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# Regulation of Heme Biosynthesis in *Neurospora crassa**

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## SUMMARY

**The mold *Neurospora crassa* does not accumulate porphyrins in iron deficiency but instead accumulates the sideramine desferricoprogen. In iron deficiency there is an accumulation of δ-aminolevulinic acid and the δ-aminolevulinic acid dehydratase level is very low. A similar situation exists in cobalt toxicity and zinc deficiency. The δ-aminolevulinic acid synthetase and ferroprotoporphyrin chelatase comparatively show only marginal changes under these conditions. Addition of iron and zinc to the respective metal-deficient cultures results in an induction of δ-aminolevulinic acid dehydratase. The induction of δ-aminolevulinic acid dehydratase is repressed by protoporphyrin and less effectively by hemin and hemoglobin. Iron deficiency, zinc deficiency, and cobalt toxicity have been found to interfere with the conversion of protoporphyrin into heme, thus rendering protoporphyrin available to repress the enzyme. This repression can be counteracted by iron and much more effectively by coprogen. A model has been proposed in which protoporphyrin has been visualized as the corepressor for the enzyme δ-aminolevulinic acid dehydratase. It is held that iron in the form of coprogen converts protoporphyrin to heme, the latter having a lesser affinity for the aporepressor. Coprogen can inhibit heme binding to the aporepressor and thus render the repressor nonfunctional. This will lead to a derepression of the enzyme δ-aminolevulinic acid dehydratase.**

δ-Aminolevulinic acid synthetase, which catalyzes the first step of the heme biosynthetic pathway, has been shown to be the regulatory enzyme of this pathway in bacterial and animal systems (1–3). For example, in the photosynthetic bacterium *Rhodopseudomonas spheroides* heme has been shown to regulate ALA[1] synthetase by feedback as well as repressive mechanisms (4). This and certain other bacteria as well as the ciliate *Tetrahymena* accumulate porphyrins in iron deficiency. In iron deficiency, the metal is not available for insertion into the porphyrin nucleus, and nonformation of heme results in a release of repression on ALA synthetase leading to a large accumulation of the porphyrins. The mold *Neurospora crassa* (5) and several other fungi (6) do not accumulate porphyrins but instead accumulate desferrisideramines (7). Sideramines have been visualized as the possible iron donors for heme synthesis in fungi (8) and recently evidence has been obtained to indicate such a role for these iron polyhydroxamates in *N. crassa* (9). If in iron deficiency, the block is only at the level of the sideramine-mediated iron incorporation into protoporphyrin, porphyrins should also accumulate along with desferrisideramine in *N. crassa*. A possible explanation for the absence of porphyrin accumulation has been offered on the basis that protoporphyrin represses ALA dehydratase, the second enzyme of the pathway. Consequently, ALA accumulates in iron-deficient *N. crassa* and addition of iron induces ALA dehydratase and heme synthesis (10, 11).

In the present investigation evidence has been obtained to establish the key role of ALA dehydratase in heme biosynthesis by *N. crassa*. A model has been proposed to explain the repression and derepression of this enzyme under different conditions.

## EXPERIMENTAL PROCEDURE

*N. crassa* Em 5297a (wild), ATCC 10816, was grown in stationary cultures in 50-ml flasks containing 10 ml of the basal medium under normal and iron-deficient conditions as described by Padmanaban and Sarma (12). After 40 hours of growth, suitable additions were made and the flasks were shaken for different intervals of time. In some experiments, the mycelia were washed, transferred to fresh media, and shaken after making the appropriate additions. The additions were generally made in 0.1- to 0.5-ml volume and the pH of the culture filtrate or fresh medium did not show any significant change after the additions. The mycelia which received identical treatment were then pooled and used for preparation of enzyme extracts.

### *Assay of Enzymes*

*δ-Aminolevulinic acid Dehydratase*—The mycelia were ground with potassium phosphate buffer (pH 7.5, 0.05 M) containing 0.01 M cysteine with a Virtis homogenizer. Very little enzyme activity could be detected in the crude extracts. However, the dehydratase activity was found localized in the 25 to 40% $(NH_4)_2SO_4$ fraction. The enzyme assay mixture in a total volume of 2 ml contained: ALA, 2 μmoles; $ZnSO_4 \cdot 7H_2O$, 0.2 μmole; potassium phosphate buffer (pH 8.2), 50 μmoles; protein, 5 to 7 mg. The enzyme preparation from *N. crassa* was found to be specifically activated by zinc.[2] After incubation for 2 hours at 37° the reaction was stopped with trichloracetic acid-

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[1] The abbreviation used is: ALA, δ-aminolevulinic acid.

