SYNTHESIS IN VIVO OF THE LECITHIN COMPONENT OF THE INNER AND OUTER MEMBRANES OF RAT LIVER MITOCHONDRIA

F.L.BYGRAVE and Th.BÜCHER
Institut für Physiologische Chemie der Universität München, Germany

Received 22 May 1968

1. Introduction

Studies on the questions concerning the origin of the complex phospholipids that constitute a basic component of the inner and outer membranes of mitochondria have recently been initiated in this laboratory. This research has shown that the energy-dependent incorporation *in vitro* of choline [1,2] and of serine [3] into phosphatidylcholine (lecithin) and phosphatidylserine respectively by rat liver mitochondria, is associated predominantly with the outer membrane [1,3,4].

An important consideration was to determine in an *in vivo* system what influence the endoplasmic reticulum and other intracellular factors might have on the apparent distribution between the two membranes of the incorporated choline. It is shown in this report that the specific radioactivity of choline in the lecithin component of the outer membrane six hours after administration of the phospholipid precursor is approximately twice that of the inner membrane and is similar to that of the microsomes. These data in conjunction with prior *in vitro* data are interpreted as being consistent with the view that mitochondria can synthesise a proportion of their own lecithin.

2. Methods and materials

Two 16-hour starved rats (each weighing about 200 g) were injected intraperitoneally each with 12 μC ^{14}C[Me] choline (2 μC/mmole from New England Nuclear Corporation). The animals were sacrificed 6 hours later, their livers pooled and the mitochondria and microsomes prepared as previously described [4]. Subfractionation of the mitochondria by "swelling-shrinking" [5] and assays of monoamine oxidase and succinate cytochrome *c* reductase and of protein were also carried out as previously described [6]. The phospholipids were extracted from each fraction with chloroform-methanol (2 : 1) and subjected to thin-layer chromatography [7]. The spots corresponding to lecithin were scraped off the glass plate and the phospholipid separated from the silica with chloroform-methanol. Portions of the chloroform-methanol eluate were assayed after acid hydrolysis for phospholipid phosphorus [8] and for radioactivity by scintillation counting [3].

3. Results

A time of 6 hours after injection of the precursor was chosen because maximal specific radioactivity of total mitochondrial phospholipid with respect to both ^{14}C and ^{32}P is attained after this period [9]. Also ^{14}C-choline is maximally incorporated *in vivo* into the lecithin component of the microsomes after about 4 hours [10].

Data from four experiments in which the specific radioactivity of choline into the lecithin component of the submitochondrial fractions from rat liver was measured are presented in table 1. The following conclusions can be drawn from these data. First, of all the mitochondrial fractions, the specific radioactivity is highest in the outer membrane. On the basis of "pure" outer and "pure" inner membrane [6] the incorporation into the outer membrane is calculated to be approximately twice that into the inner mem-

Table 1
Distribution in mitochondria of choline incorporated into lecithin in vivo.

Fraction	Activity	1	SRpo,pi	2		SRpo,pi	3		SRpo,pi	4		SRpo,pi
Microsomes	SDH	–		0			0.05			0		
	MAO	–		148			250			68		
	SR	–		206	(1.4)		483	(1.9)		400	(5.9)	
Intact mitochondria	SDH	11.5		13.9			14			20.7		
	MAO	1690		1810			980			800		
	SR	30		174	(0.1)		297	(0.3)		335	(0.4)	
Upper band from gradient (outer membrane)	SDH	9.1		8.0			7.5			20.7		
	MAO	2300		3030			3440			2080		
	SR	80	86	195	(0.06)	212	352	(0.1)	394	380	(0.2)	422
Lower band from gradient (inner membrane)	SDH	40		30.2			25.4			51		
	MAO	1258		1580			1390			800		
	SR	62	39	146	(0.1)	92	295	(0.2)	256	320	(0.4)	283
Pellet from gradient	SDH	30.2		24.0			22.8			48		
	MAO	333		307			670			340		
	SR	42		130	(0.4)		266	(0.4)		287	(0.8)	
4000 g sediment obtained prior to gradient centrifugation	SDH	43.0		29.5			25.8			44		
	MAO	107		124			263			130		
	SR	46		94	(0.8)		259	(1.0)		253	(1.9)	
Ratio SR in pure inner membrane to SR in pure outer membrane (%)			45			43			65			67

Rats were injected with ^{14}C[Me] choline and the activities indicated measured in the different mitochondrial and microsomal fractions the preparations of which were begun 6 hours after injection. SDH, MAO, SR and SRpo,pi refer to succinate cytochrome c reductase, monoamine oxidase (each μmoles per hr per mg protein), specific radioactivity (cpm per μg lecithin P) and specific radioactivity in pure outer and pure inner membrane respectively. In experiment 1, 2μC instead of 12μC ^{14}C[Me] choline were administered. Figures in parentheses indicate the ratio SR/MAO. SRpo,pi were calculated by the procedure described in ref. [6].

brane. Second, the distribution of radioactivity and monoamine oxidase (a marker for the outer membrane [11]) in the various mitochondrial fractions are, as found in our *in vitro* studies [4], approximately parallel. Third, the specific radioactivity of the outer membrane is similar to that of the microsomes.

