary more than 400-fold in response to tryptophan (12). Just as striking is the catalytic specificity of the constitutive or noninducible enzyme in comparison to the inducible kynureninase. As reported above, we found that the noninducible enzyme has a low K_m for L-3-hydroxykynurenine, which is only 0.02 of its K_m for L-kynurenine and which is also only 0.02 of the K_m for the inducible enzyme with L-3-hydroxykynurenine. On the other hand, the inducible enzyme has a relatively low K_m for L-kynurenine, which is 0.27 of its K_m for L-3-hydroxykynurenine.

As a result of these findings and the following considerations, we propose that the constitutive enzyme is specifically a ***hydroxykynureninase*** involved in the biosynthesis of NAD and that the inducible enzyme is specifically a ***kynureninase*** involved in the catabolism of tryptophan via conversion of kynurenine to anthranilate or in the reutilization of kynurenine through the anthranilate-tryptophan cycle. That the requirements of the cells for NAD probably are met completely by the noninducible enzyme (hydroxykynureninase) may be seen from the relatively insignificant level of inducible enzyme present in noninduced cells and from the noninducible enzyme's much lower K_m for L-3-hydroxykynurenine. That the inducible enzyme is involved in catabolism of tryptophan is seen by the fact that in the "induced state" anthranilate is excreted, and a large amount of the enzyme is synthesized, far in excess of that needed to meet the requirements of the cells for NAD. Thus, when tryptophan in superfluous amounts is directed toward the NAD pathway, the inducible enzyme (kynureninase) may provide a vital shunt whereby tryptophan is diverted to anthranilate and excreted or recycled.

Also of possible physiological significance is the observation that the noninducible enzyme exhibits a lower V_{max} for L-3-hydroxykynurenine than for L-kynurenine. This fact suggests a possible level of control whereby the cell limits the amount of 3-hydroxyanthranilate (and, consequently, the amount of NAD) which it produces, even in the face of excessive concentrations of L-3-hydroxykynurenine. It should be mentioned here that in *N. crassa* an additional catabolic enzyme, kynurenine transaminase, is operative and can convert L-3-hydroxykynurenine to xanthurenic acid (8). Thus, it offers a mechanism for the degradation of excess L-3-hydroxykynurenine (cf. Fig. 7).

From the foregoing data and consequences, we suggest the following regulatory features. Cellular tryptophan may enter a metabolic pool and be used for protein synthesis or, e.g., tryptophan may be directed along the pathway for biosynthesis of NAD. In its role in the maintenance of required levels of NAD, hydroxykynureninase is synthesized constitutively. Should an excess flow of tryptophan be directed along the pathway, the low V_{max} of hydroxykynureninase aids in prevention of excess NAD production, whereas the in-

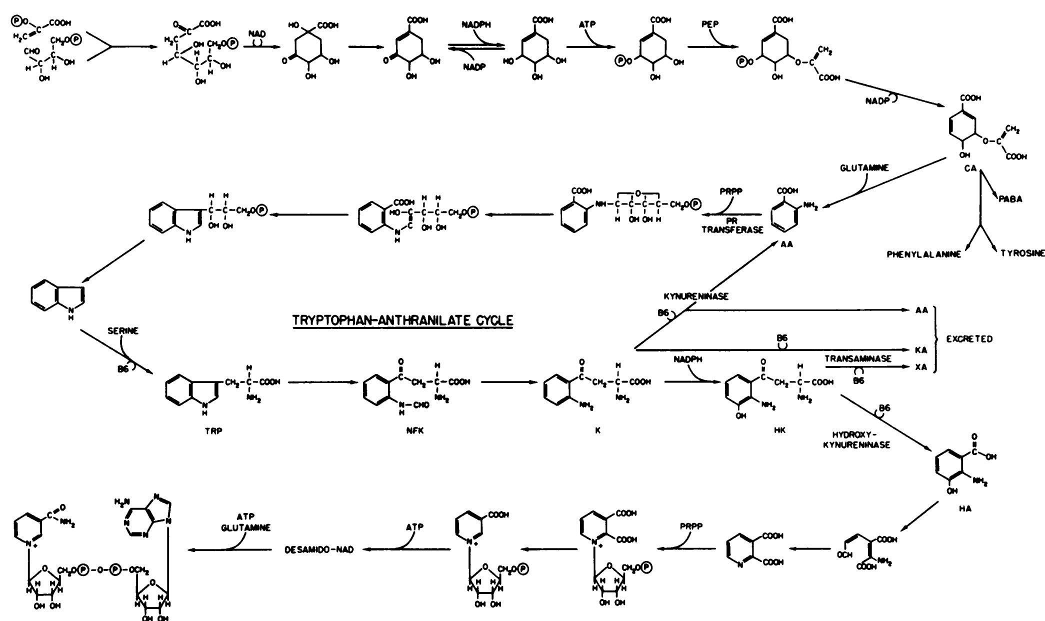

FIG. 7. *Aromatic-tryptophan-NAD pathway, showing the proposed functions of kynureninase and hydroxykynureninase. Abbreviations: CA, chorismic acid; AA, anthranilate; TRP, tryptophan; NFK, N-formylkynurenine; K, kynurenine, HK, hydroxykynurenine; HA, hydroxyanthranilate; KA, kynurenic acid; XA, xanthurenic acid; Transaminase, kynurenine-hydroxykynurenine transaminase.*