[2] G. Padmanaban, S. Muthukrishnan, and P. S. Sarma, unpublished data.

mercury reagent and the porphobilinogen formed was estimated with perchloric-Ehrlich reagent (13).

*δ-Aminolevulinic Acid Synthetase*—The enzyme preparation and assay procedures were based on the method described by Burnham and Lascelles (1). The enzyme extract was prepared with Tris-HCl buffer (0.1 M, pH 7.8) containing EDTA (2 mM) and β-mercaptoethanol (2 mM). Here also, very little activity could be detected in the crude extracts. The enzyme activity was, however, found to be localized in the 40 to 50% $(NH_4)_2SO_4$ fraction. The assay mixture in a total volume of 1.5 ml contained: succinyl-CoA, 2 μmoles; glycine, 20 μmoles; pyridoxal phosphate, 0.25 μmole; Tris-HCl buffer (pH 7.8), 25 μmoles; protein, 2 to 3 mg. After 1-hour incubation at 37° the reaction was stopped with 0.5 ml of 12.5% trichloracetic acid and supernatant was used for ALA estimation after converting it to a pyrrole derivative with ethyl acetoacetate according to the method of Mauzerall and Granick (14).

*Ferroprotoporphyrin Chelatase*—The method of assay has been described by Padmanaban and Sarma (15). The enzyme extract was prepared with potassium phosphate buffer (0.1 M, pH 7.5) containing 100 mg of Tween 20 per 50 ml of buffer. The assay mixture in a total volume of 2 ml contained: iron ($^{59}FeSO_4$), 24 mμmoles; protoporphyrin, 30 mμmoles; Tris (pH 7.8), 180 μmoles; ascorbic acid, 40 μmoles; enzyme, 0.5 ml containing 6 mg of protein per ml. After incubation for 2 hours at 37° under nitrogen, the reaction was stopped with an acetic acid-acetone mixture. Hemin was isolated from the reaction mixture after adding 5 ml of carrier blood. The recrystallized hemin was dissolved in alkaline pyridine and portions were used for measuring radioactivity and hemin content.

*Isolation of Coprogen*—The iron-binding compound accumulating in the iron-deficient or cobalt-toxic cultures of *N. crassa* has been found to be desferricoprogen and the isolation procedure has been described (5, 16). The final preparation of coprogen (iron chelate) was passed through a Sephadex column (G-15) and eluted as a single band with 50% methanol. The solution was concentrated to dryness in a vacuum, dissolved in a small quantity of absolute methanol, and precipitated with excess of acetone-ether mixture (3:1). The precipitate was dissolved in a small quantity of water and lyophilized. The sideramine preparation was pure on the basis of chromatographic, electrophoretic, and spectral data.

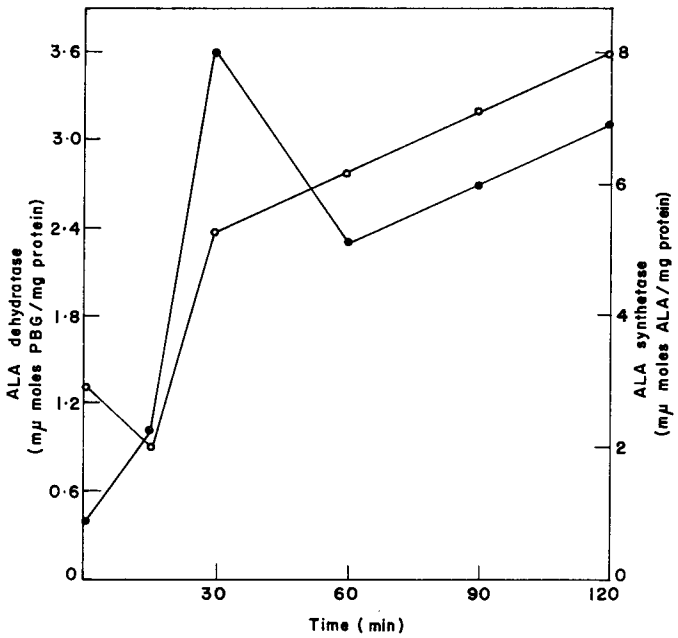


FIG. 1. Induction of ALA synthetase and ALA dehydratase by iron in iron-deficient *N. crassa* as a function of time. The experiments were repeated at least six times and the *curve* represents the results obtained in a typical experiment. The experimental details are as described in Table 1. ○——○, ALA synthetase; ●——●, ALA dehydratase; *PBG*, porphobilinogen.

