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HEMIN INHIBITS TRANSFER OF PRE-δ-AMINOLEVULINATE SYNTHASE INTO CHICK EMBRYO LIVER MITOCHONDRIA

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Pulse labelling studies in chick embryo livers show that hemin prevents the transfer of drug induced pre-δ-aminolevulinate synthase from the cytosol into the mitochondria, leading to an accumulation of precursor in the cytosol. No effect of hemin was observed on the transfer of pre-pyruvate carboxylase into mitochondria. These results eliminated a general toxic effect of hemin on mitochondrial import of proteins and are consistent with the view that hemin specifically inhibits the transfer of ALA synthase.

Chick embryo liver δ-aminolevulinate (ALA) synthase (EC 2.3.1.37), the first and rate limiting enzyme of the heme biosynthetic pathway, is normally present in liver at a low level but can be elevated up to 600-fold by administration of porphyrinogenic drugs (1). The enzyme is synthesized on cytoplasmic polysomes as a precursor of Mr = 74,000 and is subsequently processed to a mature mitochondrial matrix enzyme of Mr = 68,000 (2).

Previous studies in chick embryos (1) and in isolated hepatocytes from chick embryos (3) have shown that hemin inhibits the drug mediated induction of mitochondrial ALA synthase. Kikuchi and co-workers (4) first postulated that one role of hemin is to block the transfer of ALA synthase into mitochondria. These workers demonstrated that hemin injections into animals caused an accumulation in the liver cytosol of enzymically active ALA synthase though there is uncertainty as to the nature and molecular size of this enzyme (5,6). Recently, Ades (7) showed that in chick embryo hepatocyte cultures hemin blocked the

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processing of ALA synthase precursor but no evidence was available as to whether hemin blocked the transfer of precursor into mitochondria.

In the present study, we establish for the first time that hemin administration to chick embryos prevents the transfer of ALA synthase precursor into mitochondria and results in an accumulation of precursor in the cytosol.

It was possible that this effect of hemin results from a general impairment of mitochondrial membrane transport function and has no physiological significance. To check this possibility, the effect of hemin on the transport into mitochondria of an unrelated enzyme was investigated.

It was found that pyruvate carboxylase, a matrix enzyme, was synthesized as a precursor protein in the cytosol and that hemin had no effect on the transfer of this enzyme into mitochondria.

Materials and Methods

Materials: 2-Allyl-2-isopropylacetamide (AIA) was a generous gift from Roche, Sydney, Australia. 3,5-Diethoxycarbonyl -1, 4-dihydrocollidine (DCC) was purchased from Eastman Organic Chemicals, Rochester, New York. Hemin was a product of Porphyrin Products Incorporated, Utah.

Drug treatment of chick embryos: White Leghorn chick embryos (18 day old) were treated with a combination of AIA (2 mg) and DDC (4 mg) in 0.1 ml of dimethyl sulphoxide (2). Control embryos were treated with 0.1 ml dimethyl sulphoxide alone.

Administration of hemin to chick embryos: Hemin was dissolved in 0.2M KOH and was neutralized with 1M sodium acetate (pH 5.5) to final pH 7.8. A sample (40 µl) of hemin at 24 mg/ml was spread over the air sac membrane via a small hole in the shell above the air space. Hemin was administered at zero time and then at two 20 min. intervals. After a further 20 min. incubation 300 µCi of (S^{35})-methionine (about 120 Ci/mmole) was administered and the incubation continued for 20 min.

Preparation of antibodies. ALA synthase antibody was raised in rabbits to purified chick embryo liver mitochondrial enzyme (2,6). Chicken liver mitochondrial pyruvate carboxylase was purified in its native form by the procedure previously described (8). Subunits (Mr = 115,000) of pyruvate carboxylase were separated by SDS-polyacrylamide gel electrophoresis and the protein band extracted from the gel (9) . A rabbit was immunized initially by subcutaneous and intramuscular injections of eluted protein (1-2 mg) in 0.1% SDS mixed with an equal volume of Freund's complete adjuvant.

