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Preferential heme transport through endoplasmic reticulum associated with mitochondria in rat liver

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Abstract

The transport of de novo synthesized protoheme into the conventional microsomal fraction and endoplasmic reticulum associated with mitochondria (MAER) was studied by injecting amino[^{14}C]levulinic acid (ALA) into phenobarbital-treated rats to evaluate the role of MAER in the trafficking of heme between mitochondria and endoplasmic reticulum. In mitochondria, the specific radioactivity of the radiolabeled heme reached a maximum level at 4 min after the injection of ^{14}C-ALA. The specific radioactivity in cytosol was about 2-fold lower than that in microsomes, suggesting that the cytosolic pathway of the heme transport from mitochondria to endoplasmic reticulum is not predominant, because the specific radioactivity of heme in cytosol should be higher than that in microsomes if heme is transported mainly through cytosol. MAER showed higher specific radioactivity than the conventional microsomal fraction up to 4 min and thereafter the specific radioactivities in MAER and the conventional microsomal fraction became nearly the same. The extents of decrease in cytochrome *P*-450 and the radioactivity in microsomes by the treatment with allylisopropylacetamide which destroyed cytochrome *P*-450 but not cytochrome b_5, were essentially the same, suggesting that most of the radiolabeled heme in microsomes was incorporated into cytochrome *P*-450. These results suggest that MAER is a preferential site for the protoheme transport from mitochondria to endoplasmic reticulum.

Key words: Heme; Endoplasmic reticulum; Mitochondrion

1. Introduction

In mammalian cells, the formation of phosphatidylcholine from phosphatidylserine requires two interorganelle translocations because phosphatidylserine synthase and phosphatidylethanolamine methyltransferase are located in endoplasmic reticulum and phosphatidylserine decarboxylase is located in mitochondria [1]. Evidences that the trafficking of the phospholipids between endoplasmic reticulum and mitochondria is performed at specially associated endoplasmic reticulum-mitochondria complex are accumulated [2–4]. Recently, it has been reported that microdomains formed by closely associated endoplasmic reticulum and mitochondria are involved in controlling the Ca^{2+} concentration in the mitochondrial matrix [5]. Ferrocheratase (heme synthase), an enzyme of the final step of heme synthesis, is located in mitochondrial innermembrane and, therefore, protoheme must be transported to endoplasmic reticulum for the synthesis of microsomal cytochromes including cytochrome *P*-450. Meier et al. have proposed that rapidly sedimenting endoplasmic reticulum which is associated with mitochondria is the primary site of the apocytochrome *P*-450 synthesis [6]. The proposition was supported by Picket et al. [7]. But Padmanaban et al. have evaluated the rate of the apocytochrome *P*-450 synthesis by immunoprecipitation and have shown that endoplasmic reticulum associated with mitochondria (MAER) was not a predominant site of the synthesis [8]. Meier et al. have also

Abbreviations: MAER, mitochondria-associated endoplasmic reticulum; ER, endoplasmic reticulum; AIA, allylisopropylacetamide; ALA, δ-aminolevulinic acid; SDS-PAGE, sodium dodecylsulfate-polyacrylamide gel electrophoresis; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

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confirmed that MAER is not a preferential site of the cytochrome *P*-450 synthesis [9]. However, it remains unclear whether MAER is a preferential site of heme transport. Because the transport of protoheme between close juxtaposition of donor and acceptor membranes appears an attractive and plausible mechanism, we re-evaluated the role of MAER in the protoheme transport from mitochondria to endoplasmic reticulum. In this report, we show that MAER is a preferential site of protoheme transport and, therefore, MAER provides important and specialized microdomains for the protoheme transport and the holocytochrome *P*-450 synthesis.

2. Materials and methods

2.1. Materials

Amino[4-^{14}C]levulinic acid (ALA) (2.2 GBq/mmol) was purchased from NEN, Boston, USA. NADPH, $NADP^+$, glucose-6-phosphate dehydrogenase and glucose 6-phosphate were from Oriental Yeast, Tokyo, Japan. Steapsin and allylisopropylacetamide (AIA) from Sigma, St. Louis, MO, USA. EDTA and 2,5-diphenyloxazole (DPO) were from Dojin, Kumamoto, Japan. All other reagents were of analytical grade.

