BBA 65827

TRYPTOPHAN OXYGENASE ACTIVATION AND ASCORBATE OXIDATION IN WHOLE HOMOGENATES FROM RAT LIVER

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(Received July 1st, 1968)

SUMMARY

The measurement of tryptophan oxygenase (L-tryptophan: oxygen oxidoreductase, EC 1.13.1.12) in whole homogenates from rat liver has been modified to yield a rapid and reliable assay procedure. The assay provides optimal conditions for catalysis and for activation of the enzyme, and thus measures the total amount of enzyme present in the tissue. Additions of AMP, Mg^{2+} or ascorbate, which have been described as activators of tryptophan oxygenase under certain conditions, do not enhance the enzyme activity any further in the present system. Microsomal preparations added to cell sap have been found to increase the enzyme activity, but never beyond the activity obtained in whole homogenates.

Tryptophan does not seem to play a major part in the activation of tryptophan oxygenase observed in whole homogenates. Although a change in protein conformation during activation appears probable, no difference in molecular properties can be detected by gel filtration on Sephadex G-200. The enzyme elutes as a single, sharp peak both before and after activation.

Ascorbate is rapidly oxidized both in homogenates and in simple solutions, yielding absorbance in the 365 nm region used for tryptophan oxygenase assay. This reaction, which is accelerated by subcellular particles, may thus disturb the enzyme analysis in systems employing ascorbate as a reductant, unless the necessary precautions are taken.

INTRODUCTION

Tryptophan oxygenase (tryptophan pyrrolase, L-tryptophan: oxygen oxidoreductase, EC 1.13.1.12) has attracted considerable interest as an example of an enzyme under metabolic regulation. In mammalian liver, the amount of enzyme is controlled by both substrate levels and glucocorticoid hormone[1]. The enzymological properties of tryptophan oxygenase have consequently been extensively studied, particularly in Pseudomonas and rat liver. The enzyme appears to be a

hemoprotein[2,3]. The heme group (cofactor) of the liver enzyme is easily dissociable, the degree of conjugation being subject to regulation by tryptophan[4,5]. The presence of tryptophan is also necessary for reduction of oxidized enzyme, and for protection of this labile enzyme against various forms of inactivation[6–10]. Two binding sites for tryptophan on the enzyme have been postulated, one catalytic and one regulatory, interacting in a cooperative manner[5,10–14].

In rat liver, the enzyme occurs in several forms, differing in enzymic activity[9,15,16]. To obtain a proper measure of enzyme amount, the less active forms must be maximally activated. This seems to be the case in a recently described assay of tryptophan oxygenase in cell sap[9].

Tryptophan oxygenase may also be assayed in whole, unfractionated liver homogenates[17], but this system has not been extensively studied in recent years. Whole homogenates might prove desirable for rapid and convenient assay of the total available tryptophan oxygenase, however, provided that the enzyme could express maximal activity under these conditions. Our interest in hormonal enzyme induction[18] and our need for a more simplified procedure applicable to small tissue samples from perfused livers, prompted the present investigation.

MATERIALS AND METHODS

Animals

Male albino rats of Wistar strain were used. The animals weighed from 250 to 350 g. They were fed ordinary lab chow and water *ad libitum*, but were always fasted 18–24 h prior to use. To obtain high levels of tryptophan oxygenase, 25 mg (1 ml) of hydrocortisone acetate was injected intraperitoneally 3–6 h before the animals were killed by a sharp blow to the head. The livers were excised, and frozen or homogenized.

Tryptophan oxygenase assay

The assay for tryptophan oxygenase is a modification of the procedure employed previously[19], which measures the conversion of tryptophan to kynurenine in whole homogenates, where the enzyme formamidase (aryl-formylamine amidohydrolase, EC 3.5.1.9) is present in excess[20]. The sample of liver tissue, fresh or frozen, is homogenized (10 strokes) with a Potter–Elvehjem teflon homogenizer in a tight-fitting glass tube, with 99 parts of homogenization buffer. This consists of a 0.02 M phosphate buffer (pH 7.0), containing 2.5 mM of tryptophan and 2 μM of (met-)hemoglobin (= 8 μM of heme), freshly dissolved.

