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Mitochondrial metabolism of guanine nucleotides

Possible role of guanosine

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The catabolism of intramitochondrial guanine nucleotides was examined. During 30 min incubation of rat liver mitochondria at 37°C in the presence of oligomycin and carboxyatractyloside, guanine and xanthine were formed and appeared in the medium. Under these conditions, the direct conversion of GMP to guanine by hypoxanthine–guanine phosphoribosyltransferase is suggested to be the main catabolic route within the organelles. Only very small amounts of guanosine were produced and detected both inside and outside the organelles. [^{14}C]Guanosine and [^{14}C]inosine were taken up by the mitochondria. Therefore, guanosine is suggested to be a precursor of intramitochondrial guanine nucleotides.

Mitochondria; Purine nucleotide metabolism; Guanosine; HPLC; (Rat liver)

1. INTRODUCTION

So far, little is known about the interconversion of guanine nucleotides in mitochondria [1]. However, guanine nucleotides should at least perform important regulatory functions in these organelles owing to some particular reactions, e.g. succinate thiokinase and nucleoside-diphosphate kinase reactions [1], utilizing them as substrates. Also, it was recently shown that guanine nucleotides play an essential role in in vitro protein synthesis in mitochondria, which has not yet been elucidated [2]. The turnover of mitochondrial nucleic acids also requires the continuous supply of nucleotides [3]. In recent studies, the uptake and phosphorylation of deoxyguanosine by rat liver mitochondria [4] and properties of a purified mitochondrial deoxyguanosine kinase [5] were demonstrated. However, to date, intramitochondrial metabolism, i.e. degradation and synthesis or salvage, of (oxy-)guanine nucleotides has not been investigated. Moreover, the source of mitochondrial guanine nucleotides remains unknown, since the nucleotides themselves appear unable to penetrate the mitochondrial inner membrane [1].

The present study was aimed at elucidating the routes of guanine nucleotide degradation in mitochondria. We show here that guanine nucleotides are degraded to guanine and xanthine. In addition, guanosine is proposed to be a precursor of mitochondrial guanine nucleotides.

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Abbreviation: CAT, carboxyatractyloside

2. MATERIALS AND METHODS

2.1. *Isolation of mitochondria*

Mitochondria were isolated from the livers of fed male Wistar rats (200–250 g body wt) as in [6] in a medium consisting of 250 mM sucrose, 0.5 mM EDTA and 50 mM Tris-HCl, pH 7.4. The mitochondrial pellet was washed twice and finally resuspended in a medium containing 125 mM sucrose, 60 mM KCl, 0.5 mM EDTA and 50 mM Tris-HCl (pH 7.4) to a protein

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concentration of approx. 20 mg/ml. Protein was determined using the Biuret method with bovine serum albumin as standard.

2.2. *Catabolism of intramitochondrial guanine nucleotides*

Mitochondria were incubated in the presence of 5 μM oligomycin and 12 μM CAT at 37°C. After the time periods noted acid-soluble fractions of aliquots of the mitochondrial suspension were obtained as in [7]. Simultaneously, aliquots were centrifuged for 2 min at 11 000 × *g*. The supernatants, representing the 'mitochondria-free' medium, were extracted in the same way as the mitochondrial suspension. The neutralized extracts were stored at −20°C until analyzed.

2.3. *HPLC analysis of purine compounds*

Purine nucleotides and their catabolites were separated using a reverse-phase ion-pair HPLC technique [8]. Since IMP and GMP as well as hypoxanthine and guanine could not be separated from each other, respectively, their contribution to the joint peaks was evaluated by simultaneous peak scanning considering the differences in the absorbance spectra of these compounds.

2.4. *Incorporation of nucleosides into mitochondria*

Mitochondria (5–7 mg/ml) were incubated at 37°C in resuspension medium containing additionally (final concentrations) 1 mM ATP, 5 mM KH_2PO_4, 1 mM $MgCl_2$ and 35 μM guanosine or inosine (^{14}C-labelled, 1 μCi/ml). The assay (final volume 1 ml) was started by addition of mitochondria to prewarmed (37°C) incubation medium. After the indicated time intervals 100-μl aliquots were layered onto membrane filters (ME 26, pore size 0.6 μm), which were rinsed immediately with 20 ml ice-cold resuspension medium using a vacuum pump. The 0 min values were determined separately under the same conditions, except the temperature was maintained at 0–2°C. Filters were dried and the radioactivity subsequently quantified via standard scintillation techniques.

3. RESULTS

Fig.1 demonstrates the time courses for degradation of guanine nucleotides (+IMP), guanine + hypoxanthine and xanthine during 30 min incubation at 37°C in the presence of 5 μM oligomycin and 12 μM CAT. While guanine + hypoxanthine and xanthine increased during the first 5 min the nucleotides remained nearly constant over 15 min. After 15 min a sharp decline in the nucleotides was observed. Since in the HPLC method applied guanine and hypoxanthine co-eluted, the contribution of each compound to the joint peak was estimated by peak scanning. Quantitative evaluation was based on the differences in absorbance spectra (fig.2). The peak scans revealed that the increase in this peak was mainly due to formation of guanine. Similarly, IMP was elicited as a minor constituent of the joint peak of IMP and GMP

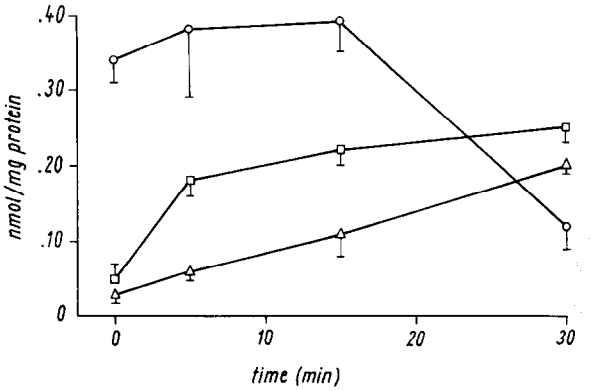

Fig.1. Degradation of guanine nucleotides in mitochondria. Mitochondria were incubated at 37°C in the presence of 5 μM oligomycin and 12 μM CAT; (○) GTP + GDP + GMP + IMP, (□) guanine + hypoxanthine, (△) xanthine. Data represent means ± SE of three experiments.

throughout the incubation period. Guanosine increased insignificantly from 0.05 to 0.09 nmol/mg protein.

