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Haeme-regulated Degradation of δ-Aminolevulinate Synthase 1 in Rat Liver Mitochondria

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Protein turnover, which occurs at various rates, is critical for the homeostasis of cellular protein levels. However, the proteolysis systems that determine the turnover rate of mitochondrial proteins are largely unknown. Delta-aminolevulinic acid synthase (ALAS) 1, a rate-limiting enzyme in the haeme biosynthesis, is one of the mitochondrial proteins that have a very short lifetime. In this study, to reveal the regulatory mechanisms for ALAS1 degradation, we examined the turnover rates of ALAS1 in rat liver under several conditions. In primary rat hepatocytes, the degradation of ALAS1 was stimulated by haeme, and suppressed by inhibition of haeme biosynthesis. Furthermore, the haeme-stimulated degradation of ALAS1 was observed in the isolated mitochondria. These results suggested that, in mitochondria, there exists an ALAS1 degradation system that is regulated by cellular haeme level and plays a crucial role in the regulation of haeme biosynthesis.

Key words: ALAS1, haeme and metabolism, metabolic regulation, mitochondria, protein degradation.

Abbreviations: ALAS, δ-aminolevulinic acid synthase; Bt_2cAMP, dibutyryladenosine 3′,5′-cyclic monophosphate; CHI, cycloheximide; DDC, 3,5-dicarbethoxy-1,4-dihydrocollidine; MSH, mannitol–sucrose–Hepes; PB, phenobarbital; SA, succinylacetone; TOM40, translocase of outer membrane.

Recent advances in the study of intracellular proteolysis brought about the various findings including the cell cycle, apoptosis, protein quality control, antigen presentation and so on (*1*). In spite of these advances, degradation of mitochondrial proteins is not well understood. It is widely recognized that mitochondrial proteins could be degraded by an autophagic process (*2*). However, since this process digests whole mitochondrion, the difference in half-lives among various mitochondrial proteins cannot be explained. Thus, the proteolytic system in which proteins are degraded individually is assumed to exist in mitochondria.

Some proteases have been found in mitochondria; for example, mitochondrial processing peptidase, mitochondrial intermediate peptidase and inner membrane peptidase remove the amino-terminal extra peptides from precursor proteins (*3*, *4*); Lon protease, AAA proteases and Clp protease digest mis-folded and non-assembled polypeptides in mitochondria, functioning in protein quality control system (*5*). These known functions, however, are unable to explain the differences in lifetimes of mitochondrial proteins.

Delta-aminolevulinic acid synthase 1 (ALAS1), which is expressed ubiquitously (*6*), catalyses the first step of haeme biosynthesis in mitochondrial matrix. ALAS1 protein has been reported to be degraded with a short half-life under certain conditions (*7*), although most of mitochondrial proteins have longer lifetime (from several hours to several days) (*8–12*). Therefore, mitochondria are supposed to have a proteolytic system that controls lifetime of ALAS1. To elucidate the molecular mechanisms underlying the determination of the lifetimes of mitochondrial proteins, we studied focusing on the degradation of ALAS1.

Since haeme biosynthesis is strictly regulated, defection of the enzymes involved in the haeme biosynthetic pathway leads to porphyria; the disease causes neurological manifestation and skin problems by accumulating porhyrins and their precursors (*13*). ALAS1 is the rate-limiting enzyme of haeme biosynthesis (*6*) and regulated by the final product, haeme. It is reported that the ALAS1 protein is regulated by intracellular free haeme, which inhibits the import of the precursor of ALAS1 in cytoplasm by attaching to the cysteine residue in the pre-sequence (*14*, *15*).

Here we report that the biosynthesis of haeme and its inhibitors affect the rate of degradation of ALAS1 in rat liver. The degradation was stimulated by hemin, and conversely, an inhibitor of haeme biosynthesis suppressed the degradation. Thus, haeme biosynthesis is regulated by the final product through the modulation of the degradation of the rate-limiting enzyme.

MATERIALS AND METHODS

Reagents—Williams' medium E, succinylacetone (SA), sodium selenite, zinc sulfate, amphotericin B, bovine

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serum albumin were purchased from Sigma. Dexamethasone was purchased from Wako (Osaka, Japan). Insulin was purchased from Seikagaku Kogyo Co. (Tokyo). Phenobarbital (PB) was purchased from Tokyo Kasei Co. (Tokyo), and 3, 5-dicarbethoxy-1, 4-dihydrocollidine (DDC) was purchased from Acros Organics. Bt_2cAMP, ampicillin, streptomycin, cycloheximide (CHI) and other general reagents were purchased from Nacalai Tesque (Kyoto, Japan). Collagenase was purchased from Nitta gelatin Co. (Osaka, Japan). Collagen was prepared from tendon of rat tails. Anti-CYP 2B was a gift from Prof. Omura (*16*).