4-ml aliquots of homogenate in glass centrifuge tubes are incubated at 37° in a gyro-rotatory metabolic shaker running at 250 rev./min. The enzyme reaction is stopped by adding 1 ml of 15% metaphosphoric acid[17]. After removal of the protein precipitate by centrifugation at 3000 rev./min, the supernatants are filtered and neutralized with 1 M NaOH.

For routine analysis of tryptophan oxygenase activity, four aliquots of the same homogenate are incubated, and the reaction is stopped (duplicate samples) after 20 and 40 min of incubation. The rate of kynurenine formation is maximal in this interval, and the change in rate is relatively small. The amount of kynurenine formed during incubation is measured spectrophotometrically in the neutralized supernatant at 365 nm. The tryptophan oxygenase activity ("TO activity") is expressed in units

(μmoles of kynurenine formed per h at 37°) per g of liver tissue (wet weight). The supernatant reveals a kynurenine spectrum comparable to a standard solution of kynurenine (Fig. 5), with no indications of other tryptophan metabolites[21]. Blanks incubated without tryptophan show no kynurenine formation.

The reaction has a sharp optimum at pH 7.0 in this system, which corresponds to previously reported pH optima[22]. The availability of oxygen seems to be sufficient, since incubations in agitated centrifuge tubes and in open 10-ml erlenmeyer flasks give identical results. Concentrations of tryptophan and (met-)hemoglobin are optimal.

Ascorbate oxidation

Ascorbate oxidation was studied by continuous measurement of $A_{345\ nm}$ in preparations incubated at 37° in the temperature-regulated cell holder of a Beckman DK-2 spectrophotometer.

Treatment of liver samples

Livers were generally frozen at − 20° after excision. No appreciable changes in tryptophan oxygenase activity were found during frozen storage of liver samples for several weeks. However, a marked increase in enzyme activity was observed in the unfrozen liver during the first 5 min following excision. From cortisol-pretreated (4 h) livers, excised and maintained in a moist atmosphere at 37°, samples were removed and frozen in liquid N_2. In one typical experiment, the following tryptophan oxygenase activities (units/g) were found (mean ± S.E. of 3 livers): 25.2 ± 0.6 (maintained for 1 min before being frozen), 30.1 ± 0.6 (5 min), and 31.3 ± 0.5 (3 h). The difference between the 1-min and 5-min values is highly significant ($P < 0.001$). The stability of the enzyme between 5 min and 3 h ($0.5 < P < 0.6$) is in agreement with previous observations[10]. We found no significant change in the dry weight/wet weight ratio of the excised liver during this period.

In order to permit full expression of this particular increase in tryptophan oxygenase activity, liver samples are routinely kept at room temperature for 5–10 min before freezing.

Differential centrifugation

A Beckman L-2 preparative ultracentrifuge with a No. 30 rotor was used for cell fractionation. The temperature was kept close to 0°. The homogenates were centrifuged at 12 500 rev./min for 15 min to remove nuclei and mitochondria, and the microsomal fraction was then sedimented from the post-mitochondrial supernatant by centrifugation at 30 000 rev./min for 60 min.

Gel filtration

Commercially available glass columns (Pharmacia) were used for gel filtration experiments with Sephadex G-200. The gel was prepared by swelling in 0.02 M phosphate buffer (pH 7.0) containing 2.5 mM tryptophan, on a boiling-water bath for 5 h. The gel was allowed to settle in the column overnight. The columns were eluted at 4° with the same tryptophan-containing buffer, and fractions were collected in a LKB linear fraction collector. A constant elution pressure was maintained by the use of a Mariotte flask.

Preparation of a tryptophan oxygenase-rich pH 5.2 precipitated fraction was performed according to CHO-CHUNG AND PITOT[23].

Protein was determined by the biuret method[24].

Chemicals

DL-Kynurenine, 5'-AMP (crystalline sodium salt prepared from yeast) and hemoglobin (twice crystallized bovine hemoglobin) were obtained from Sigma. The hemoglobin preparation may contain up to 75% (methemoglobin according to specifications, and will therefore be referred to as met hemoglobin. L-Tryptophan and hemin were from Nutritional Biochem. Corp., and hydrocortisone acetate (crystalline suspension, 25 mg/ml) from Løvens kem. fabr. (LEO). Other chemicals were reagent grade.

