Catabolism of Heme Moiety of Hemoglobin·Haptoglobin in Rat Liver Cells in Vivo*

(Received for publication, March 30, 1993, and in revised form, June 10, 1993)

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After intravenous administration of [3H-heme,14C-globin]Hb.Hp to rats, the radioactive materials extracted from the liver homogenate or its subcellular fractions were subjected to a gel filtration column. In addition to the 82-kDa component, we found three metabolites of heme moiety of the complex in the subcellular fractions. Intact hemoglobin-haptoglobin complex with 3H, 14C, and 59Fe radioactivities, an 82-kDa component with 3H, 14C, and 59Fe radioactivities, a 40-kDa component with 3H and 59Fe radioactivities, a lower molecular weight component with 3H and 59Fe radioactivities, and a component with 3H and 59Fe radioactivities bound to the microsome, which are referred to as peaks A, B, C, D, and E, respectively. Using a differential centrifugation technique, most of peak B was found in the mitochondria-lysosomal fraction. Peaks C and D were in the 82,500 x g supernatant fraction, while peak D was also found in the mitochondria-lysosomal fraction. The molecular weight of peak C was approximately 40 kDa, and the 3H radioactivity of peak C was eluted at the same fraction as glutathione S-transferases using both gel filtration and ion exchange chromatography. Peak E that was solubilized from the microsomes was found at the microsomal heme protein fraction on gel filtration chromatogram. Some of the 3H radioactivity in the microsomes was partially co-purified with cytochrome b5 fraction. These results indicate that there are at least four metabolites of heme moiety of hemoglobin-haptoglobin complex in rat liver cells during its catabolism and suggest that some of the heme derived from catabolized Hb.Hp binds to glutathione S-transferases in the cytosol and is incorporated into apoheme proteins in the microsomes or at least apocytochrome b5.

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1 The abbreviations used are: Hb-Hp, hemoglobin·haptoglobin complex; PBS, phosphate-buffered saline.
perfusion with 0.01 M phosphate-buffered saline (PBS), pH 7.1, containing heparin through the abdominal vein under anesthesia. The liver was removed, minced, homogenized in 4 volumes of PBS containing 5 mM EDTA using a motor-driven Teflon/glass homogenizer (10 strokes at 3000 rpm), and centrifuged at 740 X g for 10 min at 4°C to remove nuclei and cell debris. The post nuclear supernatant was centrifuged at 10,000 X g for 10 min and at 82,500 X g for 90 min at 4°C, successively. The pellets thus obtained were referred to as the mitochondrion-lysosomal and the cytosolic fractions, respectively. Each 0.2 ml of these specimens with 1H and 3C radioactivity was combusted in an automatic sample combustion system (Aloka ASC-112), and the radioactivity was determined in a liquid scintillation counter (Aloka LSC-900). 14Fe radioactivity in these specimens was determined in a 2-well type scintillation counter (Aloka JPC-207).

Extraction and Gel Filtration Chromatography of Labeled Hb. Hb Hp 
extracted with chloroform and acidic ether, respectively (11, 12). The microsomal fraction was approximately 70-80% and 15-20%, respectively. Water insoluble radioactive materials in the microsomal fraction were extracted with chloroform and acidic ether, respectively (11, 12). The void volume was determined by blue dextran and the elution volume by N-(2,4-dinitrophenyl)-alanine.

Extraction of Heme Metabolites from Each Peak with Organic Solvents—Since heme metabolites such as bilirubin and heme can be extracted with chloroform and acidic ether, respectively (11, 12), the heme metabolite in each peak was extracted with these organic solvents. In brief, peaks B and D were obtained by gel filtration chromatography of the extract of the homogenate. Similarly, peak C was from the cytosol fraction, and peak E was from the extract of the microsomal fraction. Each peak was concentrated by lyophilization. The concentrated sample was dissolved with 20 ml of distilled water. One-tenth volume of a trypsin solution (Mr = 24,000) was then added, and the mixture was stirred for 10 min at room temperature. Chloroform layer and the aqueous layer were collected. Radioactivities of each layer thus obtained were counted.