RESULTS AND DISCUSSION

Effect of Hemin on Synthesis of ALA Synthase

Drug treated chick embryos were administered hemin or solvent controls and pulse labelled with [^{35}S]-methionine; liver cytosol and

mitochondrial fractions were prepared and immunoprecipitated with antibody to ALA synthase. In the absence of hemin, native ALA synthase (Mr = 68,000) was detected in the mitochondria and precursor ALA synthase (Mr = 74,000) was detected in the cytosol (Fig. 1a, tracks 9 and 10 respectively). However, with hemin treatment, the level of precursor ALA synthase in the cytosol was significantly increased (Fig. 1a, track 12) but no newly synthesised ALA synthase was detected in the mitochondria (Fig. 1, track 11). These results were consistent over several experiments and established that hemin prevents the transfer of newly synthesised ALA synthase into mitochondria. In these experiments there was no effect of hemin on the synthesis of total proteins in either the mitochondrial or cytosol fractions (Fig. 1a, tracks 1-6) and this result was confirmed by determining the total radioactivity in trichloroacetic acid precipitates of each fraction (results not shown).

Effect of Hemin on Synthesis of Pyruvate Carboxylase

The effect of hemin was examined on another mitochondrial matrix protein pyruvate carboxylase.

Experiments in the absence of hemin showed that pyruvate carboxylase can be detected in the cytosol of pulse labelled chick embryos as a precursor of Mr = 117,000 and in the mitochondria as a native form of Mr = 115,000 (Fig. 2). Further details of this will be published elsewhere. The pulse labelled mitochondrial protein co-migrated with purified tritium-labelled chicken pyruvate carboxylase (result not shown).

The effect of hemin on the synthesis of pyruvate carboxylase is shown in Fig. 1b. The extent of labelling of newly synthesized pyruvate carboxylase in the mitochondria is essentially the same in the presence or absence of hemin (Fig. 1b, tracks 5 and 3). Additionally, hemin had no effect on newly synthesized cytosolic enzyme and did not cause any detectable accumulation of enzyme in the cytosol (bands visible in

