CYTOCHROME b_5 AND CO-BINDING CYTOCHROMES IN THE GOLGI MEMBRANES OF MAMMALIAN LIVERS

YOSHIYUKI ICHIKAWA and TOSHIO YAMANO

Department of Biochemistry, Osaka University Medical School, 33 Joan-cho, Kita-ku, Osaka, Japan

Received June 5, 1970

Summary

Cytochrome b_5 and cytochrome P-450 are present in the Golgi membranes of mammalian livers. In normal rabbit liver, the Golgi membranes have a similar cytochrome b_5 content on a protein basis to the smooth-surfaced and rough-surfaced microsomes, but their cytochrome P-450 content is very low. The cytochrome P-450 of the Golgi membranes was induced considerably by injections of sodium phenobarbital. On the other hand, on treatment of rabbits with 3-methylcholanthrene, cytochrome P-448 was induced not only in the smooth-surfaced and rough-surfaced microsomes, but also in the Golgi membranes of the liver.

Introduction

Cytochrome P-450 is known to be present in the smooth-surfaced and rough-surfaced microsomes and the inner membranes of mitochondria of the adrenal cortex(1,2). Cytochrome b_5 is present not only in the smooth-surfaced and rough-surfaced microsomes of various tissues, but also in the outer membranes of liver mitochondria, which are considered to possess a common origin with the microsomes(1,3,4).

Recently, Kuff and Dalton(5) and Fleischer *et al.*(6) reported the preparation of Golgi membranes. By their methods, the Golgi membranes can be separated completely from the smooth-surfaced and rough-surfaced microsomes while their results showed that the microsomal fractions obtained by the method of Hogeboom and Schneider(7) or Mitoma *et al.*(8), may be contaminated by Golgi membranes and Plasma membranes. Accordingly, in studies on the electron transport systems of microsomes, we attempted to separate the Golgi, and plasma membranes and smooth-surfaced and rough-surfaced microsomes from the whole microsomal fraction and investigated the electron transport systems of the Golgi membranes.

Methods

Golgi membranes were prepared by the method of Kuff and Dalton(5) as modified by Fleischer et al.(6). The Golgi membranes were identified by electron microscopy and by various biochemical characters such as their galactosyl transferase and succinic oxidase activities and RNA content(9). Fig.1 A and B shows the electron microscopic appearance of the Golgi and microsomal fractions, respectively, from normal rabbit liver.

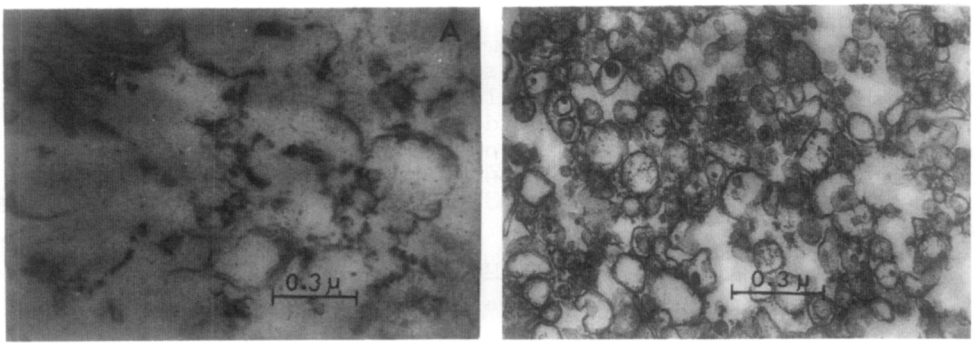

Fig.1(A,B). Electron micrographs of the Golgi and smooth-surfaced microsomal fractions prepared from normal rabbit liver. The fractions were negatively stained with 2 % Potassium phosphotungstate, pH 7.2. A, Golgi fraction ; B, smooth-surfaced microsomal fraction.

