Vol. 103, No. 1, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 16, 1981 Pages 369-375

POLY (ADP-RIBOSE) SYNTHETASE ACTIVITY IN RAT TESTIS MITOCHONDRIA

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Received September 16,1981

SUMMARY

A quite active poly (ADP-ribose) synthetase was found in isolated rat testis mitochondria. Similar levels of activity were found in mitochondria isolated from bull and hamster testis. In contrast, mitochondria isolated from rat brain or liver, and demembraned sperm, showed negligible activity. Centrifugation of testis mitochondria through a linear sucrose gradient,showed that, poly (ADP-ribose) synthetase cosediment together with succinate-cytochrome c reductase and mitochondrial proteins. Furthermore, treatment with digitonin indicated that, the enzyme is localized in the inner membrane-matrix complex. Finally, kinetic studies demonstrated that, the apparent Km for NAD^+ of the mitochondrial enzyme, was 22 μM compared with 210 μM for the nuclear enzyme.

Poly (ADP-ribose) synthetase is a well characterized enzyme, localized in the nucleus of a great variety of vertebrate and invertebrate cells, as well as in unicellular eukaryotic organisms and plants (1,2). Besides, the nuclear localization of the enzyme, a similar activity was detected in ribosomes of HeLa cells (3) and in rat liver mitochondria (4).

This report describes the presence of an active poly (ADP-ribose) synthetase in isolated mitochondria from rat testis. This finding contrast with the negligible activity detected in mitochondria from somatic tissues, such as liver and brain.

MATERIALS AND METHODS

Isolation of Mitochondria. Decapsulated rat testis was suspended in two volumes of ice-cold medium containing 0.25 M sucrose,

0006-291X/81/210369-07$01.00/0

25 mM Tris-HCl (pH 7.4) and 1 mM EDTA (Medium A). The suspension was homogenized with ten strokes in a motor-driven Potter-Elvehjem tissue grinder and diluted with Medium A, to give a 10% homogenate. The homogenate was centrifuged at 4°C for 15 min at 550 x g. The supernatant was centrifuged at 9000 x g for 10 min and, the resulting mitochondrial pellet was resuspended by hand homogenization in the original volume of Medium A and, again centrifuged at 550 x g for 15 min. About 3/4 of the supernatant was transferred to another tube and centrifuged at 9000 x g for 10 min. This washing procedure was repeated twice. The final mitochondrial pellet was resuspended in Medium A to final concentration of about 25 mg of protein per ml. This mitochondrial preparation was further purified by sedimentation through a 0.9 M to 2.1 M sucrose gradient (5). The tubes were centrifuged at 4°C (rotor SB-110) in the International Ultracentrifuge Model B-60, at 22000 rpm for 90 min. The gradients were fractionated from the bottom of the tube with a peristaltic pump.

Mitochondria from rat liver and brain, as well as from hamster and bull testis, were prepared under identical conditions. Rat sperm were obtained from the cauda epididymis as reported (6).

Treatment of isolated mitochondria with recrystallized digitonine (7) was carried out as described (8).

Enzymatic Assays. Mitochondrial poly (ADP-ribose) synthetase was assayed at 20°C for 5 min in a final volume of 0.1 ml, which contained 50 mM Hepes buffer (pH 7.5), 10 mM $MgCl_2$, 0.06 mM [^{14}C] adenosine-NAD (New England Nuclear, specific activity = 100 cpm/pmol) and 0.1 to 0.2 mg of protein. The radioactivity incorporated into the trichloroacetic acid - insoluble fraction was determined as reported elsewhere (9).

Succinate-cytochrome c reductase was assayed as described before (10). Monoamino oxidase (E.C. 1.4.3.4.) was assayed according to the procedure of Tabor et al. (11), using benzylamine-HCl as substrate. Protein was estimated by the method of Lowry et al. (12), using bovine serum albumin as standard.

RESULTS AND DISCUSSION

Table I summarized the activities of poly (ADP-ribose) synthetase measured in isolated mitochondria from rat, hamster and bull testis. A variable increase in activity of about 50 percent was found when the assay was carried out with mitochondria, previously subjected to hypotonic shock, sonicated or treated with one percent Triton X-100. In contrast, the activity measured in rat liver or brain mitochondria was low, and did not change significantly after sonication (4) or treatment with Triton X-100.

