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THE BIOSYNTHESIS OF CYTOCHROME P450 BY HEAVY AND LIGHT ROUGH ENDOPLASMIC RETICULUM OF RAT LIVER

Michael B. Cooper, John A. Craft*, Margaret R. Estall and Brian R. Rabin

Department of Biochemistry, University College London, Gower Street

London WC1E 6BT, Great Britain

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SUMMARY

The synthesis of cytochrome P450 by heavy rough endoplasmic reticulum and light rough endoplasmic reticulum has been examined in vitro, using immunochemical techniques. Contrary to previous indications the results show no evidence for preferential segregation of the cytochrome P450 m-RNA and that the presence of mitochondrial protein synthesis accounts for the differences that have previously been reported.

Two morphologically distinct types of rough endoplasmic reticulum can be isolated from rat liver. The heavy rough fraction can be prepared by centrifugation at low g-forces and consists of lamina stacks of ribosome-studded membranes closely associated with mitochondria [1]. In contrast, the light rough endoplasmic reticulum, is isolated from a post mitochondrial supernatant by centrifugation at high g-forces. This fraction consists of discrete ribosome-studded vesicles [2]. Both types of membrane are active in protein synthesis in vitro and have been shown to synthesize proteins destined for export, [3,4], and intrinsic membrane proteins [4].

Attempts to separate mitochondria from reticular membranes found in heavy rough fractions have been unsuccessful [1,5]. This close association suggests a possible functional relationship between the two organelles. Since mitochondria are the site of haem synthesis [6], whilst membranes are the site of apo-cytochrome synthesis [4,7,8], such a relationship might be expected to exist for the biosynthesis of haemo-proteins. Under

* Current address: Glasgow College of Technology, Cowcaddens Road, Glasgow, Great Britain.

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conditions of cytochrome P450 induction, a co-ordinated increase in the production of haem and the apo-cytochrome has been reported [9,10].

Various attempts have been made to locate the intracellular site of cytochrome P450 synthesis. Earlier reports of the presence of nascent cytochrome P450 associated with free polysomes [11] have been contradicted and synthesis of the cytochrome appears to exclusively restricted to membrane-bound polysomes [8,12]. A recent report has presented evidence that the cytochrome is preferentially synthesized by the light rough endoplasmic reticulum [13] whilst other workers have inferred preferential synthesis by the heavy rough endoplasmic reticulum, [14,15]. In this study we present a possible explanation for these differences and provide evidence which suggests that both types of membrane are equally active in the biosynthesis of cytochrome P450 in vitro.

METHODS

Male Sprague-Dawley rats, 200-250 g, permitted food and water adlibitum were used in this study. Animals received a single i.p;injection of phenobarbital (40 mg/kg in 0.9% w/v NaCl) on each of 4 days prior to sacrifice on day 5.

Fractions of heavy and light rough endoplasmic reticulum were isolated from the same homogenate of liver. The heavy rough endoplasmic reticulum was prepared as previously described [4]. Light rough endoplasmic reticulum was prepared from the resultant post-mitochondrial supernatant by fractionation on a discontinuous sucrose gradient system as described by Blyth et al [16]. Protein synthesis in vitro was carried out as in [4], but using [^{14}C] protein hydrolysate (50 m Ci/m atom carbon, Radiochemical Centre, Amersham) at 4μ Ci/ml and supplemented with those amino acids not present in the hydrolysate. Incorporation of radioactivity into total protein was estimated by precipitation with trichloroacetic acid [17]. Following protein synthesis in vitro, the membrane fractions were harvested by centrifugation at 105,000 g $_{(max)}$ for one hour at 4°C. The membrane pellet was resuspended to 20 mg/ml protein in a solution containing sodium phosphate (0.1M) pH 7.7, glycerol (20% v/v), EDTA (1mM), and sucrose (0.25M). The suspension of membrane was solubilized by the addition of sodium cholate (3mg/mg membrane protein) and non-solubilized material removed by centrifugation as above. The resultant supernatant, which contained greater that 90% of the incorporated radioactivity was analysed by quantitative immunoprecipitation with a specific antibody to cytochrome P450 [4]. Protein was determined by the method of Lowry [18], RNA by the method of Fleck and Begg [19]. Phospholipid was extracted by the method of Folch [20] and lipid phosphorus subsequently estimated by the method of Chen [21]. Succinate dehydrogenase was assayed by the method of Pennington [22], cytochrome P450 by the method of Omura and Sato [23] and glucose-6-phosphatase by the method of Løskes and Siekivitz [24].