### *Materials*

Protoporphyrin IX and ALA were purchased from Sigma. Hemin was a Koch-Light product. Hemoglobin was obtained from Nutrition Biochemicals. Hemin and protoporphyrin IX were dissolved in 0.02 N KOH and fresh solutions were used. In some experiments the hemin sample used was further purified by extracting an ethyl acetate solution of the sample with 3 N HCl to free it of possible protoporphyrin contamination.

## RESULTS

*Effect of Iron on Enzymes of Heme Biosynthetic Pathway*—The data presented in Fig. 1 and Table II indicate that the level of ALA dehydratase is the lowest in the iron-deficient cultures of *N. crassa* as compared to the levels of ALA synthetase and ferroprotoporphyrin chelatase which are lowered to an extent of 30%. Thus, these results can explain the accumulation of ALA in iron deficiency in this organism (10). Iron induces the synthesis of all three of these enzymes as evidenced by an increase in their levels on addition of the metal to the deficient cultures, and this increase is blocked by cycloheximide.

Fig. 1 indicates the induction pattern of ALA synthetase and ALA dehydratase on addition of iron to iron-deficient cultures. It can be seen that ALA synthetase is induced after registering an initial decrease up to the 15-min incubation period. ALA dehydratase level reaches a maximum in about 30 min. It then declines and again increases. These patterns of induction are not unusual and may represent portions of oscillatory curves observed for the same enzymes in animal systems (17). It was found that the time required for initial maximal level of ALA dehydratase to be reached varied between 25 and 45 min from experiment to experiment. However, the level of the enzyme reached after an incubation period of 2 hours was nearly the same in all of the experiments. Hence, an incubation period of 2 hours was chosen for the subsequent experiments. The pattern of results was, however, the same even when a shorter incubation period (30 min) was used.

*Repressive Effects of Protoporphyrin, Hemin, and Hemoglobin*—It has been shown earlier that protoporphyrin represses the induction of ALA dehydratase by iron in iron-deficient cultures. Hemin is less effective at a higher concentration (10). The results presented in Table I extend these observations and indicate that hemoglobin when added to the induction medium also represses ALA dehydratase and is more potent than hemin. The repressive effect of these compounds on ALA synthetase is not striking and is about 30%. Addition *in vitro* of these compounds to the assay system used for the determination of these two enzyme activities in the ammonium sulfate fractions prepared from cell-free extracts had no appreciable effect. Hemin has been shown to be a powerful feedback inhibitor and repressor of ALA synthetase from *R. spheroides* (4) and a repressor, although not a feedback inhibitor, of the same enzyme

TABLE I

*Repressive and feedback inhibitory effects of protoporphyrin, hemin, and hemoglobin on ALA synthetase and ALA dehydratase*

*N. crassa* was grown under iron-deficient conditions in 50-ml conical flasks with 10 ml of basal medium in stationary cultures. After 40 hours of growth the indicated additions were made and the flasks were shaken for 2 hours. Identically treated mycelia were then pooled and the enzyme activities were assayed as described in the text.

| Condition | ALA synthetase | ALA dehydratase |
|---|---|---|
| | *mμmoles ALA/mg protein* | *mμmoles PBG[a]/mg protein* |
| Studies *in vivo* | | |
| Iron-deficient | 3.6 | 0.5 |
| Iron-deficient + 1 μg of iron | 7.8 | 2.7 |
| Iron-deficient + 1 μg of iron + protoporphyrin ($1.8 \times 10^{-6}$ M) | 6.0 | 0.7 |
| Iron deficient + 1 μg of iron + hemin ($9.0 \times 10^{-6}$ M) | 6.1 | 1.9 |
| Iron deficient + 1 μg of iron + hemoglobin (hemin concentration, $9.0 \times 10^{-6}$ M) | 6.2 | 1.2 |
| Studies[b] *in vitro* | | |
| No addition | 7.8 | 2.7 |
| Protoporphyrin ($5 \times 10^{-5}$ M) | 6.9 | 2.5 |
| Hemin ($10^{-4}$ M) | 6.3 | 2.4 |
| Hemoglobin (hemin concentration, $10^{-4}$ M) | 7.3 | 2.5 |

[a] Porphobilinogen.