Table I. Poly (ADP-ribose) synthetase activity in mitochondria isolated from different tissues.

Mitochondria	Activity (pmol ADP-ribose/mg protein)	
	Exp. 1	Exp. 2
Rat testis	147	128
Hamster testis	160	135
Bull testis	258	308
Rat liver	3	5
Rat brain	5	1
Rat sperm (intact)	0.5	2
Rat sperm (sonicated)	2	-
Rat sperm (hypotonic lysed)	1.5	2

The enzymatic assay was performed as described in Materials and Methods.

Contrary to the high enzymatic activity found in rat testis mitochondria, the activity measured in isolated sperm from the epididymis was negligible (Table I). This poor activity did not change after hypotonic lysis or sonication of the sperm. It is well known that these treatment exposed the mitochondrial sheath (13), which can oxidized various substrates (14).

Rat testis mitochondria prepared by the procedure described in Methods, should be considered a crude preparation. Thus, the mitochondria were further purified by sedimentation in a 0.9 M to 2.1 M linear sucrose gradient, and the distribution throughout the gradient of poly (ADP-ribose) synthetase was determined and compared with the distribution of succinate-cytochrome c reductase activity and mitochondrial protein. As shown in Fig. 1, poly (ADP-ribose) synthetase was localized in the gradient in the same position of the mitochondrial enzyme and protein. This

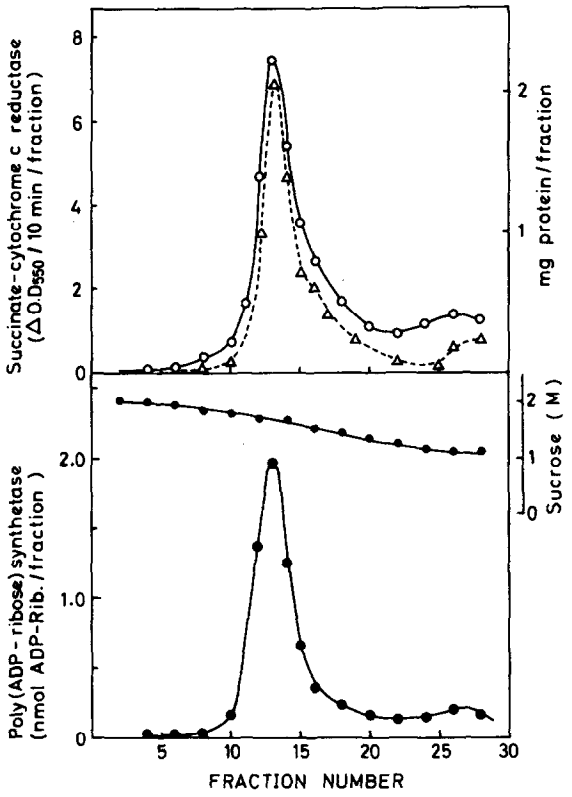

Fig. 1. **Distribution of poly (ADP-ribose) synthetase (●—●), succinate-cytochrome c reductase (○—○) and mitochondrial protein (△---△) in a linear sucrose gradient. About 8 mg of mitochondrial proteins were sedimented through a linear 0.9 M to 2.1 M sucrose gradient as described in Materials and Methods. Aliquots were taken from the different fractions for enzymatic assays and protein determination. The profile of the gradient is also shown.**

result confirmed the mitochondrial localization of poly (ADP-ribose) synthetase.

Like the nuclear activity, the rat testis mitochondrial enzyme was strongly inhibited by nicotinamide, thymidine and theophylline at a final concentration of 1 mM (Table II). These results differ markedly from the properties of the poly (ADP-ribose) synthetase associated to rat liver mitochondria. Kun et al. (4) reported that the rat liver mitochondrial enzyme was partially inhibited by nicotinamide and not inhibited by thymidine.

Table II. Effect of different inhibitors on the activity of rat testis mitochondrial poly (ADP-ribose) synthetase.

Assay conditions	Activity pmol ADP-ribose/mg protein	Inhibition %
Complete	155	0
+ Nicotinamide (1 mM)	5	97
+ Thymidine (1 mM)	15	90
+ Theophylline (1 mM)	19	88

The enzyme assay was performed as described in Materials and Methods.

