COMPARISON OF BIOTRANSFORMATION AND LIPID PEROXIDATION ACTIVITY OF MICROSOMAL FRACTIONS ISOLATED BY Ca^{2+}-BINDING AND GEL FILTRATION ON SEPHAROSE 2B AND THEIR ELECTRON MICROSCOPIC EXAMINATION

H.KOVÁŘOVÁ

Department of Chemistry and Biochemistry,
Faculty of Medicine, Charles University, 500 38 Hradec Králové

Received October 16th, 1975

Rat liver microsomal fractions were isolated by methods of Ca^{2+}-binding and gel filtration on Sepharose 2 B. The values of biotransformation activity (oxidative demethylation of amidopyrine) and lipid peroxidation activity of fractions obtained by both methods are identical and in agreement with the values reported for standard microsomes. The presence of Ca^{2+}-ions is without effect on these microsomal functions. The microsomal fraction obtained by gel filtration on Sepharose 2 B showed a significantly increased activity of glucose-6-phosphatase as compared to the standard and Ca^{2+}-bound microsomal fractions. Electron microscopic examination has shown that this fraction is an exceptionally pure "smooth" fraction free of either vesicles of the rough endoplasmic reticulum or of free ribosomes.

The term "microsomal fraction", commonly employed in biochemistry in *in vitro* experiments, designates the vesicular form of the endoplasmic reticulum obtained by differential centrifugation of the cell homogenate at 105000 *g*. Vesiculation takes place during homogenization. The endoplasmic reticulum consists of the so-called "rough" and "smooth" part. The surface of membranes of the "rough" part of the endoplasmic reticulum binds ribosomes. Both these parts can be present in the microsomal fraction[1].

Two important enzyme systems are localized in the microsomal fraction of the liver of mammals: the so-called monooxygenase system and the lipid peroxidation systems. The monooxygenase system catalyzes above all the metabolism of exogeneous compounds but also of endogeneous compounds. These biotransformation reactions require NADPH and molecular oxygen[2]. The necessity of presence of phospholipids whose components are polyunsaturated fatty acids has also been shown[3]. The lipid peroxidation systems (enzymic and monoenzymic) participate on the peroxidative degradation of polyene fatty acids. The reactions require above all molecular oxygen[4,5]. The presence of NADPH and of a certain concentration of Fe^{2+} (10^{-5}M) is essential for enzymic reactions whereas reactions of nonenzymic character can proceed without NAPDH but require a higher concentration of Fe^{2+} (10^{-3}M, ref.[6]). Some metal ions (Zn^{2+}, Mn^{2+}, Co^{2+}, Hg^{2+}) inhibit lipid peroxidation while others (Mg^{2+}, Fe^{2+}, Fe^{3+}) enhance the rate of this process. Microsomal lipids affected by peroxidation (no matter whether enzymic or nonenzymic) loose their activity and no longer exhibit their monooxygenase function[7].

Two new methods of isolation of the microsomal fraction have been reported recently. Kamath and coworkers[8] have made use of the binding of Ca^{2+}-ions to microsomes; this binding enables

aggregation and thus also the separation of the microsomal fraction by centrifugation of the postmitochondrial supernatant at low centrifugal force (30 *g*). The second method, *i.e.* the separation of microsomes from the postmitochondrial fraction by gel filtration on Sepharose 2 B, has been described by Tangen and coworkers[9]. The activities of the biotransformation reactions or their kinetic parameters and composition of the microsomal fractions thus obtained, have so far appeared in a few papers[8-11] only and are in good agreement with the values for "standard" microsomes, *i.e.* those obtained by utracentrifugation at 105000 *g*. These conclusions cannot be unambiguously generalized. It is obvious that both the Ca^{2+}-binding of microsomes and gel filtration through Sepharose columns can qualitatively and quantitatively affect the microsomal fraction obtained.

The aim of this study has been to investigate the biotransformation and lipid peroxidation activity of microsomal fractions obtained by Ca^{2+}-binding and by gel filtration on Sepharose 2 B. The results of electron microscopic observations intended to compare the fractions thus obtained with fractions isolated by the standard method are also described.

EXPERIMENTAL

Male rats weighing 150—200 *g* were sacrificed by decapitation, their livers were rapidly excised and washed with cold (4°C) 0·25M sucrose at pH 7·5. A 20% (w/v) liver homogenate was prepared by five-minute homogenation at 2000 rev. min^{-1} in 0·25M sucrose at pH 7·5. The postmitochondrial supernatant was obtained by centrifugation at 2000 *g* (10 min) and 12000 *g* (20 min). The supernatant was divided into two parts: 4—5 ml was used for the isolation of the microsomal fraction according to Tangen and coworkers[9] by gel filtration on a column of Sepharose 2 B. The remainig part was used for the isolation by Ca^{2+}-binding according to Kamath and coworkers[8] and for "standard" microsomes[12].