TABLE 1

Chemical and enzymic content of heavy and light rough endoplasmic reticulum

	Heavy rough endoplasmic reticulum	light rough endoplasmic reticulum
RNA (mg RNA/mg protein)	0.05 ± 0.02	0.13 ± 0.01
Phospholipid (µg Pi/mg protein)	3.2 ± 1.2	14.2 ± 1.5
Succinate dehydrogenase (µmol x h^{-1} x mg protein $^{-1}$)	0.08 ± 0.005 (19 ± 5)	0.04 ± 0.002 (5 ± 2)
Glucose-6-phosphatase (µmol x h^{-1} x mg protein $^{-1}$)	0.11 ± 0.01 (28 ± 5)	0.15 ± 0.02 (24 ± 4)
Cytochrome P450 (n mole x mg $Protein^{-1}$)	0.45 ± 0.05	1.40 ± 0.05

The chemical and enzymic activities of the two membrane types were determined as outlined in the Methods. The specific activity of succinate dehydrogenase is expressed as µmol 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-tetrazolium reduced x h^{-1} x mg $protein^{-1}$, and of glucose-6-phosphatase as µmoles Pi released x h^{-1} x mg protein $^{-1}$. The figures in parenthesis are the percentages of the total homogenate activity recovered in each fraction.

RESULTS AND DISCUSSION

The fractions of heavy and light rough endoplasmic reticulum were characterized by their chemical and enzymic content shown in Table 1. The presence of mitochondria in the fraction of heavy rough endoplasmic reticulum is clearly indicated by the high specific activity of succinate dehydrogenase present. A considerable proportion of the succinate dehydrogenase activity of the homogenate is recovered in this fraction. The presence of mitochondria in this fraction results in decreased RNA/protein and phospholipid/protein ratios.

Under the conditions of incubation, both membrane fractions incorporate radio-labelled amino acids into material insoluble in trichloroacetic acid, in a linear fashion (figure 1). In the data

