PURINE SALVAGE IN MAMMARY GLANDS OF MICE

J. BARANKIEWICZ, M. M. JEZEWSKA

Department of Comparative Biochemistry, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, 36 Rakowiecka St., 02-532 Warszawa, Poland

and

P. CHOMCZYŃSKI

Department of Biochemistry, Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzębiec, 05-551 Mroków, Poland

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1. Introduction

Purine salvage [1] provides the cells with a nucleotide pool, which is additional to that derived from the biosynthesis de novo. A correlation between the level of purine salvage and RNA content has been found in the breast muscle of chicken [2], and in germinating wheat seeds [3]. It seemed of interest to investigate the purine reutilization in the tissue characterized by an abrupt rise of the intensity of the RNA and DNA synthesis during the transformation from non-secretory to secretory state. This paper deals with studies of purine reutilization in the mammary glands of pregnant and lactating mice.

Both theoretically possible pathways of purine salvage were investigated:

(a) formation of nucleotides by purine phosphoribosyltransferases (EC 2.4.2.7 and EC 2.4.2.8) and

(b) two-step nucleotide synthesis by purine nucleoside phosphorylase (EC 2.4.2.1) and nucleoside kinase (EC 2.7.1.20).

2. Materials and methods

2.1. *Reagents*

[U-^{14}C]uridine was purchased from UVVVR (Czechoslovakia), [8-^{14}C]-labelled adenine, guanine and hypoxanthine were purchased from Radiochemical Center, Amersham, England. [8-^{14}C]purine nucleosides were synthesized enzymatically from labelled purines and ribose 1-phosphate, and purified by paper chromatography. The origin of all other reagents has been given in [4,5].

2.2. *Animals*

CW/P mice in their first pregnancy were used.

2.3. *Preparation of cytosol*

On an appropriate day of pregnancy or of lactation mice were sacrificed by cervical dislocation. Abdominal and thoracic mammary glands, free of lymph nodes, were minced with scissors, and homogenized in a glass homogenizer with a Teflon pestle using 4 vols of buffer (0.25 M sucrose, 2.4 mM EDTA, 0.1 M Tris–HCl, pH 7.9). The homogenate was filtered through a nylon net, and centrifuged at 1000 g for 15 min. The resulting low-speed supernatant was centrifuged at 150 000 g_{max} for 1 h. The high-speed supernatant (cytosol) was aspirated and stored at −20°C. The activities of the above-mentioned enzymes in cytosol remained unchanged after 2 weeks of storage at −20°C.

2.4. *Protein determination*

The method of Lowry et al. [6] was used.

2.5. *Determination of enzymic activities*

The following incubation mixtures were used:

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(a) for purine phosphoribosyltransferase activity: 0.1 mM [8-^{14}C]purine (spec. act. 5 Ci/mol), 1.5 mM 5-phosphorylribose-1-pyrophosphate dimagnesium salt (PRPP), 5 mM $MgCl_2$, 0.1 M Tris–HCl buffer, pH 7.4, and 0.05 mg of cytosol protein (total vol 0.2 ml);

(b) for purine-nucleoside phosphorylase phosphorolytic activity: 0.4 mM inosine or deoxyinosine, 3 mM KH_2PO_4, 0.1 M Tris–HCl buffer, pH 7.4, and 1 mg of cytosol protein (total vol 2 ml);

(c) for purine-nucleoside phosphorylase synthetic activity: 0.15 mM [8-^{14}C]purine, 1.5 mM ribose-1-phosphate (R-1-P), 0.1 M Tris–HCl buffer, pH 7.4, and 0.05 mg of cytosol protein (total vol 0.2 ml);

(d) for nucleoside kinase activity: 0.1 mM [8-^{14}C] nucleoside (specific activity 3 Ci/mol), 10 mM ATP, 5 mM $MgCl_2$, 5 mM $CaCl_2$, 0.1 M Tris–HCl buffer, pH 7.4, and 0.05 mg of cytosol protein (total vol 0.2 ml).

Reactions were carried out at 30°C for 10 min and stopped by an addition of 0.03 ml of 3 N $HClO_4$. The chromatographic resolution of the substrates and products of enzymic reactions as well as the determination of their radioactivity were performed as described previously [4,5]. The activity of the investigated enzymes was expressed in nmoles of the reaction product/mg cytosol protein per hour.

2.6. *Assays of RNA synthesis*

On an appropriate day of pregnancy or lactation mice were intraperitoneally given 20 μCi of [^{14}C] uridine in 0.5 ml of buffered saline solution. Animals were sacrificed by cervical dislocation 1 h after injection. Mammary glands (free of lymph nodes) were rapidly excised and homogenized in 10% trichloroacetic acid at 2°C. After settling of precipitate, the latter was washed twice with 10% trichloroacetic acid and once with ethanol. Following alkaline hydrolysis with 0.3 M KOH, the sample was centrifuged, and aliquots of the supernatant were taken for radioactivity determination in a liquid scintillation counter.

3. Results and discussion

Cytosol from the mammary glands of mice showed enzymic activities of purine phosphoribosyltransferases, purine-nucleoside phosphorylase and nucleoside kinase, i.e. of the enzymes allowing for both possible pathways of purine reutilization.

