THE EFFECT OF MITOCHONDRIA ON THE TERMINAL STAGES OF PYRIMIDINE NUCLEOTIDES BIOSYNTHESIS

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1. Introduction

The factors controlling the rate of free nucleotides biosynthesis in animal tissues are not well known. Since the amount of pyrimidine nucleotides in the cell is limited, their more direct involvement in regulatory mechanisms may be anticipated. In previous studies [1] a rat liver enzyme system was developed, which allows the study *in vitro* of the terminal stages of pyrimidine nucleotides biosynthesis, *i.e.* the steps: orotic acid → uridine nucleotides → cytidine nucleotides. The enzymes for these reactions are located in the cytoplasma [2,3] and therefore the enzyme system studied is constituted by the soluble cell fraction. Further, it is known that cell structures, mitochondria in particular, may influence some metabolic reactions in the hyaloplasma [4]. In this paper the action of added mitochondria on the *in vitro* synthesis of uridine and cytidine nucleotides is reported. It is shown that two mitochondrial enzymes, adenylate kinase and glutaminase, interfere in the *in vitro* synthesis of pyrimidine nucleotides catalyzed by a rat liver soluble enzyme system.

2. Methods

Rat liver cell fractions were obtained as described previously [5] with the only difference that mitochondria were centrifuged down for 10 min and this fraction was washed 2–3 fold. Pyrimidine nucleotides synthesis was determined by the incorporation of [^{14}C] orotate into the respective fractions [1]. The "standard" incubation medium contained in a final volume of 2 ml: 385 μmoles sucrose, 28 μmoles KCl, 55 μmoles Tris-HCl buffer (pH 7.6), 20 μmoles $MgCl_2$, 20 μmoles phosphoenolpyruvate, 6 μmoles ATP, 0.5 μmole GTP, enzyme preparation (the 120,000 × *g* supernatant fraction [5]) equivalent to 0.5 g fresh liver, and [^{14}C] labelled precursor as indicated in the text. Inorganic phosphate was determined by the method of Fiske and SubbaRow [6].

3. Results and discussion

As shown in table 1, an intensive conversion of [^{14}C] orotate into uridine nucleotides takes place under our conditions. As demonstrated [1], this conversion depends on the pyrophosphorylation of endogenous ribose-5-phosphate and proceeds at a rate which is 10 to 20 fold faster than the amination of uridine nucleotides to cytidine nucleotides. This latter reaction takes place with the participation of endogenous glutamine [1]. As can be seen, the addition of isolated mitochondria strongly inhibits [^{14}C] orotate incorporation. This effect is not connected with the intactness of mitochondrial structure since it is displayed also by mitochondrial fragments. The addition of oxidizable substrates, respiratory inhibitors, uncoupling agents, or raising the concentrations of either ATP or Mg^{2+}, does not abolish the inhibitory effect of mitochondria. The enzymic character of the observed inhibitory effect is shown by its disappearance after heat inactivation of mitochondria. On the other hand, the addition of fluoride restores almost completely the synthesis of UMP. Therefore the interference of two mitochondrial enzymes in pyrimidine nucleotides biosynthesis may be envisaged, namely ATPase (EC 3.6.1.4) and adenylate kinase (EC 2.7.4.3). As known, fluoride inhibits partially ATPase and com-

Table 1

The effect of mitochondria on the in vitro biosynthesis of pyrimidine nucleotides from [^{14}C] orotate.

The standard incubation medium (see "Methods") contained 0.28 μmole [6–^{14}C]orotate (specific activity 17.8 mC/mmole). Mitochondria or ultrasonic mitochondrial fragments, when present, were in amounts equivalent to 0.5 g fresh liver. The other additions listed in the table were in amounts indicated. It was demonstrated in control experiments that all these additions were *per se* without significant effect on nucleotide formation.