2.2. Treatment of animals and subcellular fractionation

Male Wistar rats, weighing 250–300 g, were used for all experiments. They were provided with a standard diet and water. The animals were pretreated with phenobarbital for 5 days (80 mg/kg of body weight a day). At 24 h after the last injection, they were administered intravenously with ^{14}C-ALA (2 μCi/250 g animal) and killed by bleeding from the carotid artery at the indicated time. The livers were quickly excised, perfused with 0.25 M sucrose and homogenized in 3 volumes of ice-cold buffer containing 0.35 M sucrose, 10 mM $MgCl_2$ and 50 mM Tris-HCl (pH 7.4) with a glass Potter-Elvehjem homogenizer. The homogenate was centrifuged for 10 min at $640 \times g$ and the obtained pellet was suspended in 2 volumes of the buffer containing 0.25 M sucrose, 5 mM $MgCl_2$, and 50 mM Tris-HCl (pH 7.4). The suspension was layered over the buffer containing 0.4 M sucrose, 5 mM $MgCl_2$, and 50 mM Tris-HCl (pH 7.4) and centrifuged at $700 \times g$ for 10 min. The resulting supernatant was centrifuged at $10\,000 \times g$ for 15 min. MAER-mitochondria complex was obtained as a pellet. This pellet was suspended in 0.35 M sucrose, 500 mM KCl, 20 mM EDTA, and 50 mM Tris-HCl (pH 7.4). The suspension was treated with polytron at the maximal output for 5 s, layered over the buffer containing 1.1 M sucrose, 500 mM KCl, 20 mM EDTA, and 50 mM Tris-HCl (pH 7.4), and centrifuged at $24\,000 \times g$ for 2 h. Mitochondria was obtained as a pellet and MAER was recovered between the border of the buffer. MAER was collected as a pellet at $105\,000 \times g$ for 1 h. For preparation of conventional microsomes, the homogenate was centrifuged at $640 \times g$ for 10 min, and then the supernatant was centrifuged at $10\,000 \times g$ for 15 min. The resulting supernatant was centrifuged at $105\,000 \times g$ for 90 min. Mitochondria, MAER, and microsomes were suspended in water.

Cytochrome b_5 was removed from membranes by the method of Omura and Sato [10]. Briefly, microsomes and MAER were incubated with 0.2% steapsin for 1 h at 37°C under nitrogen gas and pellets were obtained after centrifugation at $105\,000 \times g$ for 2 h.

2.3. Electron microscopy of MAER-mitochondria complex

MAER-mitochondria complex was fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) overnight at 4°C. Fixed pellets were cut into small fragments, washed, post fixed with OsO_4, dehydrated with graded series of ethanol, and embedded in Epon. Thin sections were cut with diamond knife, counterstained with uranyl acetate and lead citrate and examined with a Hitachi HU12A electron microscope operating at 75 kV.

2.4. Heme extraction

Heme was extracted from the fractions of mitochondria, microsomes, MAER, and cytosol. Each sample was washed with cold acetone three times and then with chloroform/methanol (2:1, v/v) once to extract lipids and washed with cold acetone once again. Finally heme was extracted with 2 ml of acetone including 90 mM HCl three times. The extracted heme in vials for a scintillation counter was shaken in a bath at 80°C for 15 min to evaporate acetone and discolored by three drops of 8.8 M hydrogen peroxide. The radioactivity of the extracted heme was measured in 5 ml of toluene/Triton X-100 (2:1,v/v) containing 4 g/l of DPO.

To confirm the purity of the extracted radiolabeled heme, we analyzed the methyl ester derivative of heme by thin-layer chromatography using 0.5-mm thick silica gel H plates developed by chloroform/methanol (94:6, v/v). Methyl ester derivative of heme was prepared as follows: The extracted heme was dried and then transformed to its methyl ester derivative by treatment with 1 ml of H_2SO_4/methanol (5:95, v/v) overnight at 4°C in the dark. After addition of a small amount of water, the derivative was extracted with 1 ml of chloroform three times.