Table 1
Activities of purine phosphoribosyltransferases and purine-nucleoside phosphorylase in the mammary glands of mice

Substrate	Enzymic activity of Phosphoribosyltransferase (nmoles of reaction product/mg protein/h) P_{12}	L_3	Purine-nucleoside phosphorylase (nmoles of reaction product/mg protein/h) P_{12}	L_3
Adenine	11	5	0.6	0.0
Guanine	210	153	1550	1200
Hypoxanthine	62	61	1482	1210
Inosine			860	
Deoxyinosine			260	

Assay condition as described in Materials and methods. Mammary glands obtained from mice on 12th day of pregnancy (P_{12}) and 3rd day of lactation (L_3).

Activities of both adenine phosphoribosyltransferase and of hypoxanthine-guanine phosphoribosyltransferase were found (table 1); similarly as in mouse spleen [7], guanine was converted to its nucleotide more rapidly than hypoxanthine. The activity towards adenine was lower than that towards guanine and hypoxanthine in the mammary glands, similarly as in other mouse tissues [8,9].

Purine-nucleoside phosphorylase of the mammary glands practically acted only on guanine and hypoxanthine, showing traces of activity towards adenine (table 1), similarly as the enzyme of other mammalian tissues [10]. Inosine was synthesized almost equally well as guanosine during pregnancy and lactation. The synthesis of inosine was about twice as rapid as its phosphorolysis, inosine being a better substrate than deoxyinosine. In contrast to the mouse spleen cells [7], the purine-nucleoside phosphorylase activity towards guanine and hypoxanthine was several times higher than the phosphoribosyltransferase activity towards these two substrates.

Mammary gland cytosol showed high adenosine kinase activity; moreover, it synthesized IMP and GMP from the respective nucleosides (table 2). These last activities were very low as compared with those of the inosine and guanosine formation by cytosol purine-nucleoside phosphorylase (table 1). In the light of the

Table 2
Activity of purine-nucleoside kinase in the mammary glands of mice

Substrate	Kinase activity (nmoles of nucleotide formed/mg protein/h)
Adenosine	820
Guanosine	35
Inosine	98

Assay conditions as described in Materials and methods. Mammary glands obtained from mice on 12th day of pregnancy.

low activity of adenine phosphoribosyltransferase and the lack of adenosine phosphorylase activity, it can be supposed that in mammary glands of mice no free adenine is utilized; degraded AMP may probably be recovered in part by the direct action of adenosine kinase. On the other hand, both guanine and hypoxanthine can be utilized in the mammary glands; probably the main way of guanine reutilization is the reaction catalyzed by hypoxanthine-guanine phosphoribosyltransferase; for hypoxanthine, both pathways of reutilization could perhaps be equally efficient.

In the final period of pregnancy the number of cell divisions increases [11], and the RNA synthesis is considerably enhanced [12,13]. Both these phenomena are related to the increased requirement of purine nucleotides. In the mammary glands of pregnant mice the purine-nucleoside phosphorylase synthetic activity and hypoxanthine-guanine phosphoribosyltransferase activity increased considerably, outpacing the rise of the RNA synthesis (fig.1); during the period of lactation, these enzymic activities decreased in parallel with the decline in the RNA synthesis (fig.1). These results point to the important role of purine salvage in the development of the mammary glands to the secretory state.

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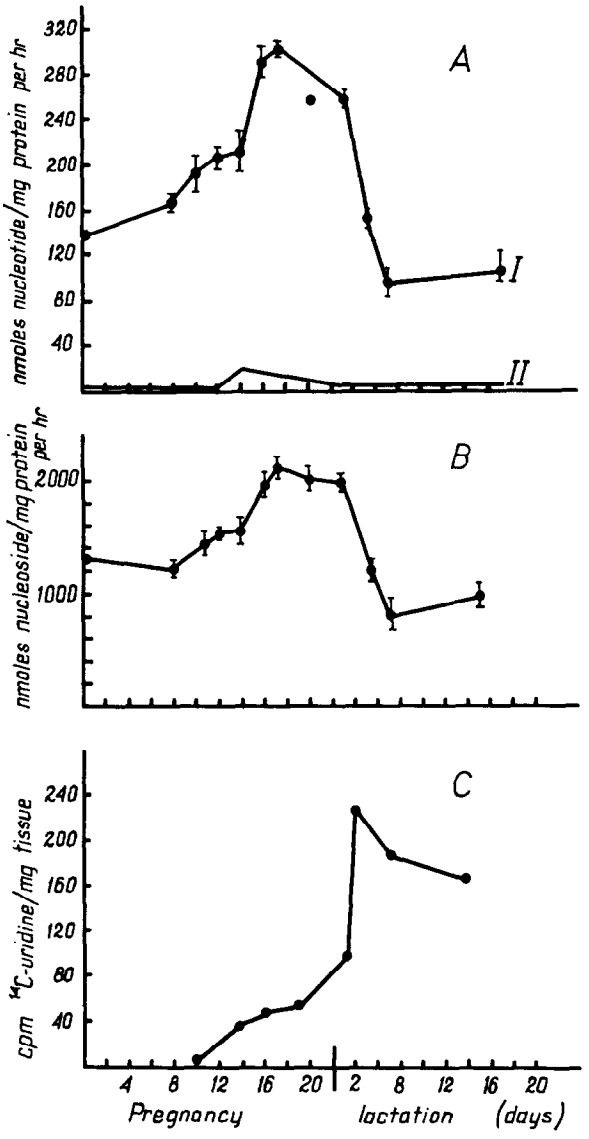

Fig.1. Changes in activities of purine phosphoribosyltransferase (A) (I, H-GPRTase; II, APRTase), purine-nucleoside phosphorylase (B), and changes in RNA synthesis (C), in mammary glands of mice during pregnancy and lactation. Assay conditions as described in Materials and methods.

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