duced kynureninase also aids by diverting kynurenine from the pathway. This shunt leads to excretion of tryptophan catabolites or to their recycling via the anthranilate-tryptophan cycle. Furthermore, inhibition of PR transferase activity by tryptophan (4) can be seen as a mechanism to prevent the cycle from operating continuously in the presence of excess tryptophan in a needless expenditure of energy. Under these conditions, it seems likely that kynureninase functions solely in the catabolic excretion of tryptophan.

Because the inducible enzyme is at a low concentration in uninduced cells and because it has a high K_m for hydroxykynurenine, the inducible enzyme (kynureninase) would not be expected to take the place of the noninducible enzyme (hydroxykynureninase) for growth on minimal medium. However, unless the inducible enzyme can not function at all in vivo as a hydroxykynureninase for the synthesis of NAD, a mutant lacking the noninducible enzyme should behave as a type of tryptophan-niacin auxotroph, growing on tryptophan or niacin but not on minimal medium. Although we have isolated and examined a tryptophan-niacin auxotroph (nt 295), as shown here, the noninducible enzyme (hydroxykynureninase) does not appear to be affected. The inducible activity does appear to be significantly lower in nt 295 than in wild type, but this is thought to be an effect rather than a cause of the auxotrophy. It has been suggested that the defect in such tryptophan-niacin auxotrophs is a faulty but partially active (leaky) tryptophan pyrrolase. It has also been found that a catabolite of tryptophan, *N*-formylkynurenine, may be the inducer of kynureninase rather than tryptophan itself (12). If these conclusions are correct, the relatively lower levels of inducible kynureninase probably reflect relatively lower levels of the inducer in this mutant. In any case, these observations do not exclude the possibility that a mutant lacking the noninducible enzyme (hydroxykynureninase) also will prove to be a tryptophan-niacin auxotroph of some kind.

A complication in genetic studies of kynureninase could arise if two genetically distinct forms of inducible enzymes exist, as described by Turner and Drucker (13). It is not yet clear what function these two activities may have in the cell, but it is conceivable that one enzyme is involved exclusively in the anthranilate-tryptophan cycle, whereas the other is involved in the catabolism of tryptophan to anthranilate or a product such as formylanthranilate (9). We have not observed two inducible enzymes, but our method of chromatography with DEAE cellulose employs a linear gradient from 0.01 M to 0.15 M phosphate,

whereas the two inducible activities reported were resolved by continuous elution with 0.07 M phosphate (13). There can be little doubt that the *noninducible* activity described here is distinct from both of the two *inducible* activities described by Turner and Drucker. Moreover, it is likely that the noninducible activity is catalyzed by a protein distinct from that catalyzing the inducible activity. This latter condition is supported (i) by the fact that the noninducible activity remains constant although the inducible enzyme varies over 400-fold in activity, (ii) by the dramatic difference in catalytic properties of the two enzymes, and (iii) by the diversity of their affinities for DEAE cellulose.

In summary, although the complete physiological roles of the inducible and noninducible enzymes must await further investigation, our findings have indicated that two "kynureninases" with clearly different specificities and functions exist in *N. crassa*. We conclude that the noninducible enzyme may be defined as a ***hydroxykynureninase*** by its low K_m for hydroxykynurenine and because it is essentially the only enzyme present in uninduced cells capable of catalyzing L-3-hydroxykynurenine to 3-hydroxyanthranilate for the indispensable synthesis of NAD. In addition, we conclude that the inducible enzyme may be defined as a ***kynureninase*** by its favorable K_m for L-kynurenine and by its highly elevated concentration in induced cells which actively excrete anthranilate.

ACKNOWLEDGMENTS

We thank J. A. DeMoss for providing mutant *tryp*-4-3, and J. Turner for mutant nt C86.

This research was sponsored by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation.

G. R. Welch was a Public Health Service trainee supported by training grant GM 1974 from the National Institute of General Medical Sciences.

F. H. G. is especially indebted to J. A. DeMoss, with whom many of the analytical methods used in this report were developed.

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