Partial Purification of Cytochrome b5 from the Microsomes Containing 3H-Heme Metabolites—Heme metabolites of [3H-heme,14C-globin] Hb. Hp in the microsomes were extracted by trypsin digestion (13), by which catalytic heme binding site of cytochrome b5 is released from microsomal membranes. In brief, microsomal pellets were suspended with the aqueous layer and solubilized with 3 ml of 0.1 M potassium phosphate buffer, pH 7.5, and sedimented as before. The final pellets were suspended in 300 ml of the same phosphate buffer to give a protein concentration of 15-20 mg/ml. One-tenth volume of a trypsin solution (3 mg/ml) in 2 mM HCl was added to the suspension of the final pellets, and the mixture was kept at 4°C for 14-16 h. The trypsin-digested suspension was then centrifuged at 82,500 X g for 60 min, and clear red supernatant was collected. The content of cytochrome b5 in microsomes and in trypsin-digested supernatant was determined by measuring the difference between the oxidized and reduced forms of the samples and assuming the extinction difference of the cytochrome between 424 and 409 nm to be 185 cm-1 mM-1 (14). Microsomal suspensions were reduced by NaN3, and the trypsin-solubilized supernatant was reduced by Na2S2O4.

Enzyme Assay—Glutathione S-transferase activity was measured with L2-dichloro-4-nitrobenzene and glutathione (reduced form) according to the method of Habig et al. (15).

Chemicals—b-Amino[2,3-3H]levulinic acid and L-[U-14C]leucine were purchased from Commissariat a l’Energie Atomique. [55Fe]Cl2 was from Du Pont-New England Nuclear. Sephadex G-75, Sephadex G-100, Sepharose CL-6B, and blue dextran were from Pharmacia LKB Biotechnology Inc. Glutathione (reduced form) and 1,2-dichloro-4-nitrobenzene were from Nakarai, Kyoto, and Wako, Tokyo, respectively.

RESULTS AND DISCUSSION

To observe sequential intracellular distribution of Hb. Hp metabolites as clearly as possible, we carried out intravenous administration of small amounts of labeled Hb. Hp (0.1 mg) in our previous studies. By using 0.1 mg of [1H-heme,14C-globin] Hb. Hp, however, we only slightly detected H-heme metabolites by gel filtration chromatography because of its low radioactivity in the eluates. We then administered 1.0 mg of labeled Hb. Hp for analysis of the radioactive heme metabolites. Before analyzing the radioactive heme metabolites, we examined the difference of the catabolism between 0.1 and 1.0 mg of [1H-heme,14C-globin] Hb. Hp administration. Fig. 1A shows the time course of the hepatic uptake of [1H-heme,14C-globin] Hb. Hp, A, the liver was removed and homogenized at the indicated times after intravenous administration of 0.1 mg (X), 0.3 mg (Y; X, Y), 1.0 mg (Z, H; Z, X, Y, Z) or 1.0 mg (Z, H; Z, X, Y, Z) of [1H-heme,14C-globin] Hb. Hp to rats. The total liver radioactivity was determined by measuring radioactivity in an aliquot of the homogenate. Each value is the mean ± S.D. of three separate experiments. B, molar ratio of [1H-heme]/[14C-globin] is plotted at the indicated time (0, 0.1 mg of Hb. Hp; 0, 1.0 mg of Hb. Hp).
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Each fraction was determined by measuring radioactivity in an aliquot administered, both \([^{3}H]\) heme and \([^{14}C]\) globin uptake attained a maximum in the liver at 40 min. By increasing the amount to 1.0 mg, the time of maximal uptake shifted from 40 to 90 min. However, the maximal uptake of both moieties was increased 10-fold in response to the increase in the amounts of Hb-Hp administered. As shown in Fig. 1B, in both cases of administration, the molar ratio of \([^{3}H]\) heme to \([^{14}C]\) globin increased gradually with time. These results suggest that 1.0 mg of Hb-Hp was taken up by the liver in the same manner as 0.1 mg of Hb-Hp, despite the shift of the time of maximal uptake, which may be caused by a restricted receptor binding capacity and/or intracellular catabolizing capacity for Hb-Hp and also suggest that the rate of catabolism of the heme moiety is different from that of the globin moiety. To determine whether there are any organelles accumulating the heme metabolites, the uptake of the radioactivity in the subcellular fraction was investigated after various amounts of doubly labeled Hb-Hp were administered. Fig. 2 shows the dose dependence of the uptake of \([^{3}H]\) heme into the subcellular fraction. The amount of \([^{3}H]\) heme taken up by each subcellular fraction increased in proportion to the amount of Hb-Hp administered. The \([^{14}C]\) globin also increased in the same manner (data not shown). The result indicates that there were no organelles accumulating the heme metabolites by increasing the amounts of Hb-Hp, suggesting that 1.0 mg of Hb-Hp may be catabolized in the same manner as 0.1 mg of Hb-Hp despite the shift in time of maximal uptake from 40 to 90 min.