2.5. Destruction of cytochrome P-450 by AIA

Microsomes were prepared from phenobarbital-treated rats at 30 min after the injection of ^{14}C-ALA. The AIA treatment of microsomes was performed essentially according to the method of De Matteis [11]. The reaction mixture (1 ml) containing 10 mM nicotinamide, 5 mM glucose 6-phosphate, 5 mM AIA, 5 mM magnesium acetate, 0.5 unit of glucose-6-dehydrogenase, 5 mM $NADP^+$, 100 mM potassium phosphate (pH 7.5), and 8.6 mg of microsomes was incubated at 37°C for 20 min. After addition of 10 volumes of cold 10 mM potassium phosphate buffer (pH 7.5) to stop the reaction, the reaction mixture was centrifuged at $100\,000 \times g$ for 2 h. Microsomes were obtained as a pellet and used for the measurements of cytochrome *P*-450, total heme, and the radiolabeled heme.

2.6. Analytical methods

Protein was determined by the method of Lowry et al. [12] with bovine serum as a standard. SDS-PAGE was done according to the method of Laemmli [13]. The activities of rotenone-insensitive NADPH–cytochrome-*c* reductase, succinate–cytochrome-*c* reductase, and glucose-6-phosphatase were assayed as previously described [1,14,15]. The activity of lactate dehydrogenase was assayed by oxidation of NADH which was measured as reduction of absorbance at 340 nm in the reaction mixture containing 50 mM potassium phosphate (pH 7.5), 10 mM NADH, and 30 mM sodium pyruvate. Cytochrome *P*-450 and cytochrome b_5 were determined by the method of Omura and Sato [16]. Heme was measured by the pyridine hemochromogen method [16].

3. Results

3.1. Separation of endoplasmic reticulum associated with mitochondria

We confirmed morphologically the presence of a single cistern structure of endoplasmic reticulum asso-