Antibody Production—Synthetic peptide (CKETPPANEKEKTAKAAVQQA) corresponding to amino terminal region of rat ALAS1 with the amino terminal cysteine added for coupling, was conjugated with keyhole limpet haemocyanin and used to immunize rabbit. The antiserum was fractionated with $(NH_4)_2SO_4$, and the IgG fraction was obtained. The IgG fraction was passed through the synthetic peptide immobilized resin, and the specific antibody was eluted with glycine–HCl (10 mM, pH 2.8). The eluate was quickly neutralized with Tris–HCl (1 M, pH 8.0). Specificity of the antibody was confirmed by immunoblotting of ALAS1-expressed cell lysate (*15*).

Immunoblotting—Samples were separated by SDS–PAGE and transferred to a polyvinylidene difluoride membrane by electroblotting. After blocking with a Tris-buffered saline (25 mM Tris–HCl, pH 7.4, 0.15 M NaCl, 0.1% Tween-20) containing 0.5% bovine serum albumin, the membrane was incubated with the anti-ALAS1 IgG in the same buffer for 1 h. After washing for three times, the membrane was further incubated with anti-rabbit IgG conjugated to a horseradish peroxidase. Immunoreactive bands were visualized by chemiluminescence using Western Lighting (PerkinElmer Life Sciences) and quantified by Cool Saver (ATTO Co., Tokyo).

Isolation and Culture of Primary Rat Hepatocytes—Male Sprague-Dawley rats of 5- to 6-week-old were obtained from Kyudo Co. (Kumamoto, Japan). The rats were fed with a commercial diet and water *ad libitum*. The hepatocytes were isolated by a modification of the two-step collagenase perfusion *in situ* (*17*). Briefly, the liver was perfused with a calcium-free balanced salt solution containing glucose (5 mM) and EGTA (0.5 mM) for 12 min at a flow rate of 40–50 ml/min and then with Williams' medium E containing collagenase (0.2 mg/ml) for 10 min at a flow rate of 25–30 ml/min. The hepatocytes were dispersed in Williams' medium E supplemented with fetal bovine serum (10%), insulin (12.5 mg/l), dexamethasone (0.1 μM), ampicillin (100 mg/l), streptomycin (100 mg/l), amphotericin B (2.5 mg/l), sodium selenite (72 nM) and zinc sulfate (13 μM). The dispersed hepatocytes were filtered successively through 150 and 70 μm meshes, and then the cells were washed for three times by low-speed centrifugation (50*g*, 1 min). The viability was estimated by a trypan blue dye exclusion test to be >85%. The isolated primary hepatocytes were cultured in Williams' medium E in collagen-coated 100 mm dishes. In the case of a serum-free condition, the medium was changed to Williams' medium E with serum-free and low concentration of insulin (0.625 mg/l) on the second day of the culture.

Induction and Degradation of ALAS1 in Primary Rat Hepatocytes—ALAS1 in the hepatocytes was induced with Bt_2cAMP (200 μM), SA (500 μM), PB (200 μM) or the combination of SA and PB on the third day of the culture under the serum-free condition. The incubations with Bt_2cAMP or the combination of SA and PB were also carried out either in the serum-free or serum-containing medium. The degradation of ALAS1 was observed after the incubation of the hepatocytes, which had been maintained in Bt_2cAMP (200 μM) or SA (500 μM) for 16 h, in the medium containing CHI (10 μg/ml). For the evaluation of the effects of hemin in the degradation, hemin (20 μM) was added to the medium containing CHI or CHI and SA. The ALAS1 protein was detected by SDS–PAGE and immunoblotting.

Induction of ALAS1 in Rat Liver—Three male Sprague-Dawley rats were intraperitoneally injected with DDC (300 mg/kg/day) for 3 days. DDC was suspended in corn oil. After about 4–6 h from the final injection, the rats were anaesthetized with sodium pentobarbital. The livers were removed and perfused with an ice-cold saline. The perfused livers were homogenized in an ice-cold MSH buffer (0.21 M mannitol, 0.07 M sucrose, 10 mM Hepes–NaOH pH 7.4) using a Potter–Elvehjem homogenizer. The homogenates were centrifuged at 600*g* for 10 min, and the supernatants were further centrifuged at 6,000*g* for 10 min. The precipitates were collected as the mitochondrial fractions and suspended in the MSH buffer. The contents of ALAS1 protein in mitochondrial fractions were analysed by SDS–PAGE and immunoblotting.