[b] The enzyme preparation was obtained from mycelia after induction with iron. Additions were made to the enzyme preparations from the cell-free extracts.

from chick embryo liver cells (2). Hemin as well as protoporphyrin exhibits feedback inhibitory effects on ALA dehydratase from *R. spheroides* (18).

*Similarity in Effects of Excess Cobalt, Zinc Deficiency, and Added Protoporphyrin*—To substantiate further that protoporphyrin can function as a corepressor for ALA dehydratase *in vivo*, experiments have been carried out to block the siderамine-mediated iron incorporation into protoporphyrin. This has been realized under two conditions. It has been shown that excess cobalt causes an accumulation of $^{59}Fe$ in the mycelial siderамine fraction and a concomitant fall in that of the heme fraction in short term tracer incorporation experiments (9). Further, this heavy metal inhibits $^{59}Fe$ incorporation into protoporphyrin in cell-free extracts (15). Zinc-deficient cells of *Ustilago sphaerogena* have been shown to accumulate the siderамine, ferrichrome, with a corresponding decrease in cytochrome *c* level (6). Addition of zinc results in a fall in the ferrichrome and an increase in cytochrome *c* contents. We have obtained similar results with zinc-deficient *N. crassa* grown with $^{59}Fe$ where there is an accumulation of the label in the mycelial siderамine fraction and a decrease in that of the heme fraction. We have also found that just as in the case of iron deficiency (10) cobalt-toxic and zinc-deficient cultures of *N. crassa* accumulate ALA but not porphyrins.

In short term incubation experiments cobalt completely blocks the induction of ALA dehydratase by iron in iron-deficient mycelia (Table II). Under these conditions ALA synthetase is depressed to an extent of only 30%. Again in zinc-deficient cultures, ALA dehydratase level is lowered. ALA synthetase level is not appreciably affected. Ferroprotoporphyrin chelatase actually shows an increase. Addition of zinc to the deficient cultures results in an increase in ALA dehydratase level which is blocked by cycloheximide. Komai and Neilands (19) have obtained similar results with zinc-deficient cells of *U. sphaerogena*. The results obtained with *N. crassa* indicate that the effects of cobalt excess, zinc deficiency, and added protoporphyrin on the levels of ALA synthetase and ALA dehydratase are similar.

In normal mycelia, after incubation for 30 min in the presence of cobalt, protoporphyrin, or cycloheximide there is a striking decrease in the ALA dehydratase level over the zero time value (Table II). ALA synthetase does not show a significant change. Thus, the decrease in ALA dehydratase under these conditions can be attributed to turnover of the enzyme, synthesis being blocked by the inhibitors.

*Counteraction of Repression due to Protoporphyrin, Hemin and Hemoglobin*—It has been proposed earlier as a working hypothesis that the inducing effect of iron may be due to the inactivation of the corepressor for the enzyme ALA dehydratase. Whereas protoporphyrin represses this enzyme considerably, hemin is effective only to a lesser extent even at a higher concentration. The suggestion has been made that iron may fix protoporphyrin as heme in heme proteins, thus rendering protoporphyrin unavailable for the corepressor function. The repressive effect of protoporphyrin on ALA dehydratase induction by iron in iron-

TABLE II

*Effect of iron, cobalt, and zinc on levels of heme biosynthetic enzymes of N. crassa*

The experimental conditions are as described in Table I.

| Condition | ALA synthetase | ALA dehydratase | Ferro-protoporphyrin chelatase |
|---|---|---|---|
| Iron-deficient | 68 | 20 | 72 |
| Iron-deficient + 1 μg of iron | 160 | 130 | 95 |
| Iron-deficient + 1 μg of iron + 5 μg of cycloheximide | 65 | 17 | 65 |
| Iron-deficient + 1 μg of iron + 1 mg of cobalt | 132 | 32 | |
| Zinc-deficient | 89 | 60 | 131 |
| Zinc-deficient + 1 μg of zinc | | 112 | |
| Zinc-deficient + 1 μg of zinc + 5 μg of cycloheximide | | 44 | |
| Normal[a] | 100 (5.5) | 100 (2.5) | 100 (0.09) |
| Normal + 1 μg of iron | 108 | 109 | |
| Normal + 1 μg of iron + 1 mg of cobalt | 90 | 44 | |
| Normal + 1 μg of iron + protoporphyrin ($1.8 \times 10^{-6}$ M) | 96 | 52 | |
| Normal + 1 μg of iron + 5 μg of cycloheximide | 90 | 35 | |

[a] The enzyme activities for normal mycelia at zero time before shaking are taken as 100. The actual values are given in parentheses. The ferroprotoporphyrin chelatase activity is expressed as millimicromoles of iron per 10 mg of hemin per mg of protein. Normal mycelia were shaken for only 30 min with the different constituents indicated.