RESULTS

Tryptophan oxygenase activity during incubation

Tryptophan in whole homogenates exhibits a characteristic time course of oxidation: initially there is a lag in kynurenine formation, lasting approx. 25–30 min, during which the reaction rate rises to a maximum. This is followed by a slow decline (Fig. 1). Increasing concentrations of substrate (tryptophan) or cofactor (heme) do

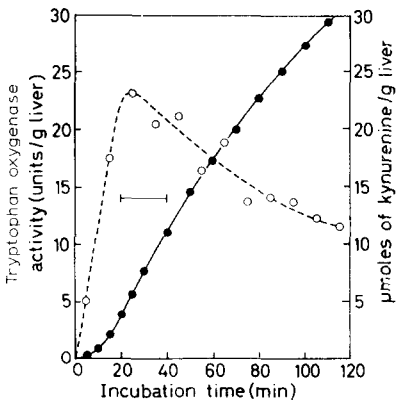


Fig. 1. Kinetics of tryptophan oxygenase catalysis in a standard incubation (1% homogenate prepared as described under MATERIALS AND METHODS). The reaction was stopped in duplicate samples at the times indicated. ●—●, amount of kynurenine; ○- - - -○, tryptophan oxygenase activity during incubation, as expressed by the mean rate of kynurenine formation in a 10-min interval. The 20-min period used for standard assay is indicated by a horizontal line segment.

not change the reaction kinetics, which may reasonably be regarded as an expression of enzyme amount. The reaction thus consists of an enzyme activation phase, followed by a phase of inactivation. At no time is the enzyme activity truly constant, but the interval 20–40 min represents a fairly close estimate to a constant, maximal reaction rate, and has been used by us to measure tryptophan oxygenase activity. The rates of activation and inactivation are both proportional to the total enzyme activity,

and preparations of different activities consequently exhibit their maximum activity during the same time period.

Tryptophan oxygenase activity is directly proportional to enzyme amount only at homogenate concentrations below 2%. At higher concentrations, a fall both in the rate of activation and in the total enzyme activity can be observed.

Tryptophan oxygenase is only 30–50% conjugated in homogenates from cortisol-treated rats, and supplementation with heme is necessary for maximal activity. Hematin and (met-)hemoglobin have been found equally effective in the concentration range 2–10 μM of heme, in contrast to the difference observed in cell sap[9]. The easily soluble (met-)hemoglobin has been preferred in the standard assay.

The liver tissue is homogenized directly in the complete incubation mixture, mainly for reasons of convenience. It makes no difference whether (met-)hemoglobin and tryptophan are added before, or immediately after, homogenization. The reported increase in enzyme activity obtained upon homogenization with tryptophan[9,16] therefore probably reflects a protective function of tryptophan during later steps in the preparative procedures. With tryptophan present in our homogenates, the enzyme activity remains stable for several hours at 4°.

The activation of tryptophan oxygenase taking place during incubation of homogenates, is similar to the activation observed in concentrated cell sap[9] or in dilute cell sap supplemented with subcellular particles[25]. The latter system, reported to yield maximal enzyme activation as compared with concentrated cell sap, approaches the conditions found in our whole homogenates, and invites a comparison.

We separated cell sap and microsomes from ordinary homogenates, and confirmed the observation that microsomes stimulate the tryptophan oxygenase activity of cell sap. Microsomes from both fresh and frozen livers were effective, whereas the freezing of isolated microsomes has been reported to destroy their activating ability[25]. Variations in microsomal amounts between 0.5 and 1.5 equiv. gave no significant difference in tryptophan oxygenase activity, suggesting maximal particle stimulation in this range. In no case, however, was the activity in microsome-supplemented cell sap higher than that found in homogenates. (Typical maximum activities: cell sap, 17; cell sap + 1 equiv. microsomes, 24; and whole homogenates, 29 units/g liver). There is thus reason to assume that tryptophan oxygenase is activated maximally in whole homogenates as compared with other systems[9,25].