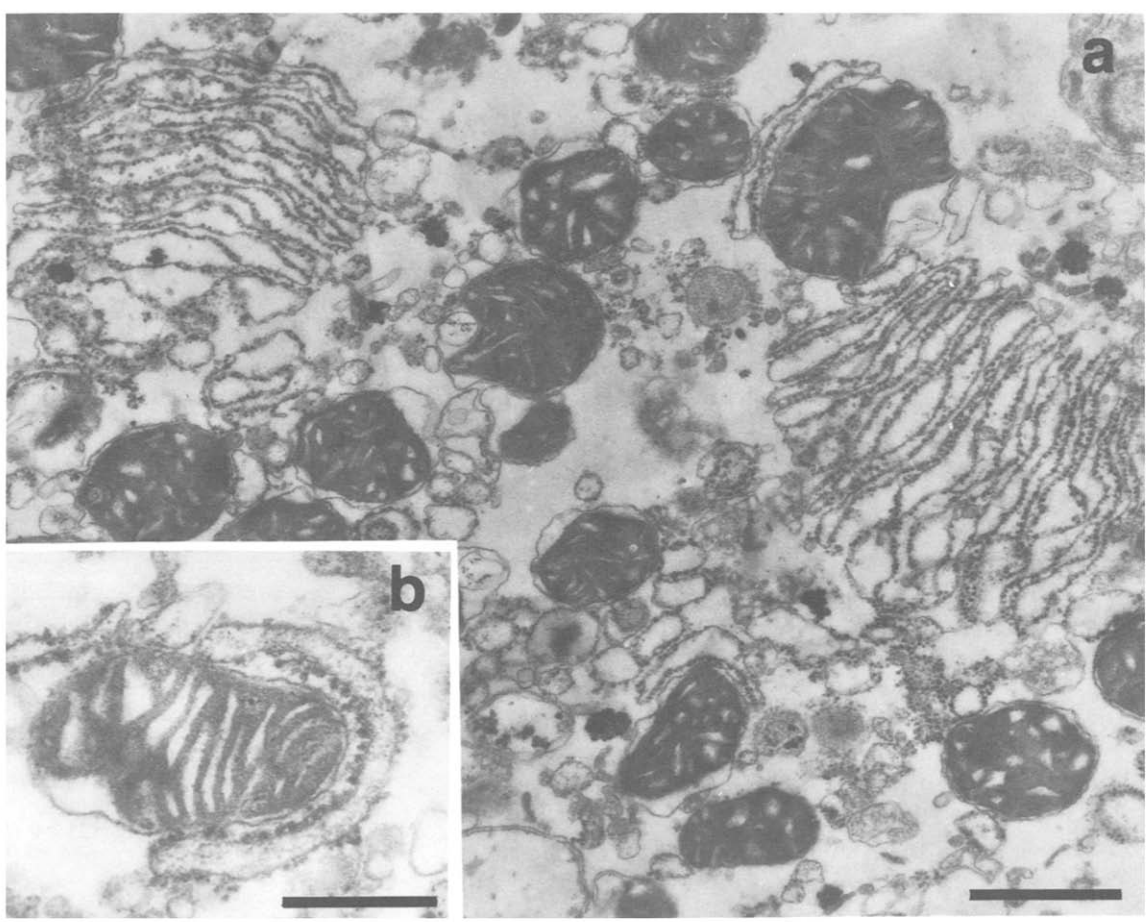

Fig. 1. Electron micrographs of MAER. The MAER-mitochondria complex was prepared as described under Materials and methods. (a) Mitochondria unassociated with ER, mitochondria-ER complex, small vesicles of ER, and ER with a multilamella structure are seen. (b) A typical mitochondria-rough ER complex is shown. Bars in (a) and (b) indicate 1 and 0.5 μm, respectively.

ciated with mitochondria (MAER) by using electron microscopy (Fig. 1). In contrast to small vesicles found in the fraction of conventional microsomes, the presence of cisternae structure of endoplasmic reticulum (ER) is characteristic for the MAER-mitochondria complex. Multi-cisternae structure and small vesicles apparently unassociated with mitochondria were also observed. The complex was separated into mitochondria and endoplasmic reticulum for the measurement of protoheme transport. MAER was well separated from the mitochondria based on the observation that the activity of succinate–cytochrome-*c* reductase, marker enzyme of mitochondria, of the MAER fraction was 1.6% of that in the mitochondrial fraction while the activities of rotenone-insensitive NADPH–cytchrome-*c* reductase and glucose-6-phosphatase in the mitochondrial fraction were 0 and 2.6% of those in the MAER fraction, respectively (Table 1). This was further confirmed by SDS-PAGE (Fig. 2). The separated MAER showed essentially the same pattern of SDS-PAGE as that of conventional microsomes while the unseparated complex showed the mixed pattern of microsomes and mitochondria. The contamination of the cytosolic fraction with ER and mitochondria was negligible (Table 1). The activity of NADPH–cytochrome-*c* reductase in MAER was lower than that in microsomes and the activity of glucose-6-phosphatase in MAER was higher than that in microsomes, which is characteristic for MAER [1].

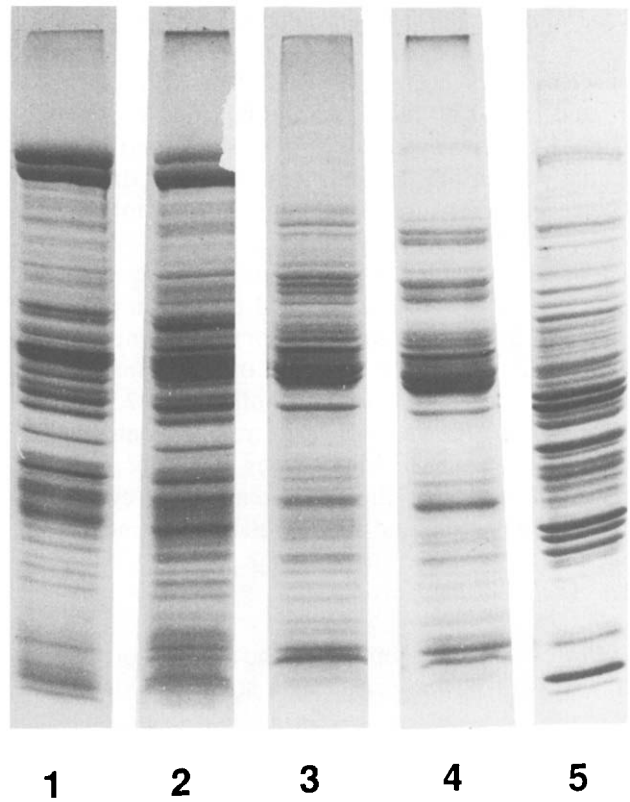

Fig. 2. SDS-PAGE pattern of subcellular fractions from rat liver. Mitochondria, MAER-mitochondria complex, microsomes, and supernatant were prepared as described under Materials and methods. 16 μg of protein for each sample was analyzed by SDS-PAGE. lane 1, mitochondria; lane 2, MAER-mitochondria complex; lane 3, MAER; lane 4, microsomes; lane 5, supernatant.