Galactosyl transferase activity was determined by a modification of the method of Babad and Hassid(10). Cytochrome b_5 content was determined by the method of Garfinkel taking the molecular extinction coefficient for the optical density between 424 and 409 nm as 165 mM^{-1} cm^{-1}(11). The content of cytochrome P-450 was determined by the method of Omura and Sato(12)and that of cytochrome P-448 from the CO-difference spectra of samples reduced with sodium dithionite using a value of 95 $mM^{-1}cm^{-1}$ for the molar extinction increment between 448 and 500 nm. The activities for hydroxylation of o-chloroaniline and demethylation of p-nitroanisole were measured as described previously (13). NADPH-cytochrome c reductase activity was measured as the in-

crease in absorbance at 550 nm of the α-band of reduced cytochrome c by the method of Ernster(14). NADH-cytochrome b_5 reductase activity was measured by the method of Strittmatter and Velick(15).

Protein content was determined by the biuret reaction(16),after addition of 1 %(w/v)sodium cholate to the sample to remove turbidity, using crystalline bovine albumin as a standard. The complication of increase in absorption at 540 nm in the biuret reaction due to heme in the test samples was avoided by using a reference containing hemoproteins in 4 %(w/v)NaOH.

The contents of manganese and copper were determined using an atomic absorption spectrophotometer(Perkin-Elmer,model 303).

Cytochrome P-450 was induced by the method of Orrenius and Ernster(17). Cytochrome P-448 was induced by intraperitoneal injection of 3-methylcholanthrene(30 mg/Kg of body weight)once daily for 10 days.

Results and Discussion

The Golgi membranes of normal liver of rabbits,rats and guinea-pigs have a high content of cytochrome b_5,whereas their cytochrome P-450 content is low.

Fig.2(a,b)shows the absorption spectra of cytochrome b_5 of whole Golgi membranes of rabbit liver in the oxidized and reduced forms at 298° and 77°K. The difference spectrum of cytochrome b_5 reduced with NADH in air minus its oxidized form in the Golgi membranes showed peaks at 424,526 and 556 nm and the absorption spectrum of the reduced α-band had a shoulder at 560 nm at room temperature. Furthermore,the absorption spectrum of cytochrome b_5 reduced with NADH in air in the Golgi membranes showed split α-,and β-bands at 77°K. This cytochrome b_5 is reduced very slowly by other reductants,such as ascorbate and cysteine. It was confirmed to be a b-type hemoprotein by the position of the absorption maxima of the α-band of its sodium dithionite-reduced pyridine hemochromogen.

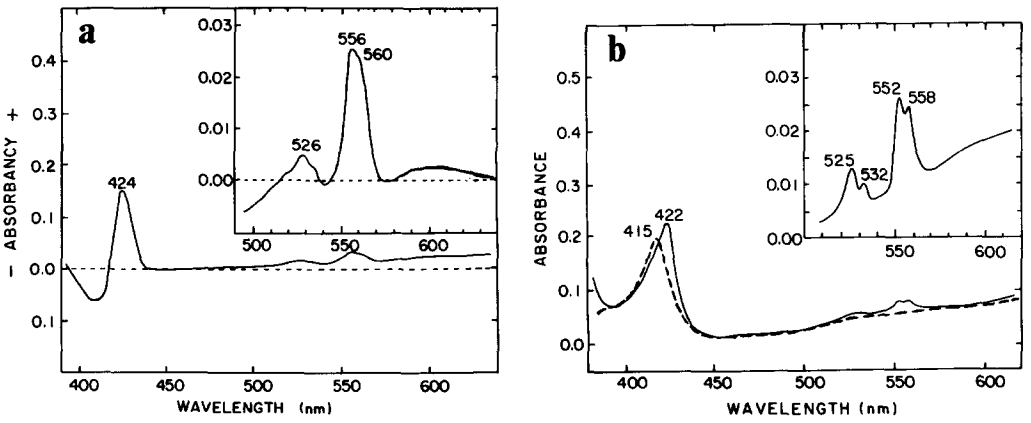

Fig.2(a). Difference spectra of NADH-reduced minus oxidized forms of cytochrome b_5 of rabbit liver Golgi membranes at 298°K.
Protein concentration, 1.5 mg/ml, 0.1 M K-phosphate, pH 7.5.
Final concentration of NADH, 0.5 mM.
———, aerobic, NADH-reduced minus oxidized forms; -----, base line.
(b). Absolute absorption spectra of NADH-reduced and oxidized forms of cytochrome b_5 of rabbit liver Golgi membranes at 77°K.
Experimental conditions as for Fig.2(a).
———, reduced form; - - - -, oxidized form.

