BBA 75572

CYTOCHROME b_5 AND P-450 IN LIVER CELL FRACTIONS

SIDNEY FLEISCHER[a], BECCA FLEISCHER[a], ANGELO AZZI[b,*] AND BRITTON CHANCE[b]

[a] *Department of Molecular Biology, Vanderbilt University, Nashville, Tenn. 37203 (U.S.A.) and* [b] *Johnson Research Foundation, University of Pennsylvania, Philadelphia, Pa. 19104 (U.S.A.)*

(Received August 31st, 1970)

SUMMARY

The contiguous membranes of liver, *i.e.* nuclei, rough and smooth microsomes, plasma membranes, and Golgi vesicles, were analyzed for cytochromes and P-450 content. Only cytochrome b_5 and P-450 were detectable. The highest cytochrome b_5 and P-450 content was in the microsome fraction. The Golgi vesicle contains cytochrome b_5 but there is little or no P-450. Thus, P-450 levels can be used to discriminate between microsomes and Golgi complex. The plasma membrane is practically devoid of cytochromes.

INTRODUCTION

In liver cells, nuclear envelopes, rough and smooth endoplasmic reticulum, Golgi complex and plasma membranes form a contiguous membrane system, a large portion of which is probably involved in the intracellular synthesis of serum proteins and their eventual secretion[1-4]. Methods for the isolation of plasma membranes[5] and Golgi membranes from bovine liver[6] and rat liver[7] have recently been described which make possible a comparison of these membranes with rough and smooth endoplasmic reticulum. The present work deals with the estimation of cytochromes P-450 and b_5 in plasma membranes, Golgi membranes, nuclei, and microsomes from bovine liver, and a comparison of microsomes and Golgi apparatus from rat liver.

MATERIALS AND METHODS

Plasma membranes, rough and smooth microsomes, nuclei[5] and Golgi membranes[6] were prepared from bovine liver as described previously. A heavy microsome fraction (R_2) was prepared from the pooled livers of 28 male Sprague–Dawley rats (200–300 g each) and Golgi-rich fraction prepared from this fraction by zonal centrifugation[7].

Difference absorption spectra were recorded at liquid nitrogen temperature with the split-beam spectrophotometer (Johnson Research Foundation). Cytochrome b_5 and P-450 were calculated from the low temperature spectra. A conversion factor for the extinction coefficients was obtained by comparing low temperature and room

* Current address: Department of General Pathology, University of Padova, Padova, Italy.