The protein content[13], the activity of glucose-6-phosphatase[14,15], the degree of amidopyrine oxidative demethylation[10] and lipid peroxidation[16] were determined in the microsomal fractions. Fresh sediments of microsomal fractions were fixed in 1% OsO_4 in phosphate buffer at pH 7·4 and scanned in Tesla EB 513 A electron microscope.

RESULTS AND DISCUSSION

The values of the individual characteristics examined in the microsomal fractions are given in Table I. As can be seen the activities of both oxidative amidopyrine demethylation as well as of lipid peroxidation are the same as regards preparations obtained by Ca^{2+}-binding and by gel filtration. It has been known that oxidative amidopyrine demethylation is inhibited by lipid peroxidation[17] (mainly by enzymic lipid peroxidation); the effect of lipid peroxidation can be observed after 15 minutes already[18]. Assuming that Ca^{2+}-ions, like any other bivalent ions, can either inhibit or enhance the degree of lipid peroxidation and thus affect amidopyrine demethylation, the effect of Ca^{2+}-ions should be reflected by the activity of aminopyrine demethylation which should be different in the Ca^{2+}-bound microsomal fraction and different in the fraction isolated by gel filtration. Since these values are identical in both cases and

TABLE I

Activities of Microsomal Functions in Microsomal Fraction Isolated by Two Different Methods

The values are means ± standard deviation calculated from 3–6 experiments. The activity of glucose-6-phosphatase is significantly increased at the probability level of $P < 0{\cdot}01$.

Method of isolation	Activity		
	glucose-6-phosphatase[a]	amidopyrine demethylase[b]	nonenzymic lipid peroxidation[c]
Ca^{2+}-binding	0·181 ± 0·003	0·023 ± 0·007	0·015 ± 0·003
Gelfiltration	0·415 ± 0·005	0·022 ± 0·006	0·018 ± 0·003

[a] μg P_i = mg prot.$^{-1}$ min^{-1}; [b] A_{412} . mg prot.$^{-1}$ 30 min^{-1}; [c] A_{535} . mg prot.$^{-1}$ 60 min^{-1}.

in agreement with the values reported for "standard" microsomes[8–10], it is obvious that Ca^{2+}-ions do not affect enzymic lipid peroxidation which proceeds during 30-min incubation of the microsomal fraction with amidopyrine in the presence of NADPH. The values of activity of nonenzymic lipid peroxidation directly determined in microsomal fractions (Table I) are also identical; this fact supports our assumption.

As obvious from the data given in Table I, the activity of glucose-6-phosphatase is significantly ($P < 0{\cdot}01$) increased in the microsomal fraction obtained by gel filtration on Sepharose 2B compared to the Ca^{2+}-bound microsomal fraction. In view of the good agreement found to exist between biotransformation and lipid peroxidation activity this enhancement of glucose-6-phosphatase activity can be explained exclusively by its structure as follows from electron microscopic examination. We have shown that microso mes isolated by Ca^{2+}-binding (Fig. 1*a**) are identical with "standard" microsomes (Fig. 1*c**), *i.e.* that they contain vesicles of the smooth and rough endoplasmic reticulum. A fraction of perfectly smooth vesicles corresponding in size to microsomes was obtained by gel filtration on Sepharose 2 B (Fig. 1*b**). This fraction represents therefore an especially pure, smooth microsomal fraction; this is evidenced also by the high content of glucose-6-phosphatase activity as marker enzyme of microsomes.

I thank Dr J. Špaček and Mrs M. Veselá for the electron microscope photographs.