ALAS1 Degradation and its Stimulation by Hemin in Isolated Mitochondria—Primary rat hepatocytes were cultured with SA (500 μM) and PB (100 μM) for 18.5 h and incubated with or without hemin (10 μM) for 20 min before harvesting. The mitochondrial fractions were isolated by aforementioned method, diluted to the protein concentration of 2 mg/ml, and incubated at 30°C. Hemin was added to the mitochondria prepared from the SA-treated hepatocytes to evaluate its effect on the mitochondrial degrading system, and the degradation was observed at 30°C. Mitochondria from the animal liver were also examined for the effects of hemin on the degradation. Sprague-Dawley rats had been intraperitoneally injected with DDC (300 mg/kg) and 18–24 h after the first injection, DDC was injected at the same dosage. The livers were collected at about 5 h after the final injection. The mitochondrial fractions prepared as described above were diluted with 15-fold volumes of Hepes–NaOH (10 mM, pH 7.4) buffer and left on ice for 15 min. The hypotonic-treated mitochondrial fractions were centrifuged at 10,000*g* for 15 min. The precipitates were again treated with the hypotonic solution and centrifuged. The pellets were collected and suspended in MSH buffer. These fractions were incubated with or without hemin (10 μM) at 30°C.

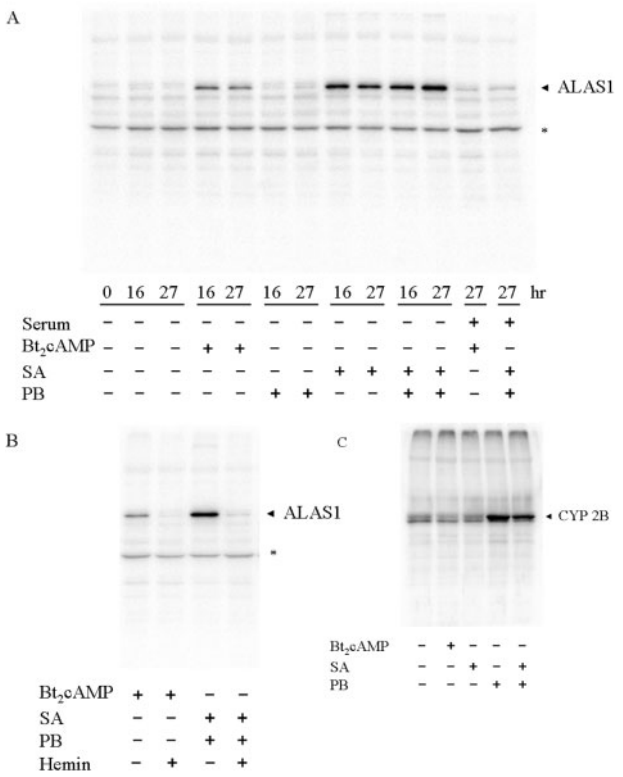

Fig. 1. **Induction of ALAS1 protein in primary rat hepatocytes.** (A) Primary hepatocytes were incubated with Bt_2cAMP (200 μM), PB (100 μM), SA (500 μM) and the combination of SA and PB for indicated times. The samples were analysed by immunoblotting using anti-ALAS1 antibody. Asterisk indicates non-specific bands. (B) The hepatocytes were incubated in SA/PB- or Bt_2cAMP-containing medium for 27 h with or without hemin (10 μM).