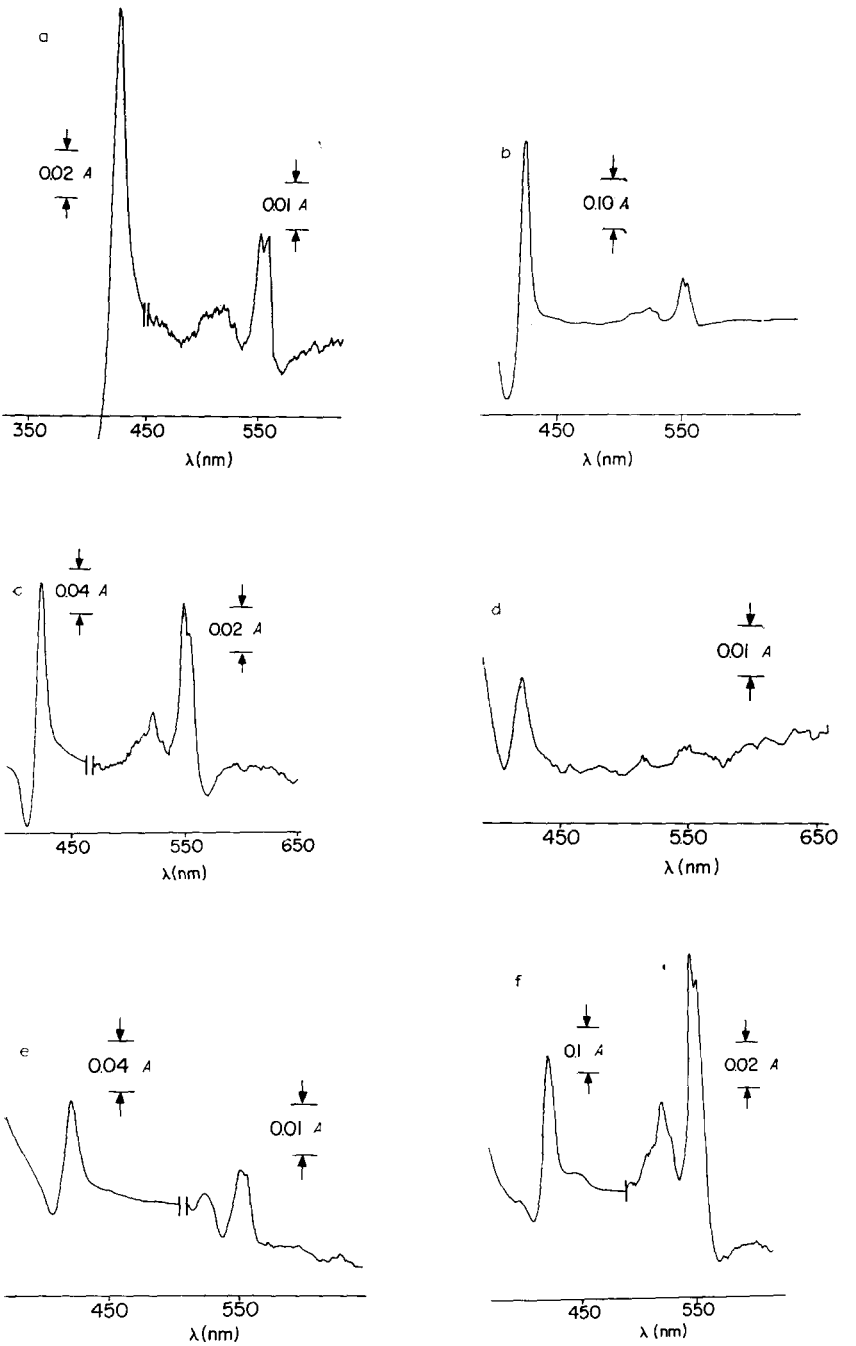

Fig. 1. Low temperature difference spectra to observe the cytochromes of liver cell fractions. The spectra are from: bovine liver (a) nuclei (9.02 mg protein per ml); (b) "rough" microsomes (2.2 mg protein per ml); (c) Golgi vesicles (2.1 mg protein per ml); (d) plasma membrane (1.29 mg protein per ml); rat liver (e) Golgi vesicles (1.04 mg protein per ml); and (f) "rough" microsome fraction (1.13 mg protein per ml).

temperature difference spectra for several of the samples. The light path for the room temperature spectra was 10 mm and for the low temperature spectra was 2 mm. A 250 mm focus Bausch and Lomb monochrometer with a grating of 600 lines/mm was used to give an effective band-width of 1.6 nm of the measuring light. The samples were frozen according to the method of CHANCE AND SPENCER[8].

Low temperature difference spectra for P-450 were obtained as the difference between the sample treated with carbon monoxide and reduced with dithionite, *vs.* the sample reduced with dithionite[9].

