SHORT COMMUNICATIONS

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Acid-soluble nucleotides of beef heart mitochondria

To establish the nature of oxidative phosphorylation it is necessary to study the state of nucleotides in mitochondria and to ascertain the mechanisms of reactions in which they take part. From this point of view, beef heart mitochondrium is the best experimental material, as its oxidative phosphorylation processes have been studied rather well. However, the data on the acid-soluble nucleotide fraction of these mitochondria are rather scarce. Only acid-soluble nucleotides of mitochondria isolated from rat liver[1–5], pigeon heart muscle[3,4], and from the yeasts *Endomyces magnussi*[6,7] and *Saccharomyces carlsbergensis*[8] have been thoroughly investigated. There are some data on the mitochondrial nucleotide content of rat heart and pig heart[9], cat brain[10], and the mold *Neurospora crassa*[7,11]. It is clear, therefore, that any data on beef heart mitochondrial nucleotides are interesting *per se*. In this paper the data are presented on the nucleotide content of the acid-soluble fraction in beef heart mitochondria and the share of these nucleotides in the total nucleotide content of the whole tissue.

Heavy beef heart mitochondria were prepared by the method of CRANE, GLENN AND GREEN[12]. An aliquot of heavy beef heart mitochondria was used to determine respiration and phosphorylation, to estimate cytochrome *a* content, as described by EVTODIENKO AND MOKHOVA[13], and for electron microscopic analysis. Mitochondrial protein was determined by the biuret method. The major portion of heavy beef heart mitochondria was fixed by liquid nitrogen.

We also studied acid-soluble nucleotides in mitochondria after their preincubation in the presence of 10 mM phosphate, 3 mM $MgSO_4$, 5 mM glutamate, 5 mM malate and 0.25 M sucrose. 250 ml of mitochondrial suspension were added to 500 ml of the above medium to a final concentration of 10 mg protein per ml. Incubation was carried out with stirring for 7 min at 25°. After that mitochondria were fixed in liquid nitrogen.

Nucleotide content of the acid-soluble fraction of mitochondria was compared to that of the whole heart muscle. The latter material was obtained from animals immediately after slaughtering. Pieces of heart were fixed in liquid nitrogen.

Acid-soluble nucleotides were studied by means of charcoal adsorption and by anion-exchange chromatography on Dowex 1 in the formic acid system, according to the procedure described earlier[7]. The identity of the compounds obtained with reference nucleotides was proved by means of chromatographic, electrophoretic, and spectrophotometric assays.

The ratios of the following components were determined: base[14], ribose[15], and total, labile and stable phosphorus[16]. NAD^+ was identified by means of alcohol dehydrogenase[17]. The amounts of NADH and NADPH were determined by the amounts of their acidic hydrolysis products: ADP-ribose and ADP-ribose phosphate[18].

Analysis of acid-soluble nucleotides of beef heart mitochondria revealed NAD^+,

NADH, $NADP^+$, NADPH, AMP, ADP, ATP, GMP, GDP, GTP, and large quantities of inosine and hypoxanthine. The amount of adenylic nucleotides (AMP + ADP + ATP) was half the total nucleotides content, and pyridine nucleotides amounted to 40% (Table I). In the case of incubated mitochondria a rather large amount of fumaric acid was detected. The NAD^+ + NADH to $NADP^+$ + NADPH ratio was equal to 3.

TABLE I

NUCLEOTIDES IN WHOLE TISSUE AND IN BEEF HEART MITOCHONDRIA

Data in parentheses are the values of nucleotides that were either not detected or not measured in the whole tissue. These values might be considered the minimum possible amounts contained in mitochondria.

Nucleotides	*μmoles/μmole of cytochrome a*	
	Beef heart	*Beef heart mitochondria*
NAD^+	4.89	3.25
AMP	16.21	6.45
$NADP^+$	(1.00)	1.00
GMP	(0.40)	0.40
NADH	(3.30)	3.30
ADP	39.46	2.65
GDP	(0.05)	0.05
NADPH	1.60	1.15
ATP	17.36	1.35
GTP + UTP	(0.20)	0.20
Total	87.17	19.80
AMP + ADP + ATP	73.03	10.45
NAD^+ + NADH	(8.19)	8.19
$NADP^+$ + NADPH	(2.15)	2.15

Liver mitochondria differ greatly from those of heart. The content of acid-soluble nucleotides, especially ATP and ADP, is significantly higher in the former. The amount of $NADP^+$ in rat liver mitochondria is higher than that of NAD^+ and the $NADP^+$ + NADPH to NAD^+ + NADH ratio varied from 1 to 2.5 (refs. 3–5). It is of interest that mitochondria from the yeast are similar to those of beef heart mitochondria in that their NAD^+ + NADH to $NADP^+$ + NADPH ratio is 3 (ref. 6).

Thus, the ratio of nicotinamide nucleotides in beef heart mitochondria is quite similar to those obtained for heart mitochondria of other animals and differs greatly from liver mitochondria. This, evidently, may be attributed to the difference in metabolism in the tissues of these organs.

Attention should be paid to the presence of large amounts of inosine and hypoxanthine in both kinds of mitochondria studied. This fact may be accounted for by very active adenylic acid desaminase contained in beef heart mitochondria. The presence of this enzyme in mitochondria was recently demonstrated by GORKIN *et al.*[19].

In the next series of experiments, the fractional amounts of mitochondrial nucleotides in the heart tissue cells were determined. It has been found that all the cytochrome *a* is localized in the mitochondria[20]. Hence, if one knows the amount of cytochrome *a* in the heart muscle and mitochondrial preparations, it is possible to

calculate the fractional amounts of certain mitochondrial compounds in the cell.

Beef heart tissue contains mainly derivatives of adenylic acids: AMP, ADP, ATP, NAD^+, NADH, $NADP^+$ and NADPH, and a high amount of adenine, adenosine, hypoxanthine and inosine. The latter compounds amounted to 60 % of the ultraviolet-absorbing material of the $HClO_4$ extract adsorbed by charcoal. Beef heart mitochondrial nucleotides were shown to amount to 20 % of the nucleotide content of the whole tissue. About 70 % of NAD was intramitochondrial, while the ATP + ADP + AMP content in mitochondria did not exceed 14% of the total nucleotide content of the tissue.

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