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SHORT COMMUNICATION

THE INTRAMITOCHONDRIAL DISTRIBUTION OF THE 3β-HYDROXYSTEROID DEHYDROGENASE-OXOSTEROID ISOMERASE. A PROBABLE REDISTRIBUTION ARTIFACT

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The 3β-hydroxysteroid dehydrogenase [E.C. 1.1.1.51] and 3-oxosteroid isomerase [E.C. 5.3.3.1] are components of the smooth endoplasmic reticulum of adrenocortical cells [1, 2]. Recently, activity of these enzymes was found in the mitochondrial fraction prepared by differential centrifugation of adrenocortical homogenates [3, 4]. This activity was not due to contamination of the mitochondrial fraction by microsomal enzymes as judged by the use of enzyme markers.

It is possible that the association of the dehydrogenase and isomerase enzymes with mitochondria is an example of a "redistribution artifact"; that is, the *in vitro* detachment of an enzyme from one site and subsequent binding to another, possibly during tissue homogenization [5]. If this were the case, the dehydrogenase and isomerase would apparently be components of the outer mitochondrial membrane.

In the present work, the intra-mitochondrial localisation of these enzymes was determined. A high speed zonal rotor (B XIV, Measuring & Scientific Equipment Limited, Crawley, U.K.) was used to separate outer and inner membrane preparations from bovine adrenocortical mitochondria. The tissue was passed through a hand Latapie mincer and then homogenized in a glass vessel with a motor driven Teflon pestle in 0·25 M sucrose, 1 mM EDTA, 30 mM Tris–HCl (pH 7·4). The homogenate was filtered through four layers of surgical gauze and centrifuged at 700 *g* ($r_{av.}$ 16·0 cm) for 10 min. The supernatant material was then centrifuged at 10,000 *g* ($r_{av.}$ 6·9 cm) for 15 min to give a crude mitochondrial fraction. This preparation was suspended in 0·25 M sucrose, 30 mM Tris–HCl (pH 7·4) without EDTA (sucrose–Tris solution) and recentrifuged at 10,000 *g* ($r_{av.}$ 6·9 cm) for 15 min. The pellet was resuspended in 20 mM phosphate buffer (pH 7·4) and subfractionation of mitochondria carried out by the method of Parsons, Williams, Thompson, Wilson and Chance [6] as modified by Satre, Vignais and Idelman [9]. The resulting membrane preparation was finally fractionated on a discontinuous sucrose density gradient in the zonal rotor. Prior to centrifugation, the rotor contained 50 ml of 5% (w/v) sucrose solution "overlay", 25 ml of sample, 150 ml of 15% (w/v) sucrose solution, 150 ml of 30% (w/v) sucrose solution, 200 ml of 40% (w/v) sucrose solution and the remaining volume 55% (w/v) sucrose solution as "cushion". The rotor was run in an MSE Superspeed 65 Mark II centrifuge at 47,000 rev./min for 1 h at 4°C. The rotor was unloaded by displacing the gradient, light end first, with 55% (w/v) sucrose solution. Material was collected from the 15–30% and 40–55% sucrose solution interfaces. The first is referred to as the "light preparation" and the second the "heavy preparation". Each preparation was diluted with an equal volume of sucrose–Tris solution

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Table 1. Distribution of marker enzymes and 3β-hydroxysteroid dehydrogenase—isomerase in mitochondrial membrane fractions

	"Light" Preparation (outer membrane)		"Heavy" Preparation (inner membrane)	
	Recovery	Specific enzyme activity	Recovery	Specific enzyme activity
Cytochrome oxidase (Inner membrane marker)	1·2%	230·00	13·2%	1,750·00
Monoamine oxidase (Outer membrane marker)	13·5%	15·00	1·2%	0·90
Steroid 21-hydroxylase (Microsomal marker)	0·4%	0·27	0·4%	0·18
Dehydrogenase/isomerase	11·8%	39·30	1·0%	2·10

Recovery is expressed as % of the enzyme activity in the crude mitochondrial fraction from which the membrane preparations were obtained.

All specific activities are expressed as nmoles product formed/min/mg protein.

and centrifuged at 20,000 g (r_{av} 6·9 cm) for 1 h in a conventional angle rotor. These preparations correspond to the outer (light) and inner (heavy) membrane fractions, respectively.

The pellets were resuspended in a small volume of sucrose–Tris solution. Aliquots of these suspensions were assayed for marker enzymes as follows: for inner membranes, cytochrome oxidase [E.C. 1.9.3.1] by the method of Smith [7]; for outer membranes, monoamine oxidase [E.C. 1.4.3.4] by the method of Wurtman and Axelrod [8]; for smooth endoplasmic reticulum fragments, steroid 21-hydroxylase [1.14.1.8] by the method of Satre, Chambaz, Vignais and Idelman [10]. Activity of the hydroxysteroid dehydrogenase and isomerase enzymes was measured by incubation of enzyme preparation (0·1 ml) in a medium containing 30 mM Tris HCl (pH 7·4), 0·5 mM NAD^+ and 10 μg pregnenolone in 10 μl N:N-dimethylacetamide (final volume 2 ml). After extraction, pregnenolone remaining and progesterone formed were measured by gas liquid chromatography on a 5 ft glass column packed with 1% OV-1 on Gas Chrom Q.

The distribution of these enzymes in the sub-mitochondrial membrane fractions is shown in Table 1. These results suggest that the hydroxysteroid dehydrogenase and isomerase enzymes found in the mitochondrial fraction are associated with the outer mitochondrial membrane rather than with the inner membrane. They may thus represent a redistribution artifact. Quantitative studies of the activity of these enzymes in whole tissue preparations and in membranes and other cellular components, to elucidate this matter further, are planned.

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