Additions to the standard incubation medium (figures in brackets represent μmoles)	Radioactivity found * in	
	Uridine nucleotides	Cytidine nucleotides
	counts/min X 10^{-3}	
None	2 550	80.2
Mitochondria	1 120	19.1
Heat inactivated ** mitochondria	2 650	89.0
Mitochondrial fragments	980	17.1
Mitochondria, $MgCl_2$ (60)	1 210	16.6
Mitochondria, ATP (6)	900	18.0
Mitochondria, ribose-5-phosphate (8)	1 240	16.3
Mitochondria, L-glutamate (30)	1 220	23.0
Mitochondria, malonate (10), rotenone (0.02)	1 100	20.5
Mitochondria, 2,4-dinitrophenol (0.2)	850	18.4
Mitochondria, NaF (50)	2 500	38.4

* The radioactivity recovered as free bases and nucleosides was in all cases negligible.
** Mitochondrial suspension heated for 5 min at 100°.

Table 2

Liberation of inorganic phosphate in the incubation medium under the conditions used for pyrimidine nucleotides biosynthesis.

Incubation medium	Additions		Inorganic phosphate liberated
	Mitochondria	NaF	
			μmoles/hour
Standard	–	–	9.2
Standard	+	–	9.2
Standard	+	+	8.3
Without ATP	–	–	2.9
Without ATP	+	–	2.4
Without ATP	+	+	3.1
Without ATP and phosphoenolpyruvate	–	–	0.3
Without ATP and phosphoenolpyruvate	+	–	0.3

The experimental details are the same as described in the legend to table 1.

pletely adenylate kinase activities of mitochondria [7,8].

The results given in table 2 indicate that there is no correlation between the inhibitory effect of mitochondria and the liberation of inorganic phosphate in the medium (compare table 1). The soluble enzyme system itself displays a rather high ATPase activity which is not influenced significantly by the addition of mitochondria with or without fluoride. Consequently, the results strongly suggest that mitochondrial adenylate kinase is the main factor determining the inhibitory action of the intact particles on pyrimidine nucleotides biosynthesis. It is likely that this enzyme changes the ratios between AMP, ADP and ATP in the medium in a direction unfavourable for phosphoribosylpyrophosphate synthesis [9]. Most likely the inhibition is due to the formation of AMP, which is the product of both adenylate kinase and ribosephosphate pyrophosphokinase (EC 2.7.6.1) reactions. As shown previously [1] the addition of AMP at a concentration of 5 μmoles per ml causes a 10 fold inhibition of [^{14}C] orotate conversion into pyrimidine nucleotides.

As it can be seen in table 1 the addition of fluoride does not relieve completely mitochondria-inhibited synthesis of cytidine nucleotides. With the use of [^{14}C]UMP as precursor it was found that the inhibitory effect of mitochondria is increased further by the addition of inorganic phosphate, while it is partly reversed by L-glutamine (fig. 1). These observations indicate that in addition to the inhibitory effect of mitochondrial adenylate kinase on pyrimidine nucleotides biosynthesis, the participation of another mitochondrial enzyme should be envisaged in the case of cytidine nucleotide formation. Most likely this enzyme is the so-called phosphate-activated glutaminase (EC 3.5.1.2) [10] which may decrease the concentration of L-glutamine in the medium. As known, this amide is an obligatory donor of amino-groups in cytidine nucleotide biosynthesis in animal tissues [3].

The observed inhibitory effect of mitochondria on the synthesis of pyrimidine nucleotides *in vitro* may reflect some regulatory mechanisms operating in the living cell.

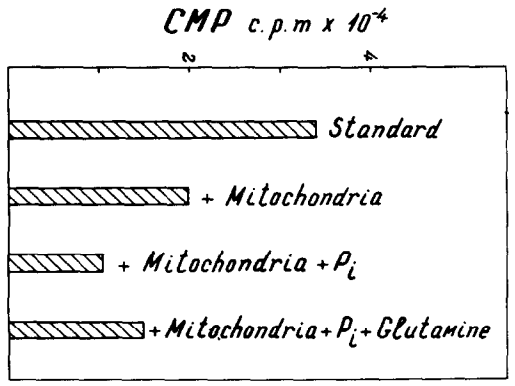

Fig. 1. *Biosynthesis of cytidine nucleotides from [^{14}C]UMP in vitro.* The standard incubation medium contained 0.65 μmole [6–^{14}C]UMP (specific activity of about 1.3 X 10^9 counts per min per mmole) as precursor. Inorganic phosphate (P_i) and L-glutamine, when present as additional components of the medium, were in amounts 40 and 10 μmoles, respectively. The other conditions were the same as indicated in table 1. The total cytidine nucleotides were converted into CMP by acid hydrolysis.

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