Miscellaneous compounds, like ascorbate and other reducing agents, divalent metal ions (Mg^{2+} and Mn^{2+}), purines and purine nucleotides have been reported to activate tryptophan oxygenase[26–31]. There is evidence indicating that both metal ions and purine derivates may act as parts of reducing systems[29,31]. In our homogenates, we found no stimulation of tryptophan oxygenase activity by $MgCl_2$ (0–30 mM) or AMP (0–5 mM). Ascorbate (0–50 mM) was likewise without any significant effect on the maximal tryptophan oxygenase activity. (The interference of the latter compound with the enzyme assay is described separately in the present report). These combined results suggest that addition of reducing agents does not further enhance tryptophan oxygenase activity in the homogenate system.

Effect of tryptophan on activation

The development of tryptophan oxygenase activity in homogenates incubated at different substrate concentrations is depicted in Fig. 2. As can be seen, the rate

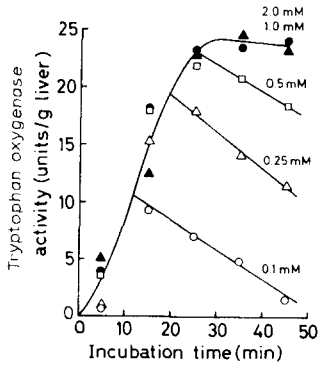


Fig. 2. Enzyme activity as a function of time, with various initial substrate concentrations. The tryptophan oxygenase activity is calculated as described in the legend of Fig. 1. At substrate levels below 1.0 mM, the enzyme is unsaturated, and the measured activity falls as substrate is consumed. Initial substrate concentrations: ○—○, 0.1 mM; △—△, 0.25 mM; □—□, 0.5 mM; ●—●, 1.0 mM; ▲—▲, 2.0 mM.

of enzyme activation is not affected by the substrate concentration in the range employed here (0.1–2.0 mM). Even 0.1 mM tryptophan permits activation to proceed maximally from the beginning of incubation. This means that if a tryptophan–enzyme interaction plays any part in the activation, the K_m for tryptophan in this reaction must be well below 10^{-4}, the approximate K_m for catalysis[4].

Relationship between tryptophan oxygenase activation and enzyme molecular structure

The observed independence of tryptophan oxygenase activation from reduction and cofactor conjugation[5], indicates that activation, of the type occurring in our

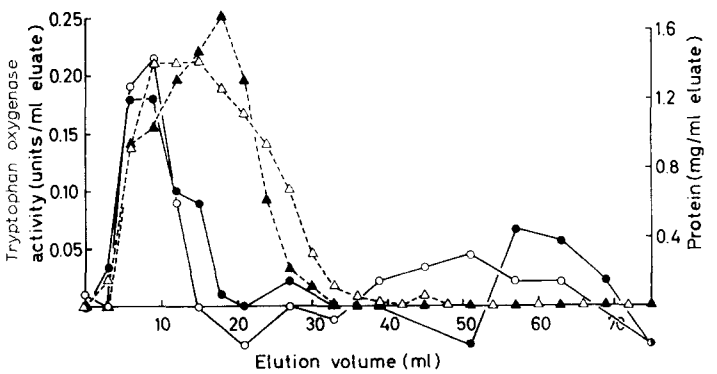


Fig. 3. Gel filtration on Sephadex G-200, of cell sap from activated (▲, ●) and non-activated (△, ○) homogenates. A 10% liver homogenate from cortisol-treated rats was prepared, and one-half of the homogenate was incubated for 30 min to achieve activation of tryptophan oxygenase. Two ml of cell sap from each homogenate half, were then applied to separate small columns of gel (1.8 cm × 30 cm, void volume 12 ml), and eluted overnight with tryptophan-containing buffer. 3-ml fractions were collected, of which 0.4 ml was added to each of the 4 tubes used for tryptophan oxygenase assay (with 3.6 ml homogenate of low enzyme activity, *i.e.*, from non-treated rats). The remainder of the eluate was used for protein determination by the biuret method. Enzyme activity (——) and protein content (— · — · —) of the eluate fractions are expressed in units/ml and mg/ml of eluate, respectively.