RESULTS

Induction of ALAS1 Protein in Primary Rat Hepatocytes—At steady state, the amount of ALAS1 protein was too small to be detected by immunoblotting (Fig. 1A). Primary rat hepatocytes were cultured with Bt_2cAMP, SA, PB or the combination of SA and PB for the induction of ALAS1 protein. Bt_2cAMP is believed to induce the ALAS1 protein by simple enhancement of the transcription (*18*). SA inhibits δ-aminolevulinic acid dehydratase (*19*, *20*), which is the second enzyme of the haeme biosynthetic pathway. SA treatment leads to the decrease of intracellular haeme (*21*). PB is the inducer of some species of cytochrome P450 (CYP) that are major haeme proteins in hepatic microsomes (*22*). PB induces mainly the expression of CYP 2B subfamily in hepatocytes (*22*), and we also confirmed the induction in this study (data not shown). Since cytochrome has a haeme moiety as a cofactor, the augmentation of CYP protein is expected to consume the intracellular free haeme. Therefore, SA and PB are expected to reduce the concentration of free haeme and release the negative feedback regulation of ALAS1, and accordingly induce the amount of ALAS1 protein. The content of ALAS1 protein was consequently increased when the hepatocytes were incubated with Bt_2cAMP. SA or the combination of SA and PB was also effective in increasing the amount of ALAS1 protein. Sole addition of PB was, however, ineffective in a significant increase of the amount of ALAS1 protein (Fig. 1A). Some repressive constituents would be included in the serum, since the induction of ALAS1 was not observed in the serum-containing medium (Fig. 1A). There are several explanations for the negative regulation of ALAS1 by haeme. For example, haeme destabilizes ALAS1 mRNA (*23*, *24*) or attaches to the pre-peptide of pre-ALAS1 preventing the precursor to translocate into mitochondria (*14*, *15*). When the hepatocytes were incubated with hemin, effects of Bt_2cAMP or the combination of SA and PB on the induction of ALAS1 were abolished (Fig. 1B). This result suggests that the ALAS1 protein level in primary rat hepatocytes is controlled by the haeme.

Effects of Hemin and Inhibitor of Haeme Biosynthesis on Degradation of ALAS1 Protein in Primary Hepatocytes—In order to examine whether ALAS1 protein was degraded in the primary hepatocytes or not, we added CHI to the medium and observed the change of ALAS1, which had been induced with Bt_2cAMP or SA. ALAS1 is known as a short-lived mitochondrial protein (*7*). When ALAS1 had been induced with Bt_2cAMP, it started to decrease with a half-life of 4–6 h under CHI-containing medium. The ALAS1 protein, which had been induced with SA, however, was stable (Fig. 2A). The difference in the stability of ALAS1 protein could be due to different modes of the induction. Bt_2cAMP induces ALAS1 protein in the transcriptional level (*18*), whereas SA reduces the intracellular free haeme (*19–21*) probably without changing the transcriptional level. ALAS1 was induced with Bt_2cAMP, and then the cells were incubated with hemin or SA in the presence of CHI. The ALAS1 protein was degraded with a half-life of 1–2 h (Fig. 2B and C) when the cells were incubated with hemin. In contrast, it was stable when cells were incubated with SA (half-life >8 h, Fig. 2B and C). The half-life of ALAS1 incubated only with CHI was intermediate between those values. Furthermore, the stability of ALAS1 protein in the presence of SA was eliminated when hemin was introduced into the culture medium (Fig. 2B and C). These results suggest that the ALAS1 reduction is regulated by the intracellular haeme level in primary rat hepatocytes.

Degradation of ALAS1 Protein and its Activation by Hemin in Mitochondrial Particles Prepared from Primary Rat Hepatocytes—In spite of numerous studies on ALAS1 enzyme, information on the degradation of ALAS1 protein is very limited. To reveal the molecular basis for the short life of the protein, we attempted to determine the intracellular localization of the ALAS1-degrading activity. The hepatocytes were first incubated with SA and PB for 19 h for the induction of ALAS1 protein, and then incubated with hemin for 20 min before harvest. ALAS1 was degraded in the mitochondria from the hemin-treated hepatocytes, while it was stable in the absence of hemin (Fig. 3A). This result indicates that ALAS1 was degraded in mitochondria and that the degradation required presence of haeme in the cells.

Next, we tried to determine the site where the degradation was activated. First, hepatocytes were