These results suggest that the absorption spectra of the cytochrome b_5 of the Golgi membranes are identical with microsomal cytochrome b_5. This might be because the Golgi membranes were contaminated with the microsomes or/and the Golgi membranes might be strongly associated with the microsomes, but this is unlikely because there was little cytochrome P-450 in the Golgi membranes of normal rabbit liver.

The cytochrome P-450 and NADPH-cytochrome c reductase of the Golgi membranes of rabbit, rat and guinea-pig liver were induced considerably by injection of sodium phenobarbital into the animals. The absorption peaks of the Soret bands of the CO-complexes of cytochrome P-450's of the Golgi membranes and the smooth-surfaced and rough-surfaced

microsomes,unlike those of the cytochrome P-448's of liver microsomes of animals after 3-methylcholanthrene treatment(18),were all at 450 nm,as shown in Fig.3. Cytochrome P-450 of liver Golgi membranes does not form a CO-complex with CO on addition of malate or succinate unlike cytochrome P-450 of adrenocortical mitochondria.

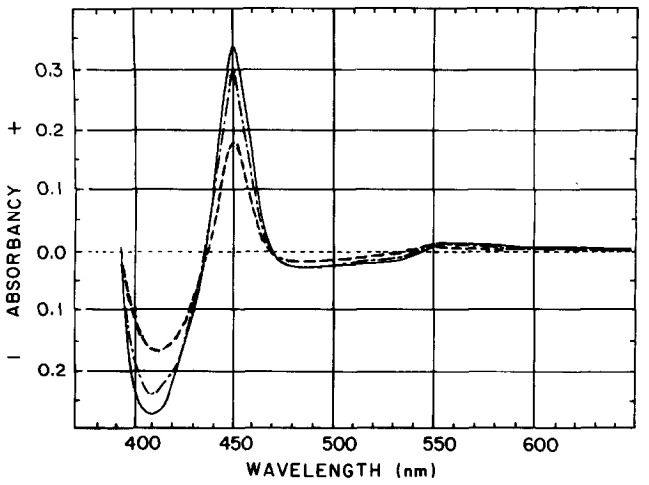

Fig.3. Carbon monoxide difference spectra of cytochrome P-450's of phenobarbital treated rabbit liver Golgi membranes and smooth-surfaced and rough-surfaced microsomes. The sample and reference cells contained a suspension of Golgi membranes or microsomes(2 mg of protein per ml,0.1 M K-phosphate,pH 7.5,20°C). CO gas was passed through the contents of the sample cell and then about 1 mg of sodium dithionite per 3 ml was added to both cells,and the difference spectra were measured 2 min later. ----,Golgi membranes; ——,smooth-surfaced microsomes; —·—·,rough-surfaced microsomes;------,base line.

Table 1 summarizes the contents of cytochrome b_5 and cytochrome P-450,and the activities of NADH-cytochrome b_5 reductase,NADPH-cytochrome c reductase,galactosyl transferase and the hydroxylation and demethylation activities for o-chloroaniline and p-nitroanisole,respectively,of the Golgi and plasma membranes and the smooth-surfaced and rough-surfaced microsomes. The Golgi membranes contained negligible

Table 1. Distributions of various enzyme activities in the Golgi, and plasma membranes and smooth, and rough-surfaced microsomes.