At 90 min after the administration of 1.0 mg of \([^{3}H]\) heme, \([^{14}C]\) globin/Hb-Hp, subcellular fractionation was carried out, and water soluble \({}^{3}H\) and \({}^{14}C\) radioactive materials were extracted from the subcellular fractions by repeated freezing and thawing. As shown in Fig. 3, the molar amount of \([^{3}H]\) heme taken up by the 750 \(\times\) g pellet, the mitochondria-lysosomal fraction, and the microsomal fraction was about \(s\)-fold that of \([^{14}C]\) globin except for the 82,500 \(\times\) g supernatant fraction, and the extraction efficiency of both \({}^{3}H\) and \({}^{14}C\) radioactivities from the 750 \(\times\) g pellet, the mitochondria-lysosomal fraction, and the microsomal fraction was approximately 40, 60, and 10\%, respectively. Labeled Hb-Hp metabolites in the extract were analyzed by gel filtration chromatography. Fig. 4, A–C, shows the gel filtration chromatogram of the radioactive materials extracted from the 750 \(\times\) g pellet, the mitochondria-lysosomal fraction, and the 82,500 \(\times\) g supernatant fraction, respectively. Most of peak \(B\) with both \({}^{3}H\) and \({}^{14}C\) radioactivities was found in the mitochondria-lysosomal fraction. Peak \(B\) is consistent with an 80-kDa protein by the elution profile of standard proteins and possessed both \({}^{3}H\) and \({}^{14}C\) radioactivities. Therefore, it was concluded that peak \(B\) is an 82-kDa subunit symmetrically dissociated from Hb-Hp, which has been reported in the previous studies (3, 4). In contrast, peaks \(C\) and \(D\) with only \({}^{3}H\) radioactivity were found in the 82,500 \(\times\) g supernatant fraction, and in both the mitochondria-lysosomal and the 82,500 \(\times\) g supernatant fraction, respectively. \({}^{14}C\) radioactive material with low molecular weight was also observed at almost the same fraction as peak \(D\) with \({}^{3}H\) radioactivity. Since the ratio of \({}^{3}H\) to \({}^{14}C\) radioactivity in this lower molecular weight fraction changed with time and in the subcellular fraction, peak \(D\) was defined as the material with only \({}^{3}H\) radioactivity. The \({}^{14}C\) radioactive materials seem to be amino acid degradation products from the protein moiety of Hb-Hp (3). Radioactive materials extracted from the microsomal fraction could not be applied to

![Fig. 2. Dose dependence of the uptake of \([^{3}H]\) heme by subcellular organelles.](image)