3.2. Identification of the extracted radioactive substance as protoheme

The radioactive heme was extracted from various subcellular fractions according to the method of heme extraction after ^{14}C-ALA was injected intravenously to rats. We examined by thin-layer chromatography whether the extract was radiolabeled heme. The radioactivities were found as a symmetric single peak where almost all radioactivities were recovered (Fig. 3). The radioactive spot had the same R_f value and color as those of authentic protoheme while ALA remained at the origin on the thin layer. The spot showed no ninhidrin reaction (results not shown). Furthermore,

Table 1
Separation of MAER from the MAER-mitochondria complex

Fraction	*P*-450 (nmol/mg of of protein)	Heme (nmol/mg of protein)	Activity (nmol/min per mg of protein)			
			T-C	S-C	G6Pase	LDH
Mitochondria	–	0.48 ± 0.19	n.d. (0)	387 ± 19.0 (100)	9.5 ± 1.1 (2.6)	0.05 ± 0.001 (0.8)
Microsomes	2.58 ± 0.70	3.36 ± 1.16	124.2 ± 30.7 (100)	1.5 ± 0.1 (0.4)	243 ± 68 (66.8)	0.26 ± 0.01 (4.0)
MAER	1.41 ± 0.36	1.67 ± 0.79	67.0 ± 21.5 (53.9)	6.0 ± 2.3 (1.6)	364 ± 50 (100)	0.04 ± 0.001 (0.6)
Supernatant	–	0.23 ± 0.11	0.7 ± 0.3 (0.6)	n.d. (0)	2.0 ± 0.2 (0.5)	6.53 ± 0.13 (100)

The subcellular fractions were prepared from phenobarbital-treated rats and the activities of marker enzymes were assayed as described under Materials and methods. The protein contents of mitochondria, microsomes, MAER, and supernatant recovered from 10 g of liver were 33.1, 126.4, 12.8, and 348.3 mg, respectively. Each value is the mean ± S.D. (four independent experiments). *P*-450, cytochrome *P*-450; T-C, rotenone-insensitive NADPH–cytochrome-*c* reductase; S-C, succinate–cytochrome-*c* reductase; G6Pase, glucose-6-phosphatase; LDH, lactate dehydrogenase; n.d., not detectable; –, not determined.

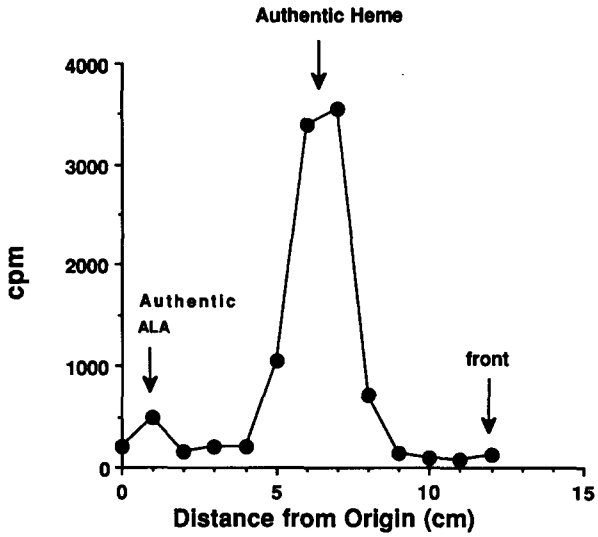

Fig. 3. The analysis of the radiolabeled heme by thin-layer chromatography. The radiolabeled heme was extracted from microsomes as described under Materials and methods. The methyl ester derivative of the extract was analyzed by thin-layer chromatography using a plate coated with silica gel H with thickness of 0.5 mm. The sample was developed with the solution of chloroform/methanol (94:6, v/v). The radioactivity in each 1 cm slice was measured. The arrows indicate the positions of methyl ester derivatives of authentic ALA and heme.