		Golgi	Smooth	Rough	Plasma
P-450*	Control	0.20	1.52	0.96	0.00
	PB.	1.10	2.84	1.70	0.00
	Control	0.20	1.55	1.01	0.00
P-448*	MC.	1.05	2.65	2.43	0.00
Cyt.b_5*	Control	0.61	0.82	0.77	0.00
	PB.	1.04	1.09	0.85	0.01
	Control	1.08	1.21	0.76	0.00
	MC.	0.76	0.85	0.76	0.00
NADPH-cyt.c reductase**	Control	0.14	0.20	0.18	0.00
	PB.	0.18	0.25	0.24	0.00
	Control	0.15	0.22	0.17	0.00
	MC.	0.17	0.25	0.22	0.00
NADH-cyt.b_5 reductase**	Control	1.25	2.25	1.75	0.11
	PB.	2.75	3.45	2.18	0.30
	Control	1.45	2.30	1.75	0.11
	MC.	1.76	1.90	2.00	0.10
p-Hydroxylation of *o*-chloroaniline***	Control	0.05	1.40	0.50	0.00
	PB.	0.30	1.70	0.80	Trace
	Control	0.04	1.30	0.50	0.00
	MC.	0.10	1.50	0.70	0.00
Demethylation of *p*-nitroanisole***	Control	0.50	0.90	0.50	0.00
	PB.	0.70	1.20	0.65	Trace
	Control	0.50	0.90	0.50	0.00
	MC.	0.70	1.10	0.70	0.00
Galactosyltransferase***	Control	1.48	0.12	0.01	Trace
	PB.	2.67	0.09	0.09	0.00
	Control	1.50	0.11	0.02	0.00
	MC.	2.33	0.06	0.01	Trace

The abbreviations used are: PB, sodium phenobarbital treated rabbit livers; MC, 3-Methylcholanthrene treated rabbit livers; P-450, Cytochrome P-450; P-448, Cytochrome P-448; Cyt.b_5, Cytochrome b_5.

Values are averages of 5 determinations of cytochrome contents and activities. *nmoles/mg protein; **NAD(P)H-cytochrome reductases are expressed as μmoles cytochrome c or b_5 reduced/min/mg protein at 30°C and pH 7.5; ***nmoles of product formed/min/mg protein, galactosyltransferase activity is expressed as nmoles galactose transferred to N-acetyl glucosamine/min/mg protein at 37°C.

succinic dehydrogenase activity and RNA content were associated with stronger galactosyl transferase activity than the smooth-surfaced and rough-surfaced microsomes,on a protein basis. They had a high cytochrome b_5 content and very low cytochrome P-450 content.

On the other hand,the Golgi membranes from liver of rabbits treated with 3-methylcholanthrene had high cytochrome b_5 and cytochrome P-448 contents like the smooth-surfaced and rough-surfaced microsomes(19). Cytochrome P-448 could be induced in rabbits only by large doses of 3-methylcholanthrene over a long period. The light absorption spectrum of the CO-complex of cytochrome P-448 in the Golgi membranes was different from that of cytochrome P-450 and the EPR signal(g values) of cytochrome P-448 in the oxidized and low spin form were very slightly, but distinctly different from those of oxidized cytochrome P-450. The cytochrome b_5 of the Golgi membranes prepared rabbit liver from after 3-methylcholanthrene treatment was identical optically and magnetically with that of the Golgi membranes of normal rabbit liver. A change from cytochrome P-450 to P-448 seemed to occur in the Golgi membranes after a similar change in the rough-surfaced and smooth-surfaced microsomes. Experiments are in progress to see whether this change is caused by synthesis of a new hemoprotein molecule,as observed in the case of two dehydroquinases in Neurospora Crassa(20),and/or is a result of a change of the environment around the protoporphyrin ring of cytochrome P-450.

The total protein,RNA and metal contents of the Golgi and plasma membranes and of smooth-surfaced and rough-surfaced microsomes are compared in Table 2. The Golgi membranes contain about 5 % of the total protein of the whole microsomal fraction of rabbit liver. They also contain high concentrations of manganese and copper on a protein basis.

From the result of Fleischer et al.(6),the physiological role of manganese in the Golgi membranes may be in activating galactosyl transferase.

Table 2. Distribution of protein, RNA, manganese and copper in the Golgi, and plasma membranes and smooth, and rough-surfaced microsomes.

