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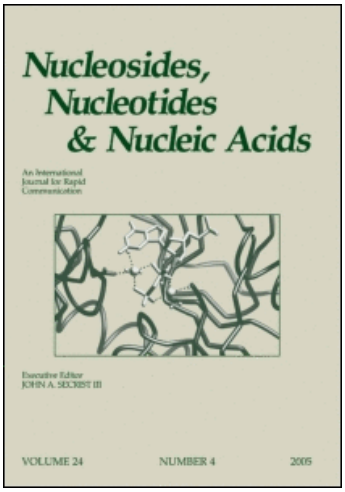

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Ribonucleic Acids of Human Milk

Elena V. Kuligina[a]; Dmitry V. Semenov[a]; Olesya N. Shevyrina[a]; Vladimir A. Richter[a]
[a] Novosibirsk Institute of Bioorganic Chemistry, Novosibirsk, Russia

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Ribonucleic Acids of Human Milk

Elena V. Kuligina,* Dmitry V. Semenov, Olesya N. Shevyrina, and Vladimir A. Richter

Novosibirsk Institute of Bioorganic Chemistry, Novosibirsk, Russia

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ABSTRACT

The milk feeding is the most essential process laying the foundation of human health at the postnatal development. However little is known about nucleic acids secreted into mother's milk during lactation. In order to investigate the composition and abundance of human milk NA we adapted the conventional isolation method to achieve high yield of total nucleic acids from milk samples. Concentration of total NA in milk samples of different donors varies from 20 to 68 mkg/ml at early stages of lactation. The average concentration tends to fall down to the end of lactation. The chain length of the major forms of NA varies from mononucleotides up to approximately 100 bases. Compositions of milk oligonucleotides are similar in samples of different donors. Major milk oligonucleotides are formed of RNA. Human milk contains the set of long-chain oligonucleotides with a developed secondary structure. Sequences of some oligo-RNAs correspond to the 3′-part of 5.8 S human ribosomal RNA and to the 3′-parts of tRNAVal and tRNATyr Primary structures of some others oligo-RNAs were related to fragments of human 18S and 28S rRNAs.

Key Words: Lactation; Human milk; Nucleic acids; RNA.

*Correspondence: Elena V. Kuligina, Novosibirsk Institute of Bioorganic Chemistry, Lavrentiev Ave. 8, Novosibirsk, 630090, Russia; E-mail: kuligina@niboch.nsc.ru.

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INTRODUCTION

Extracellular nucleic acids (NA) from human blood, liquor and urine are constituted with DNA and RNA.[1–3] The concentration of NA and appearance