TABLE III

*Iron reversal of protoporphyrin, hemin, and hemoglobin repression of ALA dehydratase*

The experimental conditions are as described in Table I.

| Condition | ALA dehydratase | | | |
|---|---|---|---|---|
| | No inhibitor[a] | Protoporphyrin, $0.9 \times 10^{-6}$ M | Hemin, $2.7 \times 10^{-5}$ M | Hemoglobin (hemin concentration, $9.0 \times 10^{-6}$ M) |
| Iron-deficient + 1 μg of iron | 100 | 40 | 44 | 42 |
| Iron-deficient + 10 μg of iron | 100 | 52 | 82 | 62 |
| Iron-deficient + 100 μg of iron | 100 | 85 | 86 | 84 |

[a] The enzyme activity in the absence of any inhibitor being added to the medium is taken as 100. The absolute value obtained in this particular experiment was 3.1 mμmoles of porphobilinogen per mg of protein.

TABLE IV

*Reversal of repression on ALA dehydratase by iron and coprogen*

Iron-deficient mycelia after 40 hours of growth were washed and suspended in fresh iron-free medium. Zinc-deficient cultures were used as such. The other experimental details are as described in Table I.

| Condition | ALA dehydratase[a] | | |
|---|---|---|---|
| | Protoporphyrin,[b] $1.8 \times 10^{-6}$ M | Hemin,[c] $2.7 \times 10^{-5}$ M | Hemoglobin[c] (hemin concentration, $9.0 \times 10^{-6}$ M) |
| Iron-deficient + 1 μg of iron | 43 | 45 | 42 |
| Iron-deficient + 25 μg of iron | 55 | | |
| Iron-deficient + 25 μg of iron (coprogen) | 82 | | |
| Iron-deficient + 5 μg of iron | | 55 | |
| Iron-deficient + 5 μg of iron (coprogen) | | 70 | |
| Iron-deficient + 10 μg of iron | | | 58 |
| Iron-deficient + 10 μg of iron (coprogen) | | | 71 |
| Zinc-deficient + 1 μg of zinc | 35 | | |
| Zinc-deficient + 100 μg of zinc | 36 | | |
| Zinc-deficient + 100 μg of iron | 32 | | |
| Zinc-deficient + 1 μg of zinc + 25 μg of iron | 42 | | |
| Zinc-deficient + 1 μg of zinc + 25 μg of iron (coprogen) | 61 | | |

[a] The enzyme activity in the absence of any inhibitor being added to the medium is taken as 100. This value ranged between 100 and 110 for all of the treatments mentioned, in the absence of the inhibitor. The absolute value for 100 corresponded to 2.9 mμmoles of porphobilinogen per mg of protein.

[b] Protoporphyrin was added 2 hours after treatment of the iron-deficient mycelia with iron or coprogen. The incubation was continued for 30 min after protoporphyrin addition.

[c] Hemin or hemoglobin was added along with iron or coprogen to the iron-deficient mycelia at zero time and incubated for 2 hours.

deficient cultures can be partially counteracted by addition of excess iron (10).

In a detailed investigation it has now been found (Table III) that excess iron not only counteracts the repression due to protoporphyrin but also that due to hemin and hemoglobin. It has not been possible to decide upon the type of interaction on the basis of the levels of iron used and the extent of counteraction obtained. The extent of counteraction obtained as a function of the iron concentration is not identical in every experiment, although the gross pattern remains the same. One reason for this may be attributed to the observation that, although the added protoporphyrin or hemin is rapidly cleared from the culture filtrate, it remains mostly adsorbed to the mycelial surface. The effective concentration of these compounds actually reaching the target site may be very low and variable. However, it has always been found that a higher level of iron is required to overcome the repression due to protoporphyrin.