		Golgi	Smooth	Rough	Plasma
Protein / liver mg/100g (total protein %)	Control	57.0 (5.3%)	345.0 (31.8%)	679.0 (62.6%)	3.5 (0.3%)
	PB.	140.0 (4.2%)	1858.5 (55.3%)	1359.0 (40.4%)	4.0 (0.1%)
	Control	55.0 (5.6%)	325.0 (33.8%)	577.5 (60.3%)	3.7 (0.3%)
	MC.	78.5 (4.0%)	902.0 (46.1%)	902.0 (46.1%)	3.0 (0.1%)
RNA / protein mg/mg	Control	0.01	0.05	0.45	0.00
	PB.	0.02	0.04	0.55	0.00
	Control	0.01	0.05	0.50	0.00
	MC.	0.01	0.07	0.46	0.00
Manganese / protein n atom/mg	Control	1.13	0.20	0.36	0.00
	PB.	0.84	0.33	0.58	0.00
	Control	0.92	0.22	0.57	0.00
	MC.	1.50	0.38	0.87	Trace
Copper / protein n atom/mg	Control	0.21	0.08	0.09	0.00
	PB.	0.17	0.08	0.14	0.00
	Control	0.21	0.08	0.09	0.00
	MC.	0.32	0.12	0.15	0.00

The abbreviations used are expressed as described in Table 1. Values are averages of 5 determinations of contents.

Acknowledgements

We thank Mr. I. Yamamoto and Mr. M. Chikata of the Medical Research Center in this University for their skillful technical assistance.

References

1. Ichikawa, Y. and T. Yamano, Arch. Biochem. Biophys., 121, 742(1967).
2. Yago, N. and S. Ichii, J. Biochem., Tokyo, 65, 215(1969).
3. Sottocasa, G.L., B. Kuylenstierna, L. Ernster and A. Bergstrand, J. Cell Biol., 32, 415(1967).
4. Schnaitman, C.A., Proc. Natl. Acad. Sci., 63, 412(1969).
5. Kuff, E.L. and A.J. Dalton, "Biochemical Studies of Isolated Golgi Membranes" In Subcellular Particles, ed. by T. Hayashi, The Ronald Press Company, New York, p. 114(1969).
6. Fleischer, B., S. Fleischer and H. Ozawa, J. Cell Biol., 43, 59(1969).
7. Hogeboom, G.H., W.C. Schneider and G.E. Palade, J. Biol. Chem., 172, 619(1948).
8. Mitoma, C., H.S. Posner, H.C. Reitz and S. Udenfriend, Arch. Biochem. Biophys., 61, 431(1956).
9. Schmidt, G. and S.J. Thannhauser, J. Biol. Chem., 161, 83(1945).
10. Babad, H. and W.Z. Hassid, "In Methods in Enzymology" ed. by S.P. Colowick and N.O. Kaplan, Academic Press. Inc., New York, Vol. 8, p. 346(1966).

11.Garfinkel,D., Arch. Biochem. Biophys.,77,493(1958).
12.Omura,T. and R.Sato, J. Biol. Chem.,239,2379(1964).
13.Ichikawa,Y. and T.Yamano, Biochim. Biophys. Acta,171,32(1969).
14.Ernster,L., Acta Chem. Scand.,12,600(1958).
15.Strittmatter,P. and S.F.Velick, J. Biol. Chem.,221,277(1956).
16.Gornall,A.G.,C.J.Bardwill and M.M.David, J. Biol. Chem.,177, 751(1949).
17.Orrenius,S. and L.Ernster, Biochem. Biophys. Res. Comm.,16,60(1964)
18.Glaumann,H.,B.Kuylenstierna and G.Dallner, Life Sci.,8,1309(1969).
19.Sladek,N.E. and G.J.Mannering, Biochem. Biophys. Res. Comm., 24,668(1966).
20.Giles,N.H.,C.W.H.Partridge,S.I.Ahmed and M.E.Case, Proc. Natl. Acad. Sci.,58,1930(1967).