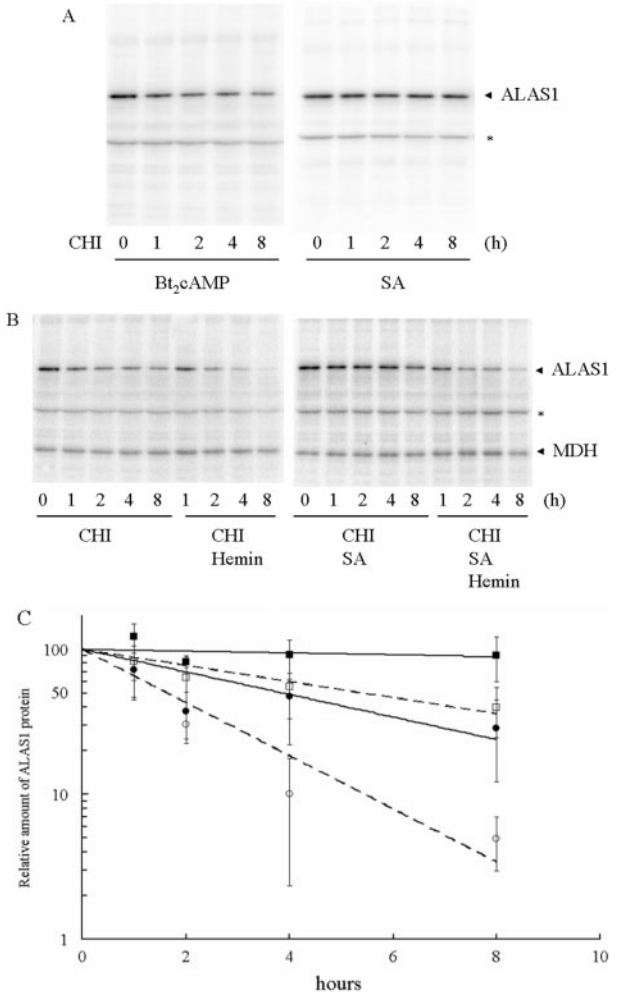

Fig. 2. **ALAS1 degradation in primary rat hepatocytes.** (A) The hepatocytes were incubated with Bt_2cAMP (200 μM) or SA (500 μM) for 16 h, and then CHI (10 μg/ml) was added to the medium. The samples were analysed by immunoblotting using anti-ALAS1 antibody. (B) Hemin (20 μM) and SA were added to the medium after the incubation with Bt_2cAMP for 15 h. The samples were analysed by immunoblotting using anti-ALAS1 and anti-MDH antibodies. (C) The bands of ALAS1 protein were quantified and normalized by the amounts of protein contained in the samples. Relative amounts of ALAS1 protein in the hepatocytes incubated with CHI/SA (Solid lines connecting filled square), CHI (Dashed lines connecting open squares), CHI/SA/hemin (Solid lines connecting filled circle) and CHI/hemin (Dashed lines connecting open circle) are shown. The data shown are the average of three independent experiments. Error bars indicate S.D.

incubated with SA and PB for 19 h, and the mitochondria were prepared. The isolated mitochondria were incubated with or without hemin for 3 h at 30°C. The amount of ALAS1 protein in the mitochondria with hemin was decreased to a trace level, whereas that in the control mitochondria did not show a significant decrease, more than 80% of the starting molecules remaining (Fig. 3B). These results suggest that hemin acts on mitochondria and provokes degradation of ALAS1 protein therein.

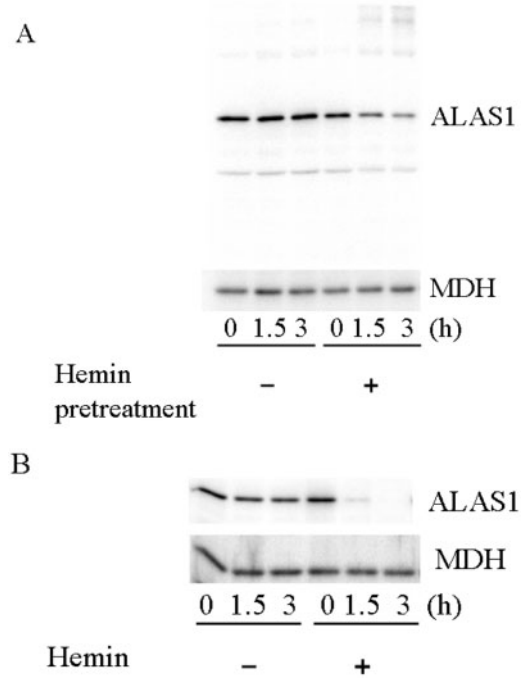

Fig. 3. **Activation of ALAS1 degradation by hemin.** Primary hepatocytes were incubated with SA (500 μM) and PB (100 μM) for 18.5 h, and then the hepatocytes were treated with or without hemin (10 μM) for 20 min before harvest. (A) The mitochondria were incubated at 30°C for indicated times, and then the samples were analysed by immunoblotting using anti-ALAS1 antibody. Anti-MDH antibody was used as internal standard. Since fragment generated from ALAS1 cannot be detected in this report, the pictures after Fig. 3A show the region of interest. (B) The mitochondria were isolated from the hepatocytes without hemin treatment before harvest, and then incubated with or without hemin at 30°C for indicated times.