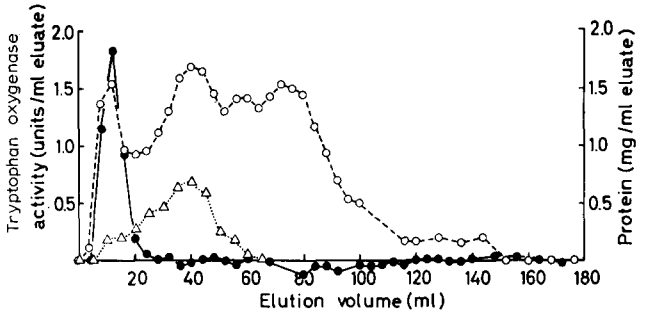


Fig. 4. Gel filtration on Sephadex G-200 of liver cell sap and partially purified tryptophan oxygenase from cortisol-treated rats. Five ml of cell sap from a 20% homogenate were applied to a large gel column (3 cm × 50 cm, void volume 45 ml) and eluted overnight. 4-ml Fractions were collected, and tryptophan oxygenase activity (●—●) and protein content (○— — —○) analyzed as described in the legend of Fig. 3. A pH 5.2 precipitate was prepared as described by CHO-CHUNG AND PITOT[23], dissolved in phosphate buffer, and 5 ml (corresponding to approx. 5 g of liver) were applied to a large Sephadex column (void volume 60 ml). 5-ml fractions were collected, and tryptophan oxygenase activity (△· · · ·△) and protein content analyzed as described in the legend of Fig. 3. No protein was detectable by the method employed.

system, may represent a change in the conformation of the enzyme protein. If a monomer–polymer transition is involved, this might be detected as a change in molecular size following activation.

In an attempt to investigate this possibility, supernatants from activated and non-activated homogenates were fractionated on Sephadex G-200, and tryptophan oxygenase activity measured in the fractions collected. This was done by adding fraction samples to an ordinary tryptophan oxygenase assay (homogenate from non-induced liver of low background enzyme activity), where we know that activation of inactive enzyme may take place. As shown on Fig. 3, tryptophan oxygenase elutes from the column as one sharp peak, immediately after the void volume. Preincubation of the homogenate for 30 min does not change this general pattern of elution. Activation thus does not seem to involve any alterations in the molecular properties of tryptophan oxygenase detectable by this procedure.

The irregularities in tryptophan oxygenase activity obtained in the later fractions (right part of elution diagram, Fig. 3) are probably not significant, since no secondary peaks of activity are found when gel filtration is performed on a larger Sephadex bead volume, giving better resolution (Fig. 4). This is the case with both preincubated and non-preincubated homogenates, and it thus seems rather unlikely that tryptophan oxygenase subunits are present in homogenates. However, we cannot exclude the possibility that subunits may elute with the main tryptophan oxygenase fraction as the result of a non-specific aggregation to proteins (including tryptophan oxygenase) or other soluble cell components, like glycogen. The sharpness of the tryptophan oxygenase peak may indeed be indicative of enzyme aggregation, since the partly purified enzyme elutes with a broader and somewhat displaced distribution, as was demonstrated by CHO-CHUNG AND PITOT[23] during the course of our investigations. Our preliminary experiments with the pH 5.2 precipitated enzyme partially confirm the results of these authors, as indicated on Fig. 4, although the peak of activity still elutes earlier than in their experiments.

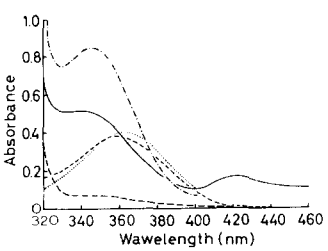
Effect of ascorbate on the assay of tryptophan oxygenase

Ascorbic acid (ascorbate) has been extensively used as a reductant in tryptophan oxygenase assays, in which it seems to effect an increase in the enzyme activity[2,9]. When we included ascorbate in our homogenates, variable results were obtained. There seemed to be an increase in the initial rate of activation in most cases, whereas the effect on maximum activity and inactivation was erratic. As the presence of ascorbate induced greater fluctuations in the analytical results, we finally suspected that a non-enzymic side reaction might be involved.

As a test of this, ascorbate-containing homogenization buffer (without any liver extract) was incubated and assayed by the usual procedure. We found a pronounced development of $A_{365\ nm}$, in the absence of any enzyme. Spectral studies revealed a product spectrum different from that of kynurenine, with a plateau at 345 nm (Fig. 5). This wavelength was used for further studies of the reaction by continuous recording in a Beckman DK-2 spectrophotometer.