![Fig. 3. Subcellular distribution of \([^{3}H]\) heme and \([^{14}C]\) globin and extraction of radioactivity from each fraction.](image)
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FIG. 4. Analysis of \[^{3}H\text{-heme,}{^{14}}C\text{-globin}\]Hb-Hp metabolites by gel filtration chromatography. Subcellular fractions were obtained at 90 min after intravenous administration of 1.0 mg of \[^{3}H\text{-heme,}{^{14}}C\text{-globin}\]Hb-Hp. The extract from the 750 \(\times\) g pellet (A) and the mitochondria-lysosomal fraction (B) by repeated freezing and thawing and the 82,500 \(\times\) g supernatant fraction (C) were applied on a Sephadex G-100 column attached to a Sephadex G-75 column. The microsomes were solubilized with 2.5% Triton X-100, and the solubilized fraction was applied on a Sepharose CL-6B column (D). Radioactivity (\(\bullet\), \(^{3}H\); \(\bigcirc\), \(^{14}C\)) in the effluent was measured.

the column because of the low yield, about 10% (Fig. 3). This indicates that most of the radioactive materials in the microsomal fraction bind to the microsomal membranes. Therefore, the radioactive materials were extracted with PBS containing 2.5% Triton X-100 and 0.2 M NaCl after removing the extract by freezing and thawing. Fig. 4D shows the Sepharose CL-6B gel filtration chromatogram of the extract. Peak E, with only \(^{3}H\) radioactivity, was eluted immediately after transferrin (\(M_{r}\) = 80,000) (data not shown).

We next examined the time course of the metabolism of these peaks. After the administration of labeled Hb-Hp (1.0 mg), the liver was removed at various times. Radioactive materials extracted from the liver homogenate by repeated freezing and thawing (yields of 50–60%) were analyzed by gel filtration through a Sephadex G-100 column. As shown in Fig. 5, peak A (intact Hb-Hp) increased up to 45–90 min and then decreased. Peak B increased gradually up to 90 min and then decreased. Peaks C and D appeared after 90 min. These results imply that Hb-Hp was degraded into peak B, and thereafter the heme moiety of peak B was transferred to peak C, D, or E. It is also suggested that the increase in the ratio of \(^{3}H\) to \(^{14}C\) radioactivity (Figs. 1 and 3) is related to the appearance of peaks C and D with only \(^{3}H\) radioactivity (Figs. 4 and 5).

An experiment using \[^{59}Fe\text{-heme}\]Hb-Hp was carried out to determine whether the heme moiety of these peaks is heme or its degradation products, such as biliverdin or bilirubin. After the administration of \[^{59}Fe\text{-heme}\]Hb-Hp (1.0 mg), the 750 \(\times\) g pellet, the mitochondria-lysosomal fraction, the microsomal fraction, and the 82,500 \(\times\) g supernatant fraction were successively obtained by differential centrifugation. Fig. 6A shows the subcellular distribution of \[^{59}Fe\]heme and the yields of the radioactivity extracted by repeated freezing and thawing. The yields were almost the same as that of \(^{3}H\) and \(^{14}C\) radioactivities (Fig. 3). These extracts were subjected to gel filtration chromatography. In the mitochondria-lysosomal fraction, peaks A, B, and D were found, but peak C was not found (Fig. 6B). In contrast, peaks A, C, and D were found in the 82,000 \(\times\) g supernatant fraction (Fig. 6C). These results are consistent with the results using \[^{3}H\text{-heme,}{^{14}}C\text{-globin}\]Hb-Hp (Fig. 4). After removing water soluble \(^{59}Fe\) radioactive material from the microsomal fraction, membrane bound \(^{59}Fe\) radioactive materials were extracted with PBS containing 2.5% Triton X-100 and 0.2 M NaCl. Fig. 6D shows the gel filtration chromatogram of the extract, indicating that \(^{59}Fe\) radioactivity was observed in the same fraction as peak E with \(^{3}H\) radioactivity (Fig. 4D). In addition, the elution dia-
performed an extraction experiment of 3H and 59Fe radioactive B, ether, as described under "Materials and Methods." As shown peak was extracted successively with chloroform and acidic most of these radioactivities in this wavelength. All peaks we found have both 3H and 59Fe peaks is heme itself, not degradation products by heme oxy-
in Table I, most of both 3H and 59Fe radioactivities with organic solvents. The heme metabolite of each
gram of peak E was consistent with that of absorbance at 410 nm (Fig. 6D); hemeproteins exhibit maximal absorption near this wavelength. All peaks we found have both 3H and 59Fe radioactivities, suggesting that the heme metabolite of these peaks is heme itself, not degradation products by heme oxygenase or biliverdin reductase. In addition, these results suggest that 3H and 59Fe-heme moiety of peak E may occur in heme proteins in the microsomes.