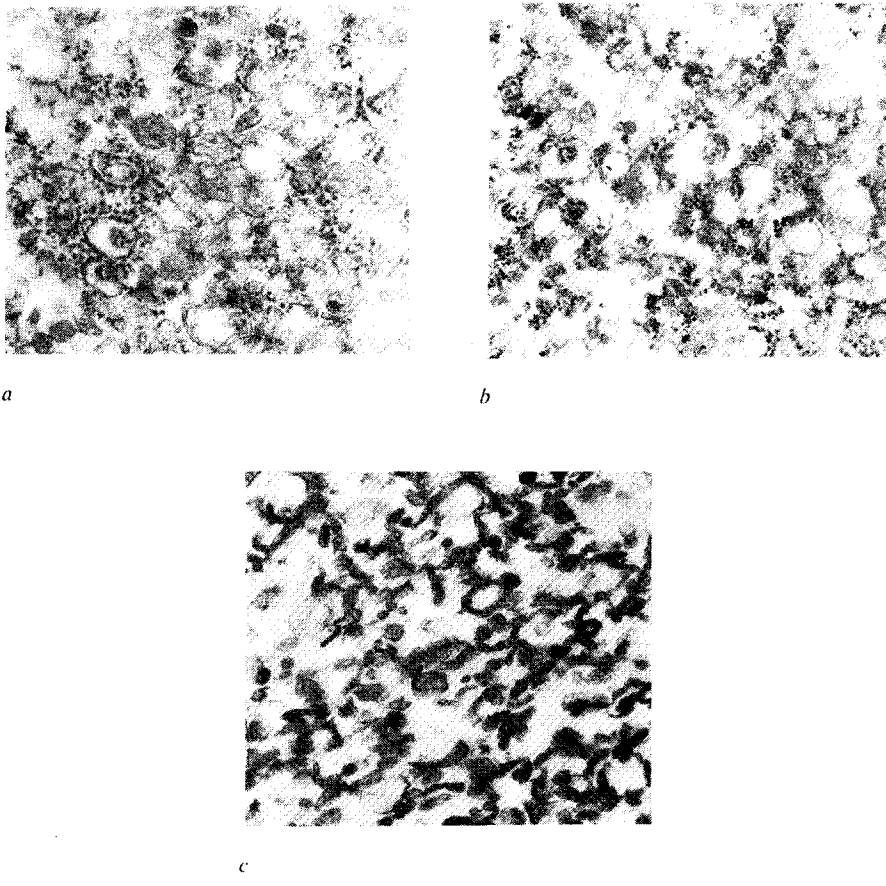
* See insert following p. 2815.

REFERENCES

1. Claude A. in the book: *Microsomes and Drug Oxidations* (J. R. Gilette, A. A. Conney, G. J. Cosmides, R. W. Estabrook, J. R. Fouts, G. J. Mannering, Eds), p. 3. Academic Press, New York/London 1969.
2. Parke D. V.: *Recent Advances in Pharmacology*, p. 29. Churchill, London 1967.
3. Coon M. J., Autor A. P., Strobel H. W.: Chem. Biol. Interactions *3*, 248 (1971).
4. Wills E. D.: Biochem. J. *113*, 315 (1969).
5. Wills E. D.: Biochem. J. *113*, 325 (1969).
6. Marks F., Hecker E.: Hoppe-Seyler's Z. Physiol. Chem. *348*, 727 (1967).
7. Lejsek K., Kvasničková E., Hais I. M.: Česk. Fysiol. *20*, 363 (1971).
8. Kamath S. A., Rubin E.: Biochem. Biophys. Res. Commun. *49*, 52 (1972).
9. Tangen O., Jonsson J., Orrenius S.: Anal. Biochem. *54*, 597 (1972).
10. Kupfer D., Levin E.: Biochem. Biophys. Res. Commun. *47*, 611 (1972).
11. Cinti D. L., Moldeus P., Schenkman J. B.: Biochem. Pharmacol. *21*, 3249 (1972).
12. Remmer H., Greim H., Schenkman J. B., Estabrook R.W. in the book: *Methods in Enzymology* (S. P. Collowick, N. O. Kaplan, Eds) 10, p. 706. Academic Press, New York 1967.
13. Lowry O. H., Rosenbrough N. J., Farr A. L., Randall R. J.: J. Biol. Chem. *193*, 265 (1951).
14. Swanson M. A. in the book: *Methods in Enzymology* (S. P. Collowick, N. O. Kaplan, Eds) 2, p. 541. Academic Press, New York 1955.
15. Fiske C. H., Subbarow Y.: J. Biol. Chem. *66*, 375 (1965).
16. Ernster L., Nordenbrand K. in the book: *Methods in Enzymology* (S. P. Collowick, N. O. Kaplan, Eds) 10, p. 574. Academic Press, New York/London 1967.
17. Orrenius S., Dallner G., Ernster L.: Biochem. Biophys. Res. Commun. *14*, 329 (1964).
18. Arstila A. U., Smith M. A., Trump B. F.: Science *175*, 530 (1972).

Translated by V. Kostka.

Comparison of Biotransformation and Lipid Peroxidation Activity of Microsomal Franctions Isolated by Ca^{2-}-Binding and Gel Filtration on Sepharose 2B and their Electron Microscopic Examination



a *b* *c*

FIG. 1

Electron Optical Photographs of Microsomal Preparations

a Preparation obtained by Ca^{2+}-binding (enlarged 28000 times), *b* preparation obtained by gel filtration (enlarged 17000 times), *c* preparation obtained by the standard method (enlarged 30000 times).