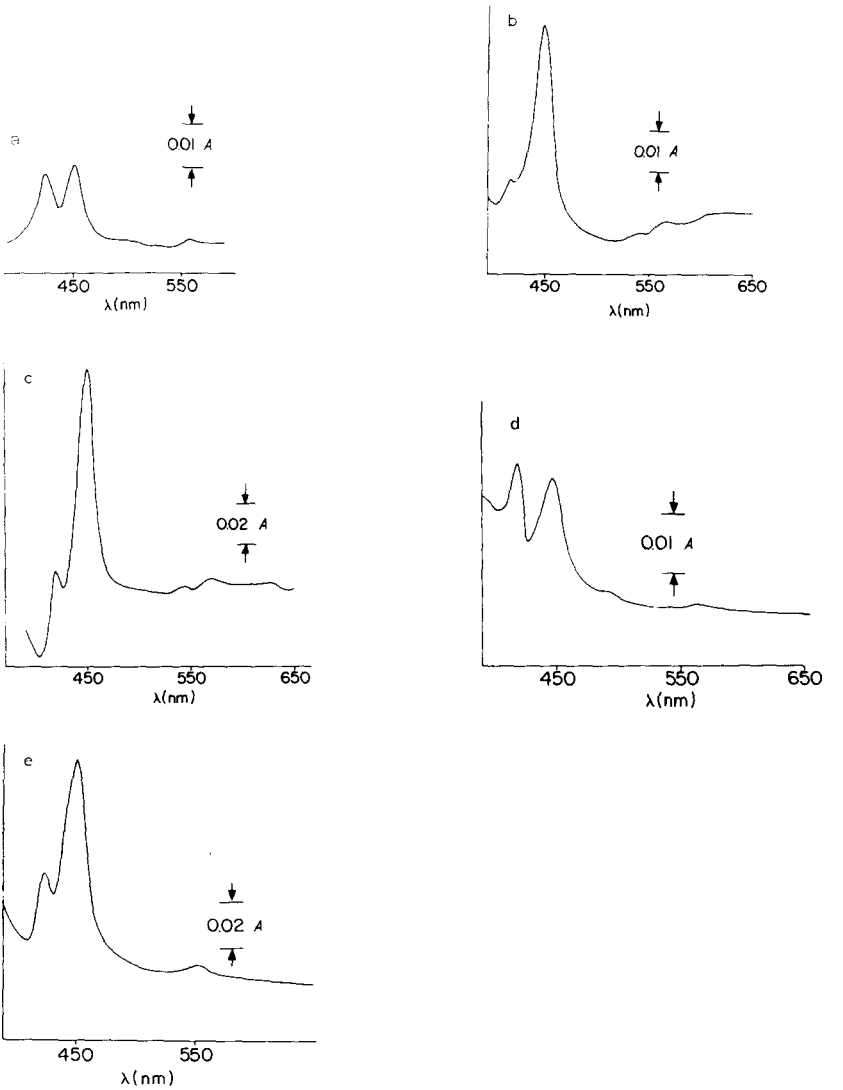

Fig. 2. Low temperature difference spectra of liver cell fractions to observe P-450 content. The spectra are from bovine liver: (a) nuclei (9.00 mg protein per ml); (b) rough microsomes (1.08 mg protein per ml); (c) smooth microsomes (1.21 mg protein per ml); (d) Golgi vesicles (0.96 mg protein per ml); and (e) rat liver "rough" microsomes fraction (0.85 mg protein per ml).

The samples were suspended in 0.25 M sucrose. In the case of nuclei, the sucrose concentration was 0.75 M. In this case a correction factor for intensification by sucrose of 0.55 was applied[10,11].

The concentration of cytochrome b_5 was calculated on the basis of an extinction coefficient of 20.1 $cm^{-1} \cdot mM^{-1}$ at 553–570 nm. Cytochrome *a* was estimated from the difference spectra at 605–630 nm using an extinction coefficient of $cm^{-1} \cdot 16$ mM^{-1} (ref. 12). The values obtained at low temperature in a 2 mm light-path cuvette were converted to room temperature and 10 mm path-length by multiplying by 0.588.

The extinction coefficient utilized for cytochrome P-450 was 91 $cm^{-1} \cdot mM^{-1}$ at 450–490 nm. The absorption peak at 420 nm was considered to be derived from P-450. An extinction coefficient of 110 $cm^{-1} \cdot mM^{-1}$ was used and the amount of this cytochrome added to cytochrome P-450 (ref. 13). The low temperature to high temperature and 2 to 10 mm light path correction factors were 0.74 and 0.97 for P-450 and P-420, respectively.

