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Electron transport activities in rat liver endoplasmic reticulum membrane fragments prepared by chromatography on Sepharose 2B

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SUMMARY

A rapid and simple method based on Sepharose 2B column chromatography of the postmitochondrial supernatant is presented for isolation of endoplasmic reticulum membrane fragments from rat liver. Microsomal electron transport activities (NADPH–cytochrome *c* reductase, NADH – cytochrome *c* reductase, NADH–ferricyanide reductase and NADPH oxidase) in preparations so obtained are fully comparable with the activities of preparations obtained by ultracentrifugation.

Endoplasmic reticulum membrane fragments (microsomes) are obtained usually by a modification of the method of Claude[1] which depends on ultracentrifugation of the postmitochondrial supernatant. Since such membrane fragments obtained by one ultracentrifugation are contaminated with soluble proteins, recentrifugation of suspended membranes is usually necessary (washed microsomes). Thus the procedure is time consuming. A short procedure for isolation of endoplasmic reticulum membranes is of great importance for investigations on microsomal electron transport, where the age of the preparation cannot be neglected for its effect on the electron transport activities[2,3].

We wish to present a rapid, simple method for the isolation of endoplasmic reticulum membrane fragments that can be used for investigations on electron transport in these membranes.

Albino male Wistar rats (200–300 g) starved for 24 h were killed by decapitation. All subsequent operations were performed at 0–4°. The livers were removed, washed with 0.25 M sucrose and then homogenized in a Potter–Elvehjem homogenizer with 10 vol. of 0.25 M sucrose (pH 7.4). After centrifugation of unbroken cells, nuclei and mitochondria at 10 000 x *g* for 10 min, 15 ml of the supernatant were applied on the Sepharose 2B column (4 cm x 12 cm), previously equilibrated with 0.1 M sodium phosphate buffer (pH 7.4). Subsequent elution was performed with the same buffer. In the eluate two distinct fractions were found (Fig. 1): one turbid which was eluted at a volume of 50 ml

and the other, red, eluting at 120 ml. Nearly all the activity of NADPH–cytochrome *c* reductase was located in the first fraction which was the basis for its identification as the microsomal membrane fraction.

Electron transport activities of microsomal membrane preparations obtained by chromatography on Sepharose 2B are given in Table I. The activities are fully comparable with the activities of preparations obtained by ultracentrifugation[8-14]. The preparation is also active in aminopyrine demethylation, at a rate comparable with that of ultracentrifugated microsomes under the conditions described by Orrenius *et al.*[12]. The level of mitochondrial contamination, which was estimated by determination of the succinate–cytochrome *c* reductase activity, was below 3%. Moreover, from the specific activities of determined enzymes, contamination of other structures or their fragments also appears to be negligible.

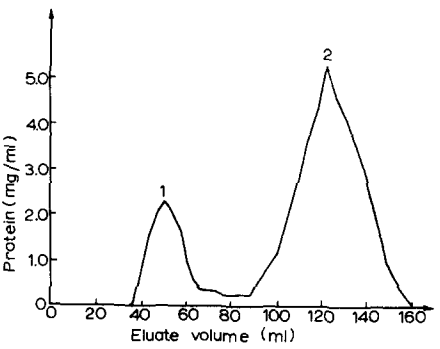

Fig. 1. Elution profile of the postmitochondrial supernatant chromatographed on Sepharose 2B. Sample containing 220 mg protein was subjected to chromatography in the conditions described in the text. Protein was determined by the method of Lowry *et al.*[4].

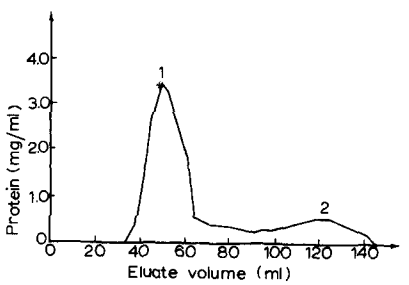

Fig. 2. Elution profile of the ultracentrifuged microsomal preparation chromatographed on Sepharose 2B. Postmitochondrial supernatant (see text) was centrifuged for 90 min at 105 000 × *g*. The pellet of microsomes was suspended in 0.1 M sodium phosphate buffer (pH 7.4) to a concentration of approx. 6 mg protein per ml. A 15-ml sample was subjected to chromatography in the conditions described in the text. Protein was determined by the method of Lowry *et al.*[4].