To further verify the heme metabolite of each peak, we performed an extraction experiment of 3H and 59Fe radioactive materials with organic solvents. The heme metabolite of each peak was extracted successively with chloroform and acidic ether, as described under "Materials and Methods." As shown in Table I, most of both 3H and 59Fe radioactivities in peaks B, C, and E were extracted with an acidic ether layer, but most of these radioactivities in peak D were recovered with a final aqueous residue. Since heme is extracted with acidic ether (12), these results strongly suggest that heme metabolite of peaks B, C, and E is heme. We do not know what the heme metabolite of peak D is, but it is not bilirubin or biliverdin, because peak D possessed 59Fe radioactivity. In a previous study (4), Oshiro and Nakajima found that only 3H-heme metabolite was present in the organelles of higher anodic mobility in carrier-free electrophoresis, in which mitochondria, lysosomal, and microsomal marker enzymes were demonstrated, at 90 min after intravenous administration of [3H-heme,14C-globin]Hb-Hp, suggesting that heme moiety that had detached from Hb-Hp binds to a certain protein other than globin in the organelles. In addition, from analysis of the radioactive heme metabolites by high performance liquid chromatography after extraction with acidic ether, the major radioactive material was identified as heme. The present results coincide with the previous study, and it may be inferred that major organelles of higher anodic mobility containing only 3H-heme are the microsomes.

It was suggested from the gel filtration chromatography that peaks C and E are proteins containing heme derived from catabolized Hb-Hp. We then performed an experiment to investigate the molecular nature of the proteins. Peak C was found in the 82,500 x g supernatant fraction and the molecular mass of peak C was estimated to be 40 kDa by the elution profile of standard proteins (data not shown). These results imply that peak C may be glutathione S-transferases, which are present in cytosol in rat liver and bind heme (16-18). We then measured 3H radioactivity and glutathione S-transferase activity in the effluents of a Sephadex G-100 gel filtration chromatography column of the 82,500 x g supernatant fraction containing 3H-heme metabolites. As shown in Fig. 7A, peak C was consistent with that of the enzyme activity. We partially purified the enzyme from the 82,500 x g supernatant. The 82,500 x g supernatant fraction with the 3H radioactivity was applied to a DEAE-cellulose column, pH 8.5. The fraction that passed through the column was further applied on a Sephadex G-100 column. As shown in Fig. 7B, peaks A and B disappeared in the effluents, and peak C and glutathione S-transferase activity were observed in the same fraction. These results suggest that glutathione S-transferases may bind heme detached from catabolized Hb-Hp. It has been reported by many investigators that glutathione S-transferases bind heme in vivo (19, 20). In particular, Senjo et al. (21) reported that some glutathione S-transferases can transfer heme "synthe-
ized" in mitochondria to apocytochrome b5 in microsomes. Unlike their result, our data suggest that glutathione S-transferases can also bind to "exogenous" heme from Hb-Hp in the liver. At least 12 glutathione S-transferase subunits have been identified and shown to be members of four families, referred to as α, µ, π, θ and δ (22). Rat liver, which contains at least 100 µM total glutathione S-transferases, is rich in subunits 1, 2, 3, and 4 (23). Different homodimeric and heterodimeric combinations of various subunits constitute the many kinds of glutathione S-transferase isozymes. Some controversy, however, exists regarding the function of glutathione S-transferases as heme carriers (24). Recently, Zilber et al. (25) also expressed doubts about the function because heme binding to glutathione S-transferases induces irreversible conformational changes in the proteins, and the protein synthesis is not linked to the need of the cell to transport heme between cell organelles (25). To solve this controversy, it is important to identify the molecular nature of peak C.