Proteins were determined by the procedure of LOWRY *et al.*[14] using bovine serum albumin as a protein standard, and glucose-6-phosphatase measured at 32° by the method of SWANSON[15].

RESULTS

Typical low temperature spectra of the organelles are shown in Figs. 1 and 2. Table I summarizes the values for P-450 and cytochrome b_5 calculated from these spectra for the purified organelles. Of the bovine liver organelles studied, smooth and rough microsomes have the highest content of P-450 and cytochrome b_5. Plasma membranes had the lowest content of cytochrome b_5, only the Soret band being detectable.

Nuclei had a small but easily detectable content of both cytochromes P-450

TABLE I

CYTOCHROME b_5 AND P-450 CONTENT AND GLUCOSE-6-PHOSPHATASE ACTIVITY OF BOVINE AND RAT LIVER CELL FRACTIONS

	Content (nmoles/mg protein)		*(b_5/P-450)*	*Glucose-6-phosphatase specific activity (μmoles P_i released/min per mg protein at 32°)*
	P-450	*Cytochrome b_5*		
Bovine liver				
"Smooth" microsomes	0.69	1.52	2.2	0.25
"Rough" microsomes	0.42	1.31	3.1	0.21
Golgi vesicles	0.16	0.95	5.9	0.070
Nuclei	0.016*	0.054*	3.4	0.026
Plasma membrane	—	<0.014**	—	0.015
Rat liver				
Microsomes (R_2)	0.88	1.11	1.26	0.114
Golgi vesicles	0.049	0.69	14.0	0.007

* Spectra made from solution of 0.75 M sucrose. Correction for intensification due to sucrose = 0.55.

** Estimated from Soret.

TABLE II

RATIO OF CYTOCHROME b_5 TO PHOSPHOLIPID IN BOVINE LIVER CELL FRACTIONS

Lipid analyses were done in collaboration with Dr. George Rouser, City of Hope Medical Center, Duarte, Calif. Lipids were extracted with chloroform–methanol[16] and freed of non-lipid contaminants by Sephadex column chromatography[17].

Bovine liver	*Total μg P per mg protein*	*μg lipid P per mg protein*	*Cytochrome b_5 / μg lipid P × 100*
Smooth microsomes	40.0	38	4.0
Rough microsomes	34.7	26	5.0
Golgi complex	39.9	37	2.6
Nuclei	32.7	3.0	1.8
Plasma membrane	16.9	9.1	0.14

and b_5. The ratio b_5 to P-450 was similar to that found for rough microsomes and is probably due to the outer nuclear envelope.

Golgi vesicles isolated from bovine liver had a level of cytochrome b_5 which was about 62 % of the level found in smooth microsomes. The level of P-450 on the other hand, was about 23 % of that of smooth microsomes. The glucose-6-phosphatase of this preparation, however, indicates contamination with microsomes to a level of about 28 % which could account for all of the P-450 found in this organelle. The ratio of cytochrome b_5 to P-450, on the other hand, is 2–3 times greater than that found in microsomes and indicates that cytochrome b_5 is present in this organelle but at about 40 % the level found in microsomes.

Golgi vesicles from rat liver contain about 62 % of the cytochrome b_5 of microsomes and only 6 % of the P-450 per mg protein. They also exhibit about 6 % of the glucose-6-phosphatase activity of microsomes, so that the low level of P-450 is probably due to contamination with microsomes.