For the purpose of detection of changes in the activity of the preparation, while on Sepharose 2B, a preparation of microsomes obtained by ultracentrifugation of a postmitochondrial supernatant was subjected to chromatography. There is a close correlation between a small increase in the specific activity of the enzymes in Fraction 1 (Table II) and a partial transfer of protein to Fraction 2 (Fig. 2). These results are in accordance with the observations of Gaylor *et al.*[15], who noted only a small decrease in the specific activity of lanosterol demethylation (approx. 5%) following the chromatography of microsomes on Sephadex G-200.

The method presented enables one to obtain microsome membranes within about 1 h after the preparation of postmitochondrial supernatant in comparison with about 2 h

TABLE I

ENZYME ACTIVITIES IN THE MICROSOMAL MEMBRANE PREPARATIONS OBTAINED BY CHROMATOGRAPHY OF POSTMITOCHONDRIAL SUPERNATANT ON SEPHAROSE 2B

Enzyme activities were determined in Fraction 1 (see Fig. 1) as follows: NADPH–cytochrome *c* reductase according to Omura *et al.*[5]; NADH–cytochrome *c* reductase and NADH–ferricyanide reductase according to Takesue and Omura[6]; NADPH oxidase according to Gillette *et al.*[7], using 0.1 M sodium phosphate buffer (pH 7.4) and $3 \cdot 10^{-5}$ M NADPH. Protein was estimated by the method of Lowry *et al.*[4].

Expt. No.	*Reductase or oxidase activity (nmoles/min per mg protein)*			
	NADPH–cytochrome c	*NADH–cytochrome c*	*NADH–ferricyanide*	*NADPH oxidase*
1	53	920	3520	6.0
2	53	800	3460	5.6
3	82	745	2570	5.9
4	41	580	2130	4.4

TABLE II

ENZYME ACTIVITIES OF MICROSOMAL PREPARATION OBTAINED BY ULTRACENTRIFUGATION BEFORE AND AFTER CHROMATOGRAPHY ON SEPHAROSE 2B (see Fig. 2)

Enzyme activities were determined as in Table I. The activities after chromatography were measured in Fraction 1 (see Fig. 2).

Enzyme	*Activity before chromatography*	*Activity after chromatography*	
	(nmoles/min per mg protein)	*(nmoles/min per mg protein)*	*(% of activity before chromatography)*
NADPH–cytochrome *c* reductase	66	77	116
NADH–cytochrome *c* reductase	890	1020	115
NADH–ferricyanide reductase	3520	3960	112
NADPH oxidase	5.6	6.6	118

required usually in the standard procedure for ultracentrifugations only. The quantity of the obtained preparation (approx. 30–40 mg protein using one liver and a column of the size mentioned above) is only limited by technical difficulties which limit the size of the column in each Sepharose chromatography procedure.

The procedure is simple, rapid and is convenient for isolation of material for investigating the enzymes involved in electron transport in microsomes.

REFERENCES

1 A. Claude, *Harvey Lectures, Ser.*, 43 (1949) 121.
2 L. Ernster, P. Siekevitz and G.E. Palade, *J. Cell Biol.*, 15 (1962) 541.
3 H. Nishibayashi and R. Sato, *J. Biochem. Tokyo*, 61 (1967) 491.
4 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, *J. Biol. Chem.*, 193 (1951) 265.
5 T. Omura, P. Siekevitz and G.E. Palade, *J. Biol. Chem.*, 242 (1967) 2389.
6 S. Takesue and T. Omura, *J. Biochem. Tokyo*, 67 (1970) 267.
7 J.R. Gillette, B.B. Brodie and B.N. La Du, *J. Pharmacol. Exptl. Therap.*, 119 (1957) 532.
8 G.L. Sottocasa, B. Kuylenstierna, L. Ernster and A. Bergstrand, *J. Cell Biol.*, 32 (1967) 415.
9 S. Orrenius, Y. Gnosspelius, M.L. Das and L. Ernster in F.C. Gran, *Structure and Function of Endoplasmic Reticulum in Animal Cells*, Academic Press, London, 1968, p. 80.
10 G. Brunner and F.L. Bygrave, *European J. Biochem.*, 8 (1969) 530.
11 P.R. Dallman, G. Dallner, A. Bergstrand and L. Ernster, *J. Cell Biol.*, 41 (1969) 357.
12 S. Orrenius, A. Berg and L. Ernster, *European J. Biochem.*, 11 (1969) 193.
13 B. Kuylenstierna, D.G. Nicholls, S. Hovmöller and L. Ernster, *European J. Biochem.*, 12 (1970) 419.
14 A. Van Tol, *Biochim. Biophys. Acta*, 219 (1970) 227.
15 J.L. Gaylor, Young-Ja Chang, M.S. Nightingale, E. Recio and B.P. Ying, *Biochemistry*, 4 (1965) 1144.