The reaction proceeds readily even when ascorbate is incubated in phosphate buffer only (Fig. 6). Sodium phosphate stimulates the reaction, and a small effect of tryptophan is also seen. Ascorbate in neutral solution is known to be oxidized by air, yielding dehydroascorbate and diketo-L-gulonic acid. The latter compound is probably responsible for $A_{345\ nm}$ (ref. 32).

The oxidation of ascorbate takes place rapidly in whole homogenates (Fig. 7), and may account for the apparent tryptophan oxygenase activating effect of the compound. The ascorbate difference spectrum of a homogenate reveals absorbance in



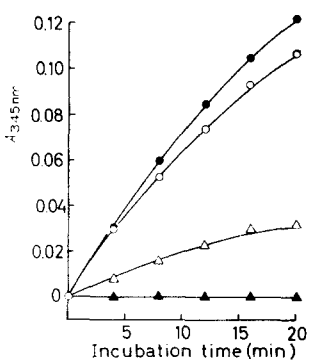


Fig. 5. Absorption spectra of various tryptophan and ascorbate metabolites, recorded with a Beckman DK-2 spectrophotometer. Deproteinized, neutralized supernatant from an ordinary tryptophan oxygenase assay (- - - -); pure kynurenine in aqueous solution (· · ·); freshly dissolved ascorbate (120 mM) in 0.02 M phosphate buffer (pH 7.0) (— — —); ascorbate solution (30 mM in phosphate buffer) oxidized by shaking with air (— · — · — · —); homogenate incubated for 20 min with 60 mM ascorbate at 37° (——). The last recording is a difference spectrum, measured directly in a 0.5 %homogenate, with an identical, but ascorbate-free homogenate as reference. In the other recordings, phosphate buffer (or water in the case of pure kynurenine) has been used as reference.

Fig. 6. Ascorbate oxidation in simple solutions. $A_{345\ nm}$ recorded continuously in a Beckman DK-2 spectrophotometer. All solutions freshly prepared. 2.5 mM tryptophan in H_2O (▲—▲); 60 mM ascorbate in H_2O (△—△); 60 mM ascorbate in 20 mM phosphate buffer (pH 7.0) (○—○); 2.5 mM tryptophan + 60 mM ascorbate in 20 mM phosphate buffer (●—●).

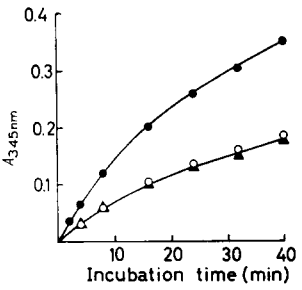


Fig. 7. Oxidation of ascorbate in a 0.5% homogenate (without tryptophan and heme) (●—●); in the corresponding cell sap (○—○); and in phosphate buffer (▲—▲). Difference reactions, recorded directly in a Beckman DK-2 spectrophotometer, with ascorbate-free homogenate, cell sap and phosphate buffer as the respective references.

several regions (Fig. 5), for instance a pronounced peak at 424 nm. Presumably ascorbate is involved in several reactions in liver homogenates.

Fig. 7 shows that ascorbate oxidation is considerably more rapid in a liver homogenate than in phosphate buffer. This is obviously due to particulate elements in the homogenate, since the rate of oxidation is not increased in cell sap.

Differences in the rate of ascorbate oxidation under various conditions may affect tryptophan oxygenase assays in rather unpredictable ways, and serious misinterpretations may occur if corrections are not made for ascorbate oxidation.

DISCUSSION

The rapid turnover of tryptophan oxygenase *in vivo* makes this enzyme particularly useful in studies of enzyme regulation. For this reason, measurements of tryptophan oxygenase activity have been performed by numerous investigators. In most cases the enzyme is assayed in unfractionated homogenates, under conditions where the full enzyme activity is not expressed[17]. The more optimal procedures available are also more laborious, since fractionation of the liver tissue is necessary[9,25]. In view of the great interest concerning tryptophan oxygenase, a simple as well as optimal assay procedure should have a certain value.