These inhibition studies demonstrated that the mitochondrial enzyme has similar properties as the nuclear enzyme (1,2). Therefore, it was interesting to compare the kinetic properties of both poly (ADP-ribose) synthetase, regarding the requirement of NAD^+. As shown in Fig. 2, the apparent Km for NAD^+ of the mitochondrial enzyme was 22 μM, compared with 210 μM for the nuclear enzyme. This result strongly suggests that the mitochondrial activity is not due to contamination with the nuclear enzyme.

To test whether, the enzyme was associated to the outer mitochondrial membrane or the inner membrane-matrix complex, rat

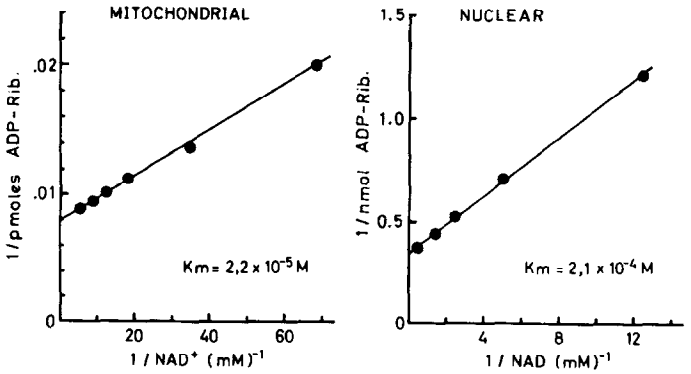

Fig. 2. Double reciprocal plot of the effect of the NAD^+ concentration on the poly (ADP-ribose) synthetase activity. The apparent Km of the rat testis mitochondrial and nuclear enzyme are shown.

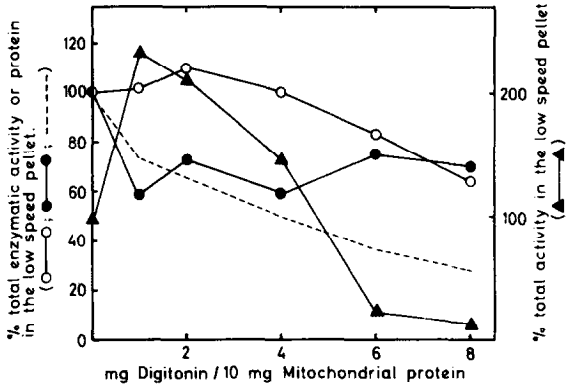

Fig. 3. **The effect of the digitonin concentration on the activity of various enzymes in rat testis mitochondrial. The percentage of total protein and enzymatic activity recovered in the 10.000 x g pellet are plotted versus the digitonin concentration. Poly (ADP-ribose) synthetase (●—●), succinate-cytochrome c reductase (○—○), monoamino oxidase (▲—▲), protein (-----).**

testis mitochondria were treated with different amounts of digitonin (8), and the activity of poly (ADP-ribose) synthetase, together with succinate-cytochrome c reductase and monoamino oxidase, was assayed in the low speed pellet (10.000 x g for 15 min). As shown in Fig. 3, of all the concentration of digitonin studied, about 70 percent of the poly (ADP-ribose) synthetase, and most of the succinate-cytochrome c reductase was found in the low speed pellet. The monoamino oxidase, on the other hand, progressively dissappear from the low speed pellet (8).

The present results demonstrated the presence of a high poly (ADP-ribose) synthetase activity in rat, hamster and bull testis mitochondria. So far, the major difference with the nuclear enzyme is the apparent Km for NAD^+. Otherwise, both enzymes have similar properties. The product of the reaction are oligomers of ADP-ribose (overage chain length between 2 to 3 residues), which are attached to mitochondrial proteins[1].

[1] Cornejo and Burzio, in preparation.

It is intriguing why testis mitochondria contains such high poly (ADP-ribose) synthetase activity, compared with the almost negligible activity in mitochondria isolated from somatic tissues. Recent developmental studies, showed that the maximal enzymatic activity (about 700 pmoles/mg of protein) was found in mitochondria isolated from testis of 20 days old rats[1]. Since at this age, the germinal epithelium is rich in primary spermatocytes (15), it is reasonable to think that, mitochondria from these cells contains the highest poly (ADP-ribose) synthetase activity.

Acknowledgments. We wish to thank Mrs. M.Angélica Espinoza for her skilful typing and Mr. Risnel Bustos for the illustrations. This work was supported by Grant S-79-20 from the Research Fund of the Universidad Austral de Chile.

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