It can be realized that, whereas the counteracting effect of iron on protoporphyrin repression can be explained as due to conversion of protoporphyrin into heme, it is difficult to explain the counteracting effects on hemin and hemoglobin repression. It may be pointed out that all of these studies have been carried out with iron-deficient cultures which contain desferricoprogen in the culture filtrate. On addition of iron, coprogen is formed which enters the mycelia as an intact molecule (12). Thus it is possible that the active form of iron is coprogen. It is thus of interest to compare the counteracting effects of inorganic iron and the iron polyhydroxamate. For this purpose *N. crassa* was grown under iron-deficient conditions. After 40 hours of growth, the mycelia were washed and transferred to a fresh iron-free medium and inorganic iron or coprogen was added. As indicated in Table IV, protoporphyrin was added after 2 hours of preliminary incubation of the mycelia with the two types of iron compounds. The incubation was continued for another 30 min and the ALA dehydratase level was assayed. It was found that hemin and hemoglobin did not produce significant repressive effects under these conditions. These two compounds were therefore added at zero time along with iron and the ALA dehydratase was assayed after shaking the mycelia for 2 hours. The results presented in Table IV indicate that coprogen is more effective than inorganic iron (or ferric citrate) in counteracting the repression due to protoporphyrin, hemin, and hemoglobin.

In the case of zinc-deficient cultures, it has been found that protoporphyrin represses the induction of ALA dehydratase by zinc. This repression has not been found to be counteracted by excess zinc or iron alone. However, excess iron in presence of zinc brings about a release of repression to the extent of 10%. Coprogen when added at the same level of iron is much more effective and the counteraction is to the extent of 50% (Table IV).

It has been found that after 2 hours of incubation the total amount of iron incorporated into the iron-deficient mycelia is the same irrespective of whether inorganic iron or coprogen has been used.

## DISCUSSION

The results obtained in the present investigation indicate an important role for the enzyme ALA dehydratase in the regulation of heme biosynthesis in *N. crassa*. This can be true as well

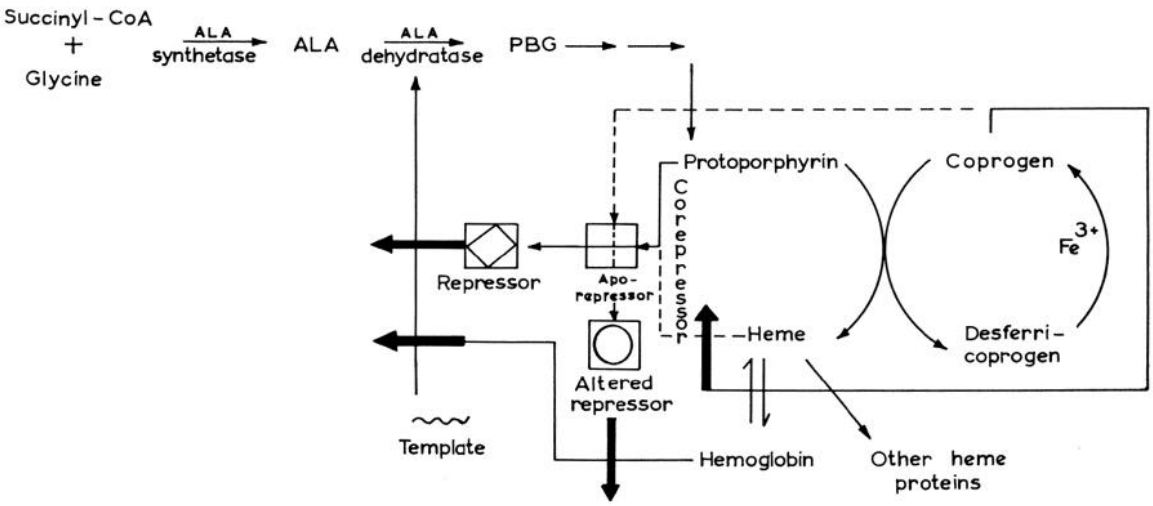


FIG. 2. A model for the regulation of ALA dehydratase in *N. crassa*. The *thick arrows* indicate blocking steps. The *dashed lines* indicate the weaker affinity. *PBG*, porphobilinogen.

of other fungi which do not accumulate porphyrins in iron deficiency but instead accumulate compounds belonging to the siderochrome series. The evidence indicating a corepressor function of protoporphyrin *in vivo* for ALA dehydratase are

1. Added protoporphyrin represses the enzyme ALA dehydratase.

2. In iron deficiency, cobalt toxicity, and zinc deficiency ALA accumulates and ALA dehydratase level is low. Under these conditions there is an interference with the sideramine-mediated iron incorporation into protoporphyrin, thus making the latter available for the corepressor function.