No nucleotides were detected in the mitochondria-free medium (see section 2.2). In contrast, guanine and xanthine were found to be present to almost the same extent in the medium as in the whole suspension, indicating that they were almost completely localized outside the mitochondria. About 50% of the guanosine was found in the medium.

In further experiments guanosine and inosine were incorporated into mitochondria (fig.3). In both cases the major portion of the nucleoside had been incorporated within 5 min. Uptake did continue ($p < 0.05$ for guanosine), but at lower rates.

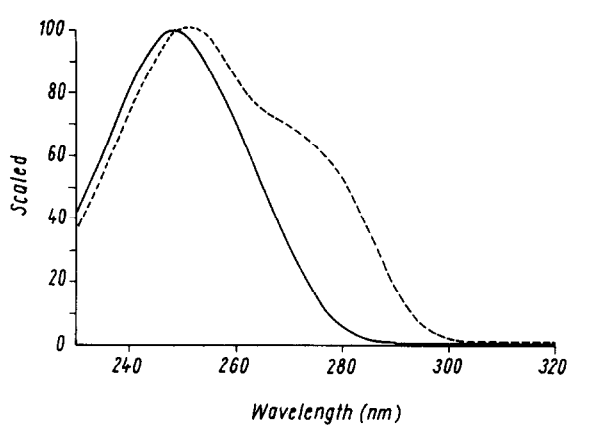

Fig.2. Overlay of peak scans obtained during separations of standard compounds. (——) Hypoxanthine, (– – –) guanine.

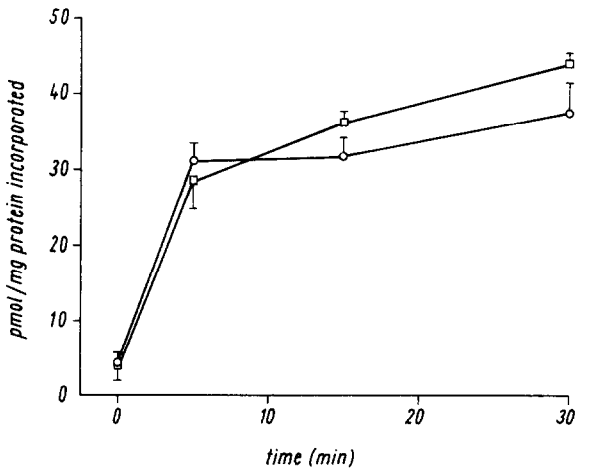

Fig.3. Incorporation of [^{14}C]guanosine (□) and [^{14}C]inosine (○) into mitochondria. Mitochondria were incubated at 37°C in the presence of 1 mM ATP, 5 mM KH_2PO_4, 1 mM $MgCl_2$ and 35 μM of the nucleoside indicated. Data represent means ± SE of three experiments.

4. DISCUSSION

In continuation of a previous study on the catabolism of mitochondrial adenine nucleotides [9], the degradation of guanine nucleotides and incorporation of nucleosides in these organelles were investigated.

The main products formed were guanine and xanthine (fig.1). Therefore, two possible routes for degradation to guanine via GMP should be considered: (i) conversion of GMP to guanosine by 5′-nucleotidase, followed by degradation to guanine by purine nucleoside phosphorylase (PNP); (ii) formation of guanine directly from GMP by hypoxanthine-guanine phosphoribosyltransferase. From the following considerations the latter pathway appears to be the major, if not exclusive, route. Purine nucleoside phosphorylase catalyzes the phosphorylytic cleavage of guanosine and inosine as well [10]. Although the accumulation of inosine exceeds that of guanosine 20-fold [9], hypoxanthine formation is not observed. Thus, rat liver mitochondria lack purine nucleoside phosphorylase activity.

Since guanine nucleotides were not detected in the medium the guanine is suggested to be produced inside the mitochondria, being released thereafter. The site of xanthine formation by guanase remains to be established.

Guanosine was almost equally distributed (per mg protein) between intra- and extramitochondrial compartments. However, it increased only very slightly and appears to be unimportant in the degradation process. Interestingly, in a study of adenine nucleotide degradation in mitochondria [9], we found that inosine, like guanosine in the present paper, was not further degraded and released into the medium. The question arises as to whether mitochondria are capable of taking up these nucleosides. This could be of great consequence for a mitochondrial salvage pathway, since the source of mitochondrial guanine nucleotides has remained unknown [1]. As demonstrated in fig.3, both guanosine and inosine were incorporated into mitochondria under conditions similar to those favouring deoxyguanosine uptake and phosphorylation by rat liver mitochondria [4]. One may assume that the continued uptake of guanosine is the result of intramitochondrial phosphorylation.

The role of incorporation of inosine into the organelles is unclear, since adenine nucleotides themselves can be taken up by mitochondria [11]. However, this does not exclude the possibility of the existence of a salvage pathway.

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