The data in table 2 show that when microsomes were placed on the gradient (see ref. [3]) the fractions obtained all had specific radioactivities which were similar to that of the original microsomes.

Table 2
Distribution in microsomes of choline incorporated into lecithin in vivo.

Fraction	Specific radioactivity
Untreated microsomes	483
Band on top of gradient	486
Band corresponding to outer membrane	496
Band corresponding to inner membrane	539

Density gradient centrifugation of the microsome fraction was carried out exactly as for the mitochondria. The specific radioactivity (cpm per μg lecithin P) in each of the bands obtained was then measured.

4. Discussion

The presence in an *in vivo* system of such unknown parameters as size and intracellular distribution of possible precursor pools, severely limit the interpretations which might be made from data of the type presented here. Also we cannot totally exclude the possibility that *exchange* of choline and not *de novo* synthesis of lecithin is being measured. In spite of these potential drawbacks however, it seems quite remarkable that the distribution of incorporated choline between the two membranes of mitochondria (in the presence of the endoplasmic reticulum) is very similar to that found in our *in vitro* studies [1,4]. In those studies it was shown that contaminating microsomes contributed to less than 1% of the incorporation by mitochondria [1,4].

The data in table 2 suggest that if contaminating microsomes in the mitochondria did contribute to the incorporation values obtained for the mitochondrial fractions, the specific radioactivity of pure inner membrane would be even less than the values we have determined experimentally. Furthermore, the similarity in the ratio SR/MAO for the outer and inner membranes and its much lower value as compared to that for the microsomes (table 1), excludes any major contribution by the microsomes. The ratio μg lecithin P/mg protein is highest in the outer membrane and lowest in the inner membrane while that for the microsomes lies between these two (see ref. [5]).

These observations as a whole are consistent with the view that mitochondria can synthesise a proportion of their own lecithin. This view which is different from that of some previous investigators [12], is strongly supported by the recent report that the enzymes in mitochondria responsible for the biosynthesis of lecithin are associated with the outer membrane [13].

It is of interest that the outer membrane of mitochondria and the microsomes have similar specific radioactivities. More data of this nature should help provide a further enlightening on the question of the origin of mitochondria and on the possible relation of the outer membrane to the endoplasmic reticulum.

Acknowledgements

The authors are grateful to Dr.W.Neupert for helpful discussion and to Mrs.U.Neumann for excellent assistance. F.L.B. is grateful to the Alexander von Humboldt-Stiftung for a research fellowship and to the Wellcome Trust (London) for a travel grant.

References

[1] F.L.Bygrave and W.Kaiser, Hoppe-Seyler's Z. Physiol. Chem. 349 (1968) 3.
[2] F.L.Bygrave and W.Kaiser, European J. Biochem., in press.
[3] F.L.Bygrave and Th.Bücher, submitted for publication.
[4] W.Kaiser and F.L.Bygrave, European J. Biochem., in press.

[5] D.Parsons, G.Williams, W.Thompson, D.Wilson and B. Chance, in: Round Table Discussion on Mitochondrial Structure and Compartmentation, eds. S.Papa, J.M. Tager, E.Quagliariello and E.C.Slater (Adriatica Editrice, Bari, Italy, 1966) p. 29.
[6] W.Neupert, D.Brdiczka and Th.Bücher, Biochem. Biophys. Research Commun. 27 (1967) 488.
[7] V.P.Skipski, R.F.Peterson and M.Barclay, Biochem. J. 90 (1964) 374.
[8] C.H.Fiske and Y.Subbarow, J. Biol. Chem. 66 (1925) 375.
[9] C.B.Taylor, E.Bailey and W.Bartley, Biochem. J. 105 (1967) 605.
[10] D.Rytter, J.E.Miller and W.E.Cornatzer, Biochim. Biophys. Acta 152 (1968) 418.
[11] C.Schnaitman, G.V.Erwin and J.W.Greenawalt, J. Cell Biol. 32 (1967) 719.
[12] G.F.Wilgram and E.P.Kennedy, J. Biol. Chem. 238 (1963) 2615.
[13] H.G.Schiefer and W.Stoffel, Hoppe-Seyler's Z. Physiol. Chem. 349 (1968) 14.