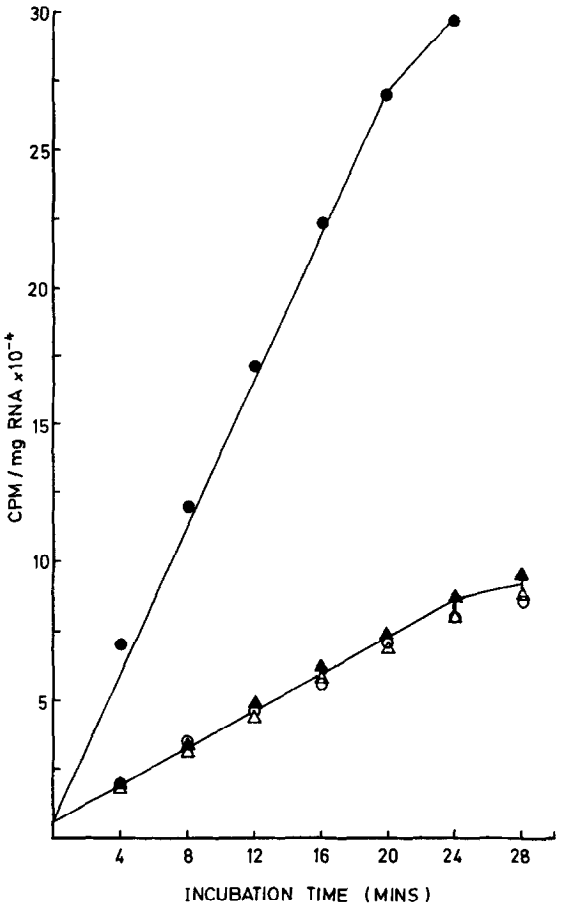

FIGURE 1

Kinetics of incorporation in vitro of [^{14}C] amino acids into trichloroacetic acid insoluble material by heavy and light rough endoplasmic reticulum. Protein synthesis in vitro was carried out as described in the Methods. Incorporation by heavy rough endoplasmic reticulum is shown in the presence (▲) and absence (●) of 5 x 10^{-6}M chloramphenicol. Incorporation by light rough endoplasmic reticulum is shown similarly in the presence (△) and absence (○) of the antibiotic.

presented in figure 1, incorporation by heavy rough endoplsamic reticulum is much greater than by light rough endoplasmic reticulum. We have considered that this discrepancy arises because of the varying levels and activities of the mitochondrial protein synthesizing system present in the heavy rough fraction.

The antibiotic chloramphenicol, selectively inhibits mitochondrial protein synthesis [25]. The inclusion of chloramphenicol in the protein

synthesizing incubation containing heavy rough endoplasmic reticulum resulted in a decreased incorporation of radio-labelled amino-acids. Further, in the presence of the antibiotic the incorporation was then the same as that found for the light rough membrane. Chloramphenicol had no effect on incorporation of radio-labelled amino acids by light rough endoplasmic reticulum (see figure 1). The protein synthesizing activity of the mitochondria in the fraction of heavy rough endoplasmic reticulum can distort comparisons of incorporation into specific proteins by the two membranes types. We have examined the biosynthesis of cytochrome P450 by both types of membrane using a specific antibody to the cytochrome. The result of these analyses are shown in table 2. Where chloramphenicol is not included in the incubation the biosynthesis of cytochrome P450 appears to be preferentially located in the light rough endoplasmic reticulum. Although the inclusion of chloramphenicol in incubations containing heavy rough endoplasmic reticulum results in diminished protein synthesis, the incorporation of radioactivity into cytochrome P450 is unaltered.

TABLE 2

The incorporation of radioactivity into cytochrome P450 by heavy and light rough endoplasmic reticulum.

$[^{14}C]$ precipitated by antibody/$[^{14}C]$ precipitated by trichloroacetic acid in fraction.

	Heavy rough endoplasmic reticulum	Light rough endoplasmic reticulum
Control	0.05 ± 0.01	0.10 ± 0.02
+chloramphenicol	0.10 ± 0.02	0.12 ± 0.01

Protein synthesis *in vitro* was carried out for 15 minutes, at which time incorporation of $[^{14}C]$ amino acids was still linear. Subsequent recovery and solubilization of the two types of endoplasmic reticulum were as described in the Methods. Immunoprecipitations using a specific antibody to cytochrome P450 were carried out on aliquots of the solubilized membranes and contained between 2000 and 4000 cpm. The data shows the fraction of radioactivity precipitated by the antibody and gives the means and standard deviations from three separate experiments. Where indicated, chloramphenicol was included in the protein synthesizing incubations in vitro at a concentration of 5×10^{-6} M.

The presence of chloramphenicol in incubations containing light rough endoplasmic reticulum has no effect on the biosynthesis of cytochrome P450. Thus under conditions where mitochondrial protein synthesis is inhibited, the incorporation of radiolabelled amino acids is identical in both membrane types.

The results of this study provide no evidence for the preferential segregation of messenger RNA for cytochrome P450 with mitochondrially associated membrane in the fraction of heavy rough endoplasmic reticulum; and is contrary to the proposal that the biosynthesis of the cytochrome is preferentially located on the light rough endoplasmic reticulum.

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