We measured an absorption spectrum of the fraction containing peak E to clarify the molecular nature of peak E with

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**Fig. 5. Time course of [3H-heme,14C-globin]Hb-Hp metabolites.** The homogenate was obtained at the indicated times after intravenous administration of 1.0 mg of [3H-heme,14C-globin]Hb-Hp. Water soluble extract from the homogenates was applied on a Sephadex G-100 column, and radioactivity (0, 3H; ·, 14C) in the effluent was measured. Vertical left and right axes represent 3H and 14C radioactivity, respectively.
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Fig. 6. Subcellular distribution of $^{59}$Fe-heme radioactivity and analysis of $^{59}$Fe-heme$\cdot$Hb$\cdot$Hp metabolites. A, the 750 $\times$ g pellet (Pe), the mitochondria-lysosomal fraction (M.L.), the microsomal fraction (Ms), and the 82,500 $\times$ g supernatant fraction (Sup) were obtained at 90 min after intravenous administration of 1.0 mg of $^{59}$Fe-heme$\cdot$Hp (open bars). Water soluble $^{59}$Fe-heme$\cdot$Hp metabolites were extracted by repeated freezing and thawing and expressed as molar amount of heme (solid bars). Each value is the mean $\pm$ S.D. of three separate experiments. Extract from the mitochondria-lysosomal fraction (B) and the 82,500 $\times$ g supernatant (C) were applied on a Sephadex G-100 column attached to a Sephadex G-75 column. The microsomes were solubilized with 2.5% Triton X-100, and the solubilized fraction was applied on a Sepharose CL-6B column (D). $^{59}$Fe-Radioactivity (O) and an absorbance at 410 nm (D) in effluent were measured. B.D., blue dextran; DNP-Ala, dinitrophenyl alanine.

Table I

<table>
<thead>
<tr>
<th>Peak</th>
<th>CHCl$_3$</th>
<th>Acidic ether</th>
<th>Aqueous residue</th>
<th>% total radioactivity</th>
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<tr>
<td>B $^3$H</td>
<td>5.5</td>
<td>75.0</td>
<td>19.5</td>
<td>75.0</td>
</tr>
<tr>
<td>$^{59}$Fe</td>
<td>64.6</td>
<td>73.0</td>
<td>20.6</td>
<td>73.0</td>
</tr>
<tr>
<td>C $^3$H</td>
<td>5.5</td>
<td>75.5</td>
<td>19.0</td>
<td>75.5</td>
</tr>
<tr>
<td>$^{59}$Fe</td>
<td>6.0</td>
<td>80.0</td>
<td>14.0</td>
<td>80.0</td>
</tr>
<tr>
<td>D $^3$H</td>
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<td>6.4</td>
<td>88.5</td>
<td>6.4</td>
</tr>
<tr>
<td>$^{59}$Fe</td>
<td>0.1</td>
<td>2.6</td>
<td>97.3</td>
<td>2.6</td>
</tr>
<tr>
<td>E $^3$H</td>
<td>7.0</td>
<td>71.0</td>
<td>22.0</td>
<td>71.0</td>
</tr>
<tr>
<td>$^{59}$Fe</td>
<td>0.7</td>
<td>78.0</td>
<td>20.3</td>
<td>78.0</td>
</tr>
</tbody>
</table>