In the case of zinc deficiency this type of a result can be obtained even if the metal is assumed to be directly involved in the synthesis of ALA dehydratase. The argument can be advanced that a decreased ALA dehydratase in zinc deficiency leads to decreased levels of protoporphyrin. This would result in the accumulation of $^{59}Fe$ in the sideramine fraction because of the unavailability of protoporphyrin which accepts the iron. However, it has been found that the porphyrin levels in zinc deficiency are not decreased as compared to the normal even though ALA dehydratase level is low and there is accumulation of ALA. This indicates at least an additional involvement of zinc at the site of iron incorporation into protoporphyrin. In this context, it may be pointed out that the effects of zinc deficiency are not as striking as those of iron deficiency or cobalt toxicity. The accumulation of ALA and the fall in ALA dehydratase levels are brought about only at severe stages of zinc deficiency, indicating a probable indirect involvement of this metal in ALA dehydratase synthesis. The requirement of iron and zinc for the synthesis of ALA dehydratase protein can be interpreted in terms of the inactivation of the corepressor protoporphyrin, by iron through a zinc-dependent reaction. The ferro protoporphyrin chelatase level, however, does not decrease in zinc deficiency. It is known that reducing agents such as GSH, NADH, and ascorbic acid are necessary for the enzymic incorporation of iron into protoporphyrin *in vitro*. It is possible that in zinc deficiency the necessary reducing potential in the cell is not maintained for the adequate manifestation of ferroprotoporphyrin chelatase activity *in vivo*, thus rendering protoporphyrin available for corepressor function.

The results of the present investigation indicate that the formation of heme is, however, not sufficient to explain completely the release of repression due to protoporphyrin. The features to be explained are:

1. Hemin also represses ALA dehydratase although to a smaller extent at a higher concentration. Hemoglobin also represses and is more effective than hemin.

2. Iron counteracts the repressive effects due to protoporphyrin, hemin, and hemoglobin.

3. Coprogen is more effective than inorganic iron (or ferric citrate) in counteracting the repression due to protoporphyrin, hemin, and hemoglobin.

All of these results can be fitted into a model (Fig. 2), the basis of which is similar to the one proposed by Granick (2) for the regulation of ALA synthetase. Heme has been assigned the role of corepressor for the enzyme ALA synthetase. Compounds which induce porphyria are visualized as competing with heme for the corepressor site, thereby inactivating the repressor and thus leading to an induction of the enzyme. In the present model it is visualized that the repressor for ALA dehydratase is active only in presence of the corepressor protoporphyrin. The repressor has a weaker affinity for hemin. Coprogen is capable of inhibiting the heme binding to the repressor. The model takes into account the superiority of coprogen as compared to iron in overcoming the repressive effects. Coprogen can also overcome the repression due to hemin. Hemoglobin may act by giving rise to heme or may itself mimic the function of the repressor. In this context it is interesting to record that in *Neurospora* 50% of the heme is present as hemoglobin (4) or at least a closely related protein. Recently, Marver, Schmid, and Schutzel (20) have emphasized the functional importance of hemin and methemoglobin as repressors *in vivo* of microsomal cytochrome synthesis. The progressive counteracting effect on protoporphyrin repression due to the addition of increasing concentrations of iron to iron-deficient cultures can be explained as a result of increasing iron saturation of desferricoprogen and an increase in the net coprogen content of the mycelia. Even in experiments in which the iron-deficient mycelia have been washed and suspended in a fresh medium containing only inorganic iron some coprogen would still be formed and the counteracting effect of inorganic iron

may be determined by the amount of the iron chelate formed. It has recently been found in the case of itoic acid, 2,3-dihydroxy benzoyl glycine, that, once the iron-deficient cells are committed to the production of the iron-binding compound, addition of the metal does not have an immediate effect. Significant synthesis continues for some more time and then only stops (21). The lack of desferricoprogen accumulation in zinc-deficient cultures can account for the poor counteraction of the protoporphyrin repression when excess iron is added along with zinc. However, added coprogen brings about a substantial counteraction of the repression due to protoporphyrin.

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