ALAS1 Degradation in Rat Liver Mitochondria—ALAS1 degradation and its stimulation were also analysed in rat liver mitochondria.

The level of ALAS1 protein in rat liver mitochondria was too low to be detected by immunoblotting (Fig. 4A), as well as in the primary hepatocytes. Thus, we attempted to increase the amount by injection of DDC. DDC is known as the inhibitor of ferrochelatase, and its administration promotes accumulation of ALAS1 protein (*25–27*). In the mitochondria from DDC-treated rats, the content of ALAS1 protein was increased drastically, although the degree varied from rats to rats.

As shown in the hepatocytes, significant degradation was not observed in the mitochondria from DDC-treated rats. Again, addition of hemin to the incubation buffer facilitated the degradation (Fig. 4B). The effect of hemin indicates that DDC depleted haeme and that the reduction of haeme concentration stabilized ALAS1.

Since we prepared the mitochondria by a different centrifugation method, the fraction contained a significant amount of lysosomes. Actually, a high activity of cathepsin B was detected in the fraction. To eliminate the influence of the lysosomal proteases on the degradation, the mitochondrial fraction was swollen by dilution

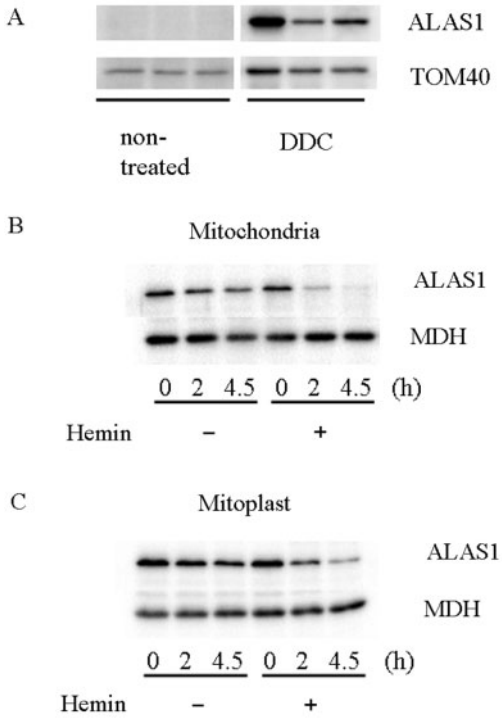

Fig. 4. **ALAS1 degradation in mitochondria and mitoplast from rat liver.** (A) Three rats were injected with DDC (300 mg/kg/day) for 3 days. After 4–6 h from the final injection, the rat was killed and the liver was removed. The mitochondria were isolated from the liver, and then confirmed the content of ALAS1 protein by immunoblotting using anti-ALAS1 antibody. Anti-TOM40 antibody was used as internal standard. (B) The mitochondria from DDC-treated rat liver were incubated with or without hemin (10 μM) at 30°C for indicated times. (C) The mitochondria were diluted with hypotonic buffer and incubated on-ice for 15 min, and then the dilute was centrifuged at 10,000*g* for 15 min. The precipitate was treated with hypotonic buffer again and centrifuged, and then the precipitate was collected as a mitoplast fraction. The mitoplast was incubated with or without hemin (10 μM) at 30°C for indicated times. The incubated mitochondria and mitoplast were analysed by immunoblotting using anti-ALAS1 antibody.

with a hypotonic buffer, and more than 90% of the contaminated cathepsin B was removed. Degradation of ALAS1 stimulated by hemin was reproduced in the mitoplast fraction. This result shows that ALAS1 protein is degraded in mitochondria from a rat liver, and the activation of the degradation by hemin is unaffected by the hypotonic treatment.

DISCUSSION

In this study, we attempted to unveil the mitochondrial proteolysis through the degradation of ALAS1 protein. ALAS1 is a representative short-lived mitochondrial protein and localized at mitochondrial matrix. This enzyme plays a key role in the control of haeme biosynthesis and is regulated by the concentration of intracellular haeme as a negative feedback (*6*).

Some studies have so far proposed the mechanisms of the negative feedback regulation of ALAS1 by haeme. The intracellular free haeme binds to the cysteine residue of the pre-peptide of pre-ALAS1 in cytoplasm and prevents the precursor from translocating to mitochondria (*14*, *15*). It also possibly reduces the stability of mRNA for ALAS1 (*23*, *24*). The amount of ALAS1 protein in mitochondrial matrix could be regulated by both mechanisms. In this study, we did not verify these mechanisms for induction of ALAS1 protein although we did not detect the precursor form in cytoplasm from the cultured hepatocytes that had been incubated with the combination of SA and PB together with hemin (Fig. 1B). Here we present that haeme accelerates degradation of ALAS1 protein and that its depletion leads to accumulation of ALAS1 protein. We suppose that Bt_2cAMP increases the transcription of ALAS1 mRNA (*18*) and that the reduction of intracellular free haeme as the result of SA or DCC treatment leads to suppression of the degradation of ALAS1 protein. How haeme acts in ALAS1-degrading system? Though haeme is reported to degrade proteins by generating reactive oxygen species (*28*), MDH, a matrix enzyme, was stable when the cultured hepatocytes or isolated mitochondria were incubated with hemin (Fig. 3A and B). Haeme is known to act as a modulator on proteins such as transcription factor Bach1 (*29*). Bach1 is a basic leucine zipper protein and forms heterodimers with small Maf proteins to repress a gene expression in a manner dependent on a Maf recognition element. Haeme binds to the haeme regulatory motif of Bach1, and the haeme-Bach1 complex is released from the Maf recognition element. As the result, gene repression is cancelled (*29*). Haeme could act as an activator for an ALAS1-sepecific protease or a modulator for ALAS1 itself. Haeme regulatory motif has a Cys–Pro dipeptide sequence whose cysteine residue is required for haeme binding. In the acceleration of ALAS1 degradation, haeme is assumed to act on the ALAS1-degrading enzyme or the associated proteins. ALAS1 has several Cys–Pro sequences not only in the pre-sequence but also the mature protein. The cysteine residue in the pre-sequence are believed to be involved in retarding the translocation of the precursor form. When haeme bound to the mature protein, the haeme–ALAS1 complex could change the conformation to a form that is susceptible to specific or general proteases.

In conclusion, ALAS1 protein is physiologically degraded by the final products, haeme, and the amount is increased as the result of inactivation of the proteolysis, which is caused by haeme depletion. We demonstrated that the rate of ALAS1 degradation was altered in response to haeme in rat liver mitochondria. This finding supports the novel mechanism of regulation of haeme biosynthesis via the degradation of rate-limiting enzyme.

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REFERENCES

1. Glickman, M.H. and Ciechanover, A. (2002) The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. *Physiol. Rev.* **82**, 373–428
2. Takeshige, K., Baba, M., Tsuboi, S., Noda, T., and Ohsumi, Y. (1992) Autophagy in yeast demonstrated with proteinase-deficient mutants and conditions for its induction. *J. Cell Biol.* **119**, 301–311
3. Gakh, O., Cavadini, P., and Isaya, G. (2002) Mitochondrial processing peptidases. *Biochim. Biophys. Acta* **1592**, 63–77
4. Ito, A. (1999) Mitochondrial processing peptidase: multiple-site recognition of precursor proteins. *Biochem. Biophys. Res. Commun.* **265**, 611–616
5. Käser, M. and Langer, T. (2000) Protein degradation in mitochondria. *Semin. Cell Dev. Biol.* **11**, 181–190
6. Ferreira, G.C. and Gong, J. (1995) 5-Aminolevulinate synthase and the first step of heme biosynthesis. *J. Bioenerg. Biomembr.* **27**, 151–159
7. Hayashi, N., Terasawa, M., and Kikuchi, G. (1980) Immunochemical studies of the turnover of δ-aminolevulinate synthase in rat liver mitochondria and the effect of hemin on it. *J. Biochem.* **88**, 921–926
8. Brunner, G. and Neupert, W. (1968) Turnover of outer and inner membrane proteins of rat liver mitochondria. *FEBS Lett.* **1**, 153–155
9. Kobayashi, K., Kamimura, T., and Tuboi, S. (1982) Studies on the turnover rates of cytosolic and mitochondrial fumarases in rat liver. *J. Biochem.* **91**, 1511–1518
10. Kawajiri, K., Harano, T., and Omura, T. (1977) Biogenesis of the mitochondrial matrix enzyme, glutamate dehydrogenase, in rat liver cells. I. Subcellular localization, biosynthesis, and intracellular translocation of glutamate dehydrogenase. *J. Biochem.* **82**, 1403–1416
11. Wallace, R., Knecht, E., and Grisolia, S. (1986) Turnover of rat liver ornithine transcarbamylase. *FEBS Lett.* **208**, 427–430
12. Grisolia, S., Hernandez-Yago, J., and Knecht, E. (1985) Regulation of mitochondrial protein concentration: a plausible model which may permit assessing protein turnover. *Curr. Top. Cell. Regul.* **27**, 387–396
13. Sassa, S. (2006) Modern diagnosis and management of the porphyrias. *Br. J. Haematol.* **135**, 281–292
14. Lathrop, J.T. and Timko, M.P. (1993) Regulation by heme of mitochondrial protein transport through a conserved amino acid motif. *Science* **259**, 522–525
15. Munakata, H., Sun, J.Y., Yoshida, K., Nakatani, T., Honda, E., Hayakawa, S., Furuyama, K., and Hayashi, N. (2004) Role of the heme regulatory motif in the heme-mediated inhibition of mitochondrial import of 5-aminolevulinate synthase. *J. Biochem.* **136**, 233–238
16. Harada, N. and Omura, T. (1983) Phenobarbital- and 3-methylcholanthrene-induced synthesis of two different molecular species of microsomal cytochrome P-450 in rat liver. *J. Biochem.* **93**, 1361–1373
17. Seglen, P.O. (1976) Preparation of isolated rat liver cells. *Methods Cell Biol.* **13**, 29–83
18. Giono, L.E., Varone, C.L., and Canepa, E.T. (2001) 5-Aminolaevulinate synthase gene promoter contains two cAMP-response element (CRE)-like sites that confer positive and negative responsiveness to CRE-binding protein (CREB). *Biochem. J.* **353**, 307–316
19. Tschudy, D.P., Hess, R.A., and Frykholm, B.C. (1981) Inhibition of δ-aminolevulinic acid dehydrase by 4,6-dioxoheptanoic acid. *J. Biol. Chem.* **256**, 9915–9923
20. Sassa, S. and Kappas, A. (1983) Hereditary tyrosinemia and the heme biosynthetic pathway. Profound inhibition of δ-aminolevulinic acid dehydratase activity by succinylacetone. *J. Clin. Invest.* **71**, 625–634
21. Giger, U. and Meyer, U.A. (1983) Effect of succinylacetone on heme and cytochrome P450 synthesis in hepatocyte culture. *FEBS Lett.* **153**, 335–338
22. Sueyoshi, T. and Negishi, M. (2001) Phenobarbital response elements of cytochrome P450 genes and nuclear receptors. *Annu. Rev. Pharmacol. Toxicol.* **41**, 123–143
23. Drew, P.D. and Ades, I.Z. (1989) Regulation of the stability of chicken embryo liver delta-aminolevulinate synthase mRNA by hemin. *Biochem. Biophys. Res. Commun.* **162**, 102–107
24. Hamilton, J.W., Bement, W.J., Sinclair, P.R., Sinclair, J.F., Alcedo, J.A., and Wetterhahn, K.E. (1991) Heme regulates hepatic 5-aminolevulinate synthase mRNA expression by decreasing mRNA half-life and not by altering its rate of transcription. *Arch. Biochem. Biophys.* **289**, 387–392
25. Granick, S. and Urata, G. (1963) Increase in activity of δ-aminolevulinic acid synthetase in liver mitochondria induced by feeding of 3,5-dicarbethoxy-1,4-dihydrocollidine. *J. Biol. Chem.* **238**, 821–827
26. Hayashi, N., Terasawa, M., Yamauchi, K., and Kikuchi, G. (1980) Effects of hemin on the synthesis and intracellular translocation of δ-aminolevulinate synthase in the liver of rats treated with 3,5-dicarbethoxy-1,4-dihydrocollidine. *J. Biochem.* **88**, 1537–1543
27. Cole, S.P.C. and Marks, G.S. (1984) Ferrochelatase and N-alkylated porphyrins. *Mol. Cell. Biochem.* **64**, 127–137
28. Aft, R.L. and Mueller, G.C. (1984) Hemin-mediated oxidative degradation of proteins. *J. Biol. Chem.* **259**, 301–305
29. Ogawa, K., Sun, J., Taketani, S., Nakajima, O., Nishitani, C., Sassa, S., Hayashi, N., Yamamoto, M., Shibahara, S., Fujita, H., and Igarashi, K. (2001) Heme mediates derepression of Maf recognition element through direct binding to transcription repressor Bach1. *EMBO J.* **20**, 2835–2843