The present results suggest that with some modifications of the original procedure[17], the activity of tryptophan oxygenase may be maximally expressed in whole homogenates. The important modifications are: (1) Addition of heme (as (met-)hemoglobin) to the homogenate. It is generally recognized that addition of heme is needed for full tryptophan oxygenase activity in cell sap and purified preparations[3,4], and the necessity of heme addition has also been demonstrated with whole homogenates[33]. However, it has been stated that heme is not required in homogenates[26], and it is our impression that some investigators add heme to their homogenates, and others do not. It should be stressed that the heme content of whole homogenates is not sufficient for full conjugation of cortisol-induced tryptophan oxygenase, and that supplementation with heme is an absolute necessity. (2) The enzyme should be assayed in dilute (1%) homogenates, and the activity measured at the time when it is maximal, *i.e.*, between 20 and 40 min of incubation at 37°. (3) Tryptophan should be present

from the beginning of the assay procedure (most conveniently included in the homogenization medium) to protect the enzyme from any inactivation during subsequent handling.

When these precautions are taken, tryptophan oxygenase is activated maximally during incubation (as compared with the particle-supplemented cell sap system), and no further addition of activating substances (ascorbate, AMP, Mg^{2+}) is necessary.

Analysis of the assay conditions in whole homogenates may also reveal something about the mechanism of tryptophan oxygenase activation. The similarities between homogenates and cell sap (especially particle-supplemented cell sap) makes it reasonable to assume that we are dealing with the same type of activation in all these crude systems[9,25]. A mechanism was recently suggested by the observation that activation in particle-supplemented cell sap seemed to be dependent on xanthine oxidase activity[31]. The function of this enzyme may be to provide reducing equivalents in the form of H_2O_2. The supply of reducing power is apparently not limiting in whole homogenates, however, since neither substrates for xanthine oxidase (AMP) nor other reductants or components of reducing systems (ascorbate, Mg^{2+}) enhance the rate of activation. This is also the case in freshly prepared cell sap[9]. Other evidence further indicates a difference between simple reductions and activation in crude systems: reduction of oxidized, purified tryptophan oxygenase preparations occurs instantaneously[2,34], while activation in crude systems develops relatively slowly. This is particularly well demonstrated when purified tryptophan oxygenase is mixed with cell sap; in this case an added reductant (ascorbate) immediately activates a fraction corresponding to the amount of pure enzyme, while another fraction, presumably the endogenous cell sap enzyme, is activated slowly during incubation[9]. It also seems unlikely that interactions of the enzyme with tryptophan or heme are limiting in activation. Our data indicate that the requirement for tryptophan in the activation reaction is low, and other investigators have found that under anaerobic conditions considerable activation may occur in the absence of tryptophan[5]. Activation may still be required by enzymes fully conjugated with heme[16], and the rapidity of conjugation as seen with purified tryptophan oxygenase[4], further tends to rule out conjugation as the limiting reaction in activation.

We are thus left with the possibility that activation may involve a change in the enzyme protein proper. The "allosteric" nature of tryptophan oxygenase[5,10–14] could indicate a subunit structure of the enzyme, and a mechanism involving subunit association would be an attractive hypothesis for the explanation of enzyme activation. However, our experiments with gel filtration on Sephadex G-200 provided no evidence in favour of this assumption. Tryptophan oxygenase eluted as a homogeneous peak both before and after activation, thus revealing no information about the quaternary structure. It is still possible that a relatively subtle change in enzyme conformation is involved in activation, but the detection of such a change apparently requires more refined methods.

In many investigations concerning tryptophan oxygenase, ascorbate has been used in the assay of the enzyme. The results presented in this paper demonstrate that oxidation of ascorbate in liver homogenates and cell sap may disturb the assay of tryptophan oxygenase seriously. This can be partly corrected by employing appropriate blank reactions including ascorbate. The possibility remains, however, that

ascorbate oxidation may be stimulated by tryptophan in the assay system. Our experiments reveal only a slight stimulation by tryptophan in simple buffer solution, but it has been shown that tryptophan may stimulate ascorbate oxidation considerably under more complex conditions[35]. The use of ascorbate in tryptophan oxygenase assays must thus be considered rather unfortunate, and results obtained with this reductant ought to be regarded cautiously.

ACKNOWLEDGEMENTS

The present work was supported by The Norwegian Council for Science and The Humanities. The skillful technical assistance of Miss L. SKUTLE and Mrs. T. GANGNÆS is greatly appreciated.

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