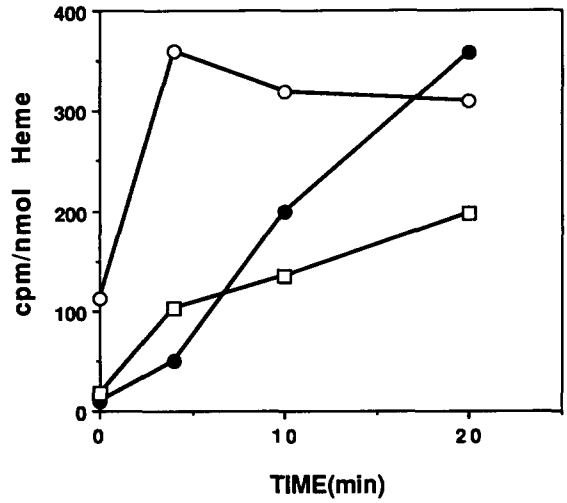

Fig. 4. Time-course of the distribution of the radiolabeled heme. The total heme content and the radiolabeled heme in each fraction were measured as described under Materials and methods. The specific radioactivity is expressed as cpm/nmol heme. Each point is mean of three experiments. The open circle, closed circle, and open square indicate mitochondria, microsomes, and supernatant, respectively.

we observed the same distribution of the radioactivity when heme was labeled with ^{59}Fe (results not shown). These results indicate that the radioactivity, present in the acetone-containing HCl extract, is derived from heme.

3.3. Time-course of the incorporation of radioactivity into subcellular fraction

The radiolabeled heme was expressed as the specific radioactivity per heme (cpm/nmol of heme) instead of

Table 2
Relative distribution of radiolabeled heme

Time	Expt.	Relative distribution (cpm/nmol heme)			
		Mt	Ms	MAER	supernatant
0 min	1	84	3.6	9.8 (2.7)	23
	2	22	1.1	2.2 (2.3)	20
	3	16	1.0	2.8 (2.8)	8
	Mean	41	1.9 [4.6]	5.0 [12.2]	17 [41.5]
4 min	1	207	25	60 (2.4)	137
	2	200	20	46 (2.3)	93
	3	184	12	28 (2.3)	49
	Mean	197	19 [9.6]	45 [22.8]	93 [47.2]
8 min	1	286	188	231 (1.2)	142
	2	240	82	97 (1.2)	57
	3	313	172	214 (1.2)	146
	Mean	280	147 [52.5]	181 [64.6]	115 [41.1]
24 h	1	120	97	108 (1.1)	53
	2	125	120	122 (1.0)	60
	3	133	110	130 (1.2)	60
	Mean	126	109 [86.5]	120 [95.2]	59 [47.1]

Rats were killed at the indicated time after the injection of ^{14}C-ALA and the subcellular fractions were prepared as described under Materials and methods. In the case of 0 min, rats were killed immediately after the injection (within 30 s). Values for microsomes, MAER and supernatant are expressed as percent of the specific radioactivity of heme in mitochondria. The data show values of three independent experiments. Values in round parentheses are ratios of MAER to microsomes. Values in hooked parentheses are% of cpm in mitochondria. Mt, mitochondria; Ms, microsomes; MAER, mitochondria-associated endoplasmic reticulum.

Table 3
Effect of the treatment of microsomes with AIA

	Cytochrome *P*-450 (nmol/mg of protein)	Radioactivity of heme (cpm/mg of protein)
NADPH−(*B*)	2.43	325.3
NADPH+(*A*)	1.63	218.2
Loss (*B* − *A*)	0.80 (33)	107.1 (33)

Microsomes were treated with 5 mM AIA for 20 min at 37°C as described in Fig. 5. Each value is mean of two experiments. Values in parentheses indicate % of loss.

