UNEQUAL ^{32}P-LABELING OF 5'NUCLEOTIDES IN RIBONUCLEIC ACIDS OF DIFFERENT MAMMALIAN TISSUES

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1. Introduction

We have previously reported that rat liver RNA labeled for a short time by ^{32}P and hydrolyzed by snake venom phosphodiesterase (orthophosphoric diester phosphohydrolase, EC 3.1.4.1.) into 5'-nucleotides, shows a specific radioactivity always higher for AMP than that found for GMP, UMP and CMP. The difference in labeling is found for ribosomal RNA (rRNA) and nuclear RNA as well as for the internal chain of transfer RNA (tRNA) [1]. This fact is related to the high labeling of the αP of ATP in the acid soluble precursor pool of the liver [1, 2]. In this paper we have examined to what extent this phenomenon may be found in other tissues of the rat or in the liver of other species.

2. Materials and methods

Animals received an intraperitoneal injection of 100 μCi of $^{32}PO_4HNa_2$ per 100 g of body weight before killing by decapitation. The liver, the heart and the kidneys were rapidly removed and chilled at 0°C.

Ribosomes and supernatant were prepared from a homogenate in 0.25 M sucrose [1]. Extraction of transfer RNA and ribosomal RNA by phenol has been previously described [3, 4] as well as the degradation of RNAs by phosphodiesterase and the isolation of the so-formed 5'-nucleotides [1, 3].

The extraction of the acid soluble fraction from liver has been performed in 10% trichloroacetic acid according to a method reported elsewhere [1]. Since prolonged ischemia produces a rapid and marked decrease of free nucleotides in kidney [5, 6], we have adopted the following procedure: the rats were anesthetized with ether and the kidneys were rapidly removed and plunged into a bath of alcohol containing solid CO_2 (the second kidney must be removed not more than 5 or 6 sec after the first one).

The fractionation of the acid soluble fraction by ion exchange chromatography in the Dowex 1 X 4 formate system, the purification of the 5'-nucleotide monophosphates and triphosphates, and the obtention of the αP group from ATP, GTP and UTP have been reported in detail in a previous paper [1].

The specific radioactivity of the nucleotides was determined by counting radioactivity in a liquid scintillation spectrometer Packard and by UV absorption determination.

3. Results and discussion

The specific radioactivities of 5'-nucleotides derived from ribosomal and transfer RNA of rat liver by phosphodiesterase hydrolysis are similar for UMP, GMP and CMP whereas that of AMP is always much higher, especially after a short time of incorporation (table 1). These facts are correlated to the very high labeling of the αP of the ATP in comparison with that of the other nucleoside triphosphates in the acid soluble precursor pool; on the other hand, the αP group of the nucleoside triphosphate is always in equilibrium with the phosphate group of the homologous nucleoside monophosphate (table 2).

Similar results are obtained for 5'-nucleotides of

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Table 1

Specific radioactivity of 5′-nucleotides derived from snake venom phosphodiesterase degradation of ribosomal and transfer RNA extracted from different tissues of rat and from liver of different species after 4 hr of incorporation of 100 μCi $^{32}PO_4Na_2H$ per 100 g body weight.

5′-nucleotides obtained after phosphodiesterase degradation of RNAs	cpm per μmole of 5′-nucleotide							
	Liver						Kidney	Heart
	Rat		Guinea-pig		Rabbit		Rat	Rat
	rRNA	tRNA[a]	rRNA	tRNA[a]	rRNA	tRNA[a]	rRNA	rRNA
AMP	5,750	6,810	3,800	5,050	420	480	3,950	530
GMP	1,148	1,860	700	1,120	85	124	2,300	530
CMP	2,020	1,910	560	885	75	96	5,200	1,960
UMP	564	1,350	680	1,090	146	118	2,800	840
AMP/GMP	5.1	3.7	5.4	4.5	4.9	3.9	1.7	1
AMP/CMP	2.8	3.6	6.8	5.7	5.6	5	0.7	0.3

[a] Internal chain of tRNA after complete removal of the terminal sequence pCpCpA [1, 3].

Table 2

Specific radioactivities of the nucleotides of the acid soluble precursor pool fraction after one hour incorporation of 100 μCi of $^{32}PO_4H\ Na_2$ per 100 g of body weight.

Nucleotides from the acid soluble pool	cpm per nmole of P	
	Rat liver	Rat kidney
αP group from		
ATP	231	126
GTP	24.2	54
UTP	12	–
5′-nucleotides		
AMP	236	108
GMP	22.4	61
GMP	–	180
UMP	12.6	78
$\frac{\alpha P \text{ group of ATP}}{\alpha P \text{ group of GTP}}$	9.5	2.3

rRNA and tRNA extracted from liver of other species such as guinea pig and rabbit (table 1).

On the contrary, in rRNA extracted from rat heart and kidney and degraded by the same enzyme reaction, 5′-AMP does not show the high labeling observed in liver (table 1). Consequently, it must be postulated that the ratio between specific activities of the αP groups of ATP and GTP in the acid soluble fraction of kidney should be lower than that of liver. Experiments performed on nucleotides isolated from precursor pool of kidney show clearly that results are in agreement with the expected values (table 2).

The hypothesis that we have proposed for the high labeling of the αP of ATP in liver is the existence of metabolic pathways which are in addition to the *de novo* synthesis and which also lead to the synthesis of 5′nucleotides [1]. Thus, the *in vivo* conversion of a free purine to a nucleoside by means of a reaction with riboso-1-phosphate [7, 8], followed by the direct phosphorylation of the so-formed nucleoside to a 5′-nucleotide in the presence of ATP as cofactor [9, 10], would correspond to what we call the "parallel synthesis". This is able to explain the high labeling of the αP of 5′-nucleotides since, by such a reaction, the nucleoside receives the γP group of ATP acting as cofactor, which is well known for its high radioactivity [11]. Although the last step is thought to be functional for the different tissues [12], the present results show clearly that, as far as 5′-AMP formation is concerned, the relatively high contribution of the "parallel synthesis" with regard to the *de novo* synthesis appears as a specific property of the liver tissue whatever the animal species concerned.

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