Nuclei have a very small content of membranes. It was of interest, therefore, to also determine the amount of membrane in each organelle and express the cytochrome content in terms of a membrane parameter. Table II shows the ratio of cytochrome b_5 to phospholipid phosphorus found for bovine liver organelles. It can be seen that microsomes have the highest content of cytochrome b_5 per μg phospholipid phosphorus, whereas Golgi membranes and nuclei have about half as much. The value for nuclei is consistent with the idea that the two membranes surrounding the nucleus are different, the inner one being devoid of these components. The amount of cytochrome b_5 per μg lipid P in plasma membranes is less than 1/30 the value found for microsomes and is consistent with the idea that this is not a component of the plasma membrane but is a contaminant.

Cytochrome oxidase was not present in significant amount in any of the fractions studied. The limit of detection of cytochrome *a* was less than 0.04, 0.05, 0.007, 0.03, and 0.05 nmole/mg protein for bovine liver plasma membrane, Golgi and nuclei, and rat liver Golgi and microsomes, respectively.

DISCUSSION

Cytochrome P-450 and b_5 in liver microsomes form an electron transport system involved in the metabolism of various drugs, steroids, *etc.*[9,13,18]. The ratio of cyto-

chrome b_5 to P-450 for rough microsomes after sonication yields membranes with a wide variation (from 0.51 to 3.3) in this ratio indicating some heterogeneity in the distribution of these two components within endoplasmic reticulum.[19] It would appear that Golgi membranes are related to endoplasmic reticulum membranes in that they contain considerable amounts of cytochrome b_5. The content of P-450, on the other hand, is very small or absent in Golgi fractions which are not contaminated with endoplasmic reticulum. Bovine liver Golgi show a significant activity of NADH–cytochrome *c* reductase activity[6]. However, rat liver Golgi vesicles which contain even higher levels of cytochrome b_5 are devoid of this activity[7]. Thus in this case cytochrome b_5 is not related to NADH oxidation. It will be of interest to see what role, if any, cytochrome b_5 plays in the Golgi membranes.

Glucose-6-phosphatase is generally used as a marker enzyme for endoplasmic reticulum in liver. The specific activity of glucose-6-phosphatase activity of the plasma membrane was 6–7 % of that found for purified microsomes. We do not, however, detect a comparable level of contamination with microsomal cytochrome b_5. This discrepancy could be explained by heterogeneity of the microsomal fraction in terms of cytochrome b_5 and glucose-6-phosphatase content or to the presence of glucose-6-phosphatase in liver plasma membranes.

Carefully purified liver plasma membranes do not appear to contain significant amounts of cytochromes. If cytochromes are associated with the plasma membrane, they are not firmly attached so that they are lost in the isolation procedure. Cytochromes have been postulated as ion carriers in transport in yeast membranes[20]. The very low levels or apparent absence of cytochromes in highly purified plasma membranes cast doubt on such a role for cytochromes in liver.

Isolated nuclear membranes from rat liver have been shown to have about 50 % of the level of glucose-6-phosphatase and rotenone-insensitive NADH–cytochrome *c* reductase of endoplasmic reticulum[21]. Our analyses also show that, on a lipid phosphorus or membrane basis, cytochrome b_5 in the nucleus is about 50 % of the level found in endoplasmic reticulum. These results support the hypothesis[21] that the outer membrane of the nucleus, which is contiguous with endoplasmic reticulum, resembles endoplasmic reticulum in its enzymic activities whereas the inner membrane differs from endoplasmic reticulum. Our results with bovine liver nuclei are in agreement with previous results of CONOVER[22], who showed that rat liver nuclei contain little or no cytochromes ($a + a_3$) or *b*. This is in contrast to calf thymus nuclei which contain an entire respiratory chain and are capable of oxidative phosphorylation.

ACKNOWLEDGEMENTS

This work was supported in part by U.S. Public Health Service Grants AM 14632 and GM 12202, a Grant-in-Aid from the American Heart Association, and a National Institutes of Health fellowship to A. A. (FO5-TW-1291-02). Miss Ute Lhotke and Mrs. Christine Rotgers provided capable technical assistance in the preparation of some of the cell fractions. We are also grateful to Dr. Dave Wilson for advice and help.

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