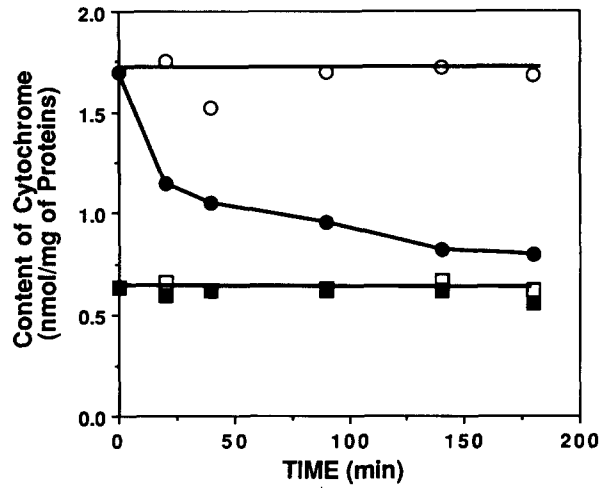

Fig. 5. Effect of AIA on cytochromes b_5 and *P*-450. Microsomes were treated with 5 mM AIA at 37°C with or without NADPH. An aliquot of the reaction mixture (1 ml) was diluted with 10 volumes of cold 10 mM potassium phosphate buffer (pH 7.5) at the indicated time and centrifuged at 100000× *g* for 2 h. The resulting pellet was used for the measurements of cytochromes b_5 and *P*-450. The closed and open symbols indicate the reactions with and without NADPH, respectively. The circle and square indicate contents of cytochrome *P*-450 and cytochrome b_5, respectively.

the specific radioactivity per protein (cpm per mg of protein) for the purpose of examining the chronological relationship of heme incorporation into the subcellular fractions. The radiolabeled heme rapidly appeared in mitochondria and the specific radioactivity reached plateau at 4 min after the injection of ^{14}C-ALA while the specific radioactivity in microsomes continued to increase for 20 min (Fig. 4). The specific radioactivity in MAER was about 2-fold higher than that in the microsomal fraction up to 4 min (Table 2). The tendency of the higher specific radioactivity in the former than that in the latter continued although the difference became smaller with time (Table 2).

The radioactivity per mg of protein in microsomes was also slightly lower than that in MAER up to 4 min and increased gradually to a level approx. 2-fold higher than that in MAER after 20 min (results not shown). Because the specific contents of heme and cytochrome *P*-450 in microsomes were also about 2-fold higher than those in MAER (Table 1), the observation that the ratio of the radioactivity per mg of protein in microsomes to that in MAER was about 2 after 20 min would suggest that most of the radiolabeled heme is incorporated into cytochromes at 20 min. The specific radioactivity per heme, or the newly synthesized heme per cytochrome *P*-450, in MAER was higher than that in microsomes at early time, suggesting that more heme than that required for the synthesis of holocytochrome *P*-450 is transported to MAER initially and, therefore, MAER rather than microsomes seems to be the initial site of heme transport.

The specific radioactivity in the cytosolic fraction was approx. 2-fold lower than those in MAER and microsomes at 20 min while it was higher than that in MAER and microsomes at the early stage (Fig. 4 and Table 2).

3.4. Incorporation of the radioactivity into cytochrome P-450

We studied whether the protoheme incorporated into microsomes is a moiety of cytochrome *P*-450. Allylisopropylacetamide (AIA), the xenobiotic substrate of the mixed function oxidase system, selectively destroys the heme moiety of cytochrome *P*-450 but not that of cytochrome b_5 [11]. Microsomes which were

Table 4
Effect of steapsin on microsomes

Steapsin	% of each cytochrome				% of radioactivity	
	b_5		*P*-450 + *P*-420		heme	
	−	+	−	+	−	+
Supernatant	0	98.9 ± 0.8	0	7.6 ± 1.8	2.0 ± 1.0	13.5 ± 2.1
Pellet	100	1.1 ± 0.8	100	92.4 ± 1.8	98.0 ± 1.0	86.5 ± 2.1

Microsomes were prepared from phenobarbital-treated rats which received 2 μCi/ 250 g body weight and were killed 30 min after the injection. Microsomes (5 mg/ml) in 0.1 M potassium phosphate buffer (pH 7.4) were treated with 0.2% steapsin at 37°C for 1 h. After centrifugation at 100000 × *g* for 2 h, the resulting supernatant and pellets were used for measurements of cytochrome b_5, cytochrome *P*-450/*P*-420 and the radioactivity of heme. The contents of cytochrome b_5 and *P*-450/*P*-420 were 0.87 and 2.82 nmol/mg of protein, respectively. Each value is the mean ± S.D. (four independent experiments).

prepared at 30 min after the injection of ^{14}C-ALA were incubated with AIA in the presence of the NADPH-generating system to destroy the heme moiety of cytochrome *P*-450 (Fig. 5). The loss of the radioactivity in microsomes was essentially the same as the extent of the decrease of cytochrome *P*-450 (Table 3), strongly suggesting that almost all radioactivity was incorporated into cytochrome *P*-450. Even after the treatment of microsomes with steapsin, which released most of cytochrome b_5 from membranes [10], the radioactivity in microsomes remained in the membranes (Table 4). This was consistent with the fact that almost all radiolabeled heme is a moiety of cytochrome *P*-450.

4. Discussion

In this report, we showed that almost all protoheme transported to microsomes might be efficiently incorporated into cytochrome *P*-450. The observation strongly suggests that the synthesis of apocytochrome *P*-450 and the incorporation of heme into apocytochrome *P*-450 is tightly coordinated as previously reported [9] and that the heme transport observed in this report is closely relevant to the physiological events but not to the nonspecific attachment of heme to the membranes.

We found higher specific radioactivity in MAER than that in conventional microsomes at very early stage of the heme transport. Meier et al. have also observed similar results [9]. Although the preferential transport of heme to MAER was observed only transiently, the preferential transport may continue steadily because the synthesis and transport of heme are considered to be under the steady state during the short range of time-course in this study based on the facts that we used rats which were treated successively with phenobarbital for 5 days and the injected ^{14}C-ALA was a trace amount. It is uncertain why the preferential transport of heme to MAER was observed only at early stage. One possibility is that the heme transport through cytosol might mask the preferential transport in the MAER-mitochondria complex. Another possible mechanism may be the rapid translocation of heme from MAER to endoplasmic reticulum (ER). If the translocation of heme from MAER to ER occurs rapidly, the microsomal fraction may have less newly synthesized heme than the MAER fraction only at early stage.

The existence of the transient pool of heme in cytosol and the possible involvement of cytosolic component in the heme transport have been reported [17,18]. We also observed that the specific radioactivity in cytosol is comparable with those in microsomes and mitochondria (Fig. 4). The specific radioactivity in ER should not exceed that in cytosol if all heme is transported to ER from mitochondria via cytosol. The specific radioactivity in microsomes, however, continued to increase over that in cytosol (Fig. 4). This observation contradicts the hypothesis that all heme is transported to ER through cytosol. Because the total heme content of microsomes is much larger than that in cytosol (Table 1), it is unlikely that a large and stable heme pool in cytosol, which is not involved in the heme transport, makes the specific radioactivity in cytosol lower than that in microsomes. Therefore, there must be organelles which have the higher specific radioactivity than ER. Mitochondria are most plausible candidates, although the specific radioactivity in the mitochondrial fraction became slightly lower than that in ER at 20 min (Fig. 4). Because mitochondrial cytochromes are barely induced by phenobarbital, the newly synthesized radiolabeled heme should be mainly transported to other organelles. In contrast, the radiolabeled heme in microsomes was incorporated into cytochrome *P*-450 (Table 3 and 4). Thus, it is reasonable that the specific radioactivity in mitochondria is slightly lower than that in microsomes at later stage. The changes of specific radioactivities with time in the three fractions raise the possibility that considerable amounts of heme are transported directly from mitochondria to ER without passing through cytosol. But we can not rule out the possibility at present that the space between MAER and mitochondria forms the specially confined compartment of cytosol where the heme transport occurs predominantly and such compartmentalization of cytosol makes the observed specific radioactivity of heme in cytosol lower than that in microsomes. In any case, the MAER-mitochondria complex is the most plausible site for the transport of heme to ER.

Although the quantitative contribution of MAER to total heme transport has been estimated to be about 3% judged by the radioactivities incorporated into the MAER and microsomal fractions [9], the true contribution might be higher than 3% if the heme translocation from MAER to microsomes could be considered. Based on the result that heme was about 2-fold more preferentially transported to the MAER fraction than to microsomes (Table 2), we may estimate that the contribution of the MAER-mitochondria complex to the total heme transport could be more than 50%.

It has been reported that MAER shows different distribution of enzymes, amino acid composition and lipids compared with those of endoplasmic reticulum unassociated with mitochondria [1,3]. Thus, MAER has been recognized as a highly specialized subfraction of endoplasmic reticulum for the trafficking of lipids between endoplasmic reticulum and mitochondria. The precise mechanism of the trafficking remains to be solved. It is quite unknown whether the heme transport is performed through the same machinery as that of

the phospholipid transport. Heme synthase is located at the innermembranes of mitochondria, as is phosphatidylserine decarboxylase. Therefore, the contact site of inner and outer membranes of mitochondria might play an important role in the heme transport as in the phospholipid transport [2,4].

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