$^3$H and $^{59}$Fe radioactivity. As shown in Fig. 8, the fraction exhibited a typical absorption spectrum of heme protein, namely an absorption maximum at 410 nm as the Soret band and $\alpha, \beta$ band between 500 and 600 nm. The elution diagram of $^{59}$Fe radioactivity was consistent with that of the absorbance at 410 nm (Fig. 6D). Almost the same result was observed in peak E with $^3$H radioactivity in Fig. 6D (data not shown). These results suggest that heme of Hb$\cdot$Hp may be incorporated into apoheme proteins in microsomes. Therefore, we tried to separate heme protein, or cytochrome $b_6$, from the microsomes containing the $^3$H radioactive materials. The microsomes containing the $^3$H radioactivity were treated with trypsin under the conditions in which the heme binding fragment of cytochrome $b_6$ is released (13). Fig. 9 shows a gel filtration chromatogram of the fraction treated by trypsin. Cytochrome $b_6$ was demonstrated at the same fraction as $^3$H radioactive material. By means of trypsin digestion, 70–80% of cytochrome $b_6$ was recovered from the microsomes, while the yield of $^3$H and $^{59}$Fe radioactivities was approximately 30%. This result indicates the possibility that other microsomal apoheme protein or apocytochrome P-450, may also bind the heme derived from catabolized Hb$\cdot$Hp. This possibility must be left for further investigation. Our finding that heme of Hb$\cdot$Hp may be incorporated into apoheme protein in the microsomes is very important to understand heme metabolism.
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1.0
5.0
0.0
0 20 40 60 80 100
Fraction No.

2.0 1.0 0.5 0.0
0 20 40 60 80 100

2.0 1.0 0.5 0.0
0 20 40 60 80 100

FIG. 7. Partial purification of glutathione S-transferase (GST) from the supernatant fraction containing [3H-heme, 14C-globin]Hb. Hp metabolites. The 82,500 x g supernatant fraction was obtained at 90 min after intravenous administration of 1.0 mg [3H-heme, 14C-globin]Hb. Hp. The fraction was divided in two portions. One portion was applied on a Sephadex G-100 column (A). The other portion was applied on a DEAE-cellulose column, pH 8.5, and the fraction passed through the column was concentrated by lyophilization. The concentrated sample was further applied on a Sephadex G-100 column (B). Glutathione S-transferase activity (C) and 3H radioactivity (D) in effluent was measured as described under “Materials and Methods.” B.D., blue dextran; DNP-Ala, dinitrophenyl alanine.

In liver cells. Our data indicate two possibilities of heme metabolism. 1) Some of the heme derived from catabolized Hb. Hp may be incorporated into apoheme proteins in the microsomes and function as the prosthetic group of hemeprotein. 2) Some of the heme derived from catabolized Hb. Hp may be temporarily incorporated into apoheme protein and then degraded into bilirubin and excreted into the bile duct.

In the present study we found three Hb. Hp metabolites (peaks B, C, and D) using the extract from organelles destroyed by repeated freezing and thawing. Therefore, we cannot rule out the possibility that heme moiety may be artificially released from catabolized Hb. Hp and further bind a certain heme carrier during the preparation. Even if so, however, the heme moiety seems not to be derived from peak B because peak B always possessed the same ratio of 3H to 14C radioactivity as intact doubly labeled Hb. Hp (Figs. 4 and 5). Since 3H radioactivity in the lower molecular weight fraction was observed as peak D in Fig. 7B and Fig. 9, the 3H radioactive material of peak D may be one released from catabolized Hb. Hp, for example peaks C and E, during the preparation. We isolated peak E from the extract with 2.5% Triton X-100. Since a significant amount of 3H-heme and 59Fe-heme metabolite was incorporated into the microsomal fraction and most of these radioactive materials could not be extracted by the freezing and thawing, the heme moiety of peak E firmly binds apoheme proteins, suggesting that peak E is not an artifact of the preparation. Peak C also does not seem to be an artifact, because heme carrier in cytosol is
necessary to carry heme derived from catabolized Hb·Hp to apoheme proteins in the microsomes.

REFERENCES