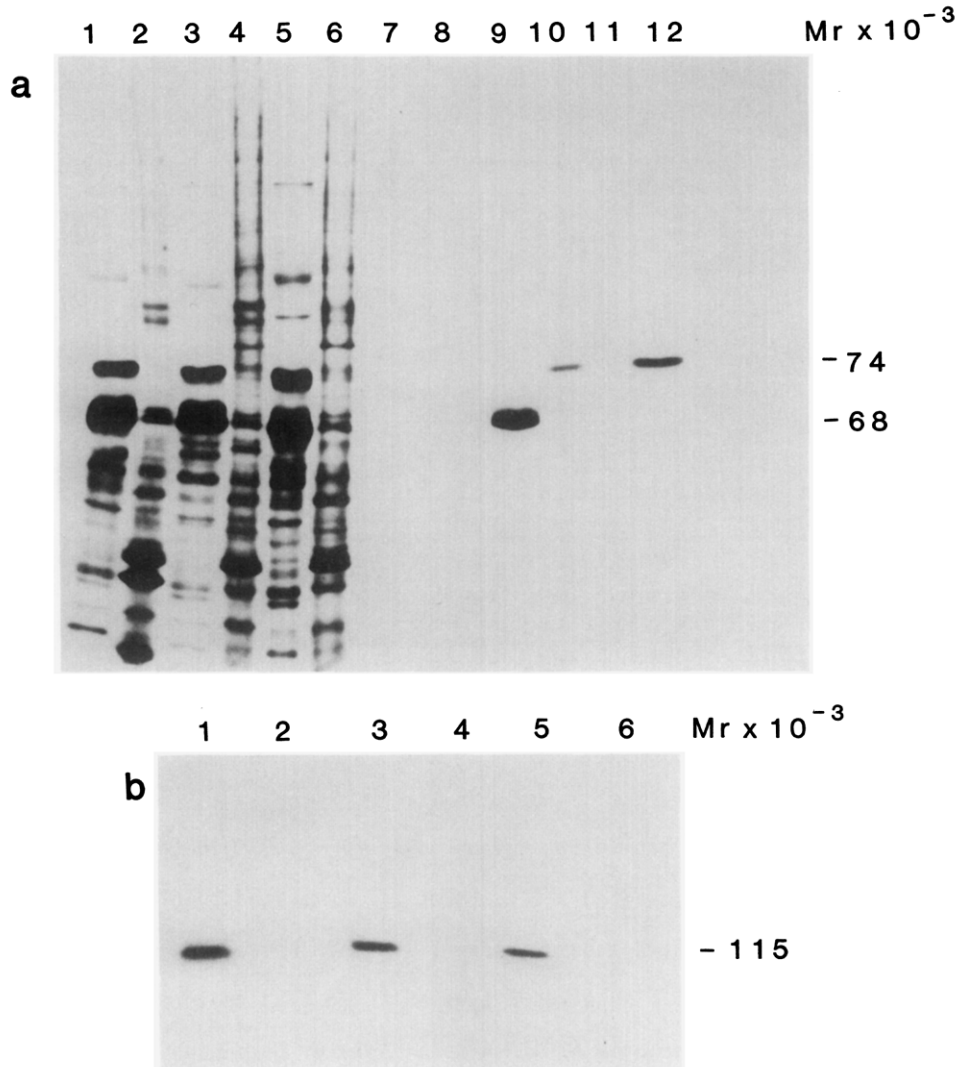

Fig. 1

Effect of hemin on the synthesis of ALA synthase (a) and pyruvate carboxylase (b). Drug treated or control chick embryos were administered hemin (or its solvent) and [^{35}S]-methionine as described in Materials and Methods. Cytosol and mitochondrial fractions were prepared and immuno-precipitated with antibody to either chick embryo ALA synthase or chicken pyruvate carboxylase. Total proteins and immuno-precipitates were analysed by SDS-polyacrylamide gel electrophoresis. Each immuno-precipitation track represents 45% of the respective cell extract from a single chick embryo.

a. Total proteins of mitochondria from control, drug induced and drug induced plus hemin embryos (tracks 1,3,5, respectively); total proteins of cytosol from control, drug induced and drug induced plus hemin embryos (tracks 2,4,6, respectively); immuno-precipitates of ALA synthase from mitochondria and cytosol respectively of control embryos (tracks 7 and 8); drug treated embryos (tracks 9 and 10) and drug treated embryos plus hemin (tracks 11 and 12).

b. Immuno-precipitates of pyruvate carboxylase obtained from mitochondria and cytosol respectively of control embryos (tracks 1 and 2); induced embryos (tracks 3 and 4); induced embryos plus hemin (tracks 5 and 6).

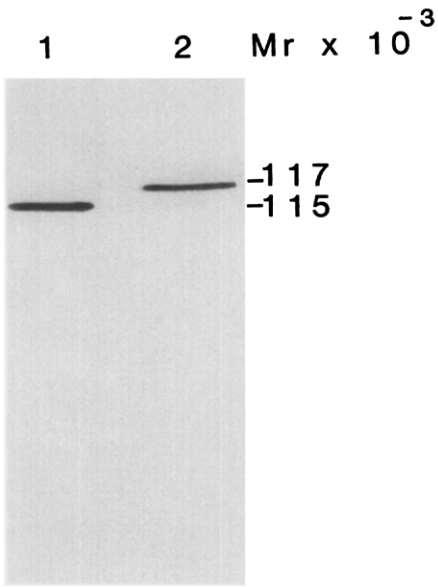

Fig. 2 Immunological identification of mature and precursor forms of pyruvate carboxylase. Five chick embryos were pulse labelled for 20 min. each with 300 μCi of [^{35}S] methionine (about 120 Ci/mmole) and the livers removed and combined. Mitochondrial and cytosol extracts were prepared and immuno-precipitated with pyruvate carboxylase antiserum (2). The immuno-precipitates were heated at 95°C for 2 min. with 2% SDS and analysed by SDS-polyacrylamide gel electrophoresis. Immuno-precipitates of mitochondria (track 1) and cytosol (track 2).

autoradiography but too faint to show up in Fig. 1b). In the same experiment, hemin prevented the transfer of ALA synthase into mitochondria and caused accumulation of labelled precursor enzyme in the cytoplasm (Fig. 1a). These results established that hemin prevents the transfer into mitochondria of ALA synthase precursor but not that for pyruvate carboxylase. This eliminates a general toxic or non-specific effect of hemin on mitochondrial import of proteins and raises the possibility that hemin specifically affects the transfer of ALA synthase into mitochondria.

It has long been known that hemin represses the formation of ALA synthase (10). More recent work (3) has established that in isolated hepatocyte suspensions hemin concentrations as low as 50nM inhibit synthesis of ALA synthase. At this concentration the inhibition mimicked the effect of cordycepin, suggesting that the hemin was acting as a transcriptional inhibitor. Other effects of hemin at the post-

transcriptional level have been suggested (11) but no definitive evidence for this exists. The present work shows that hemin may specifically control the level of mitochondrial ALA synthase by an entirely separate mechanism, that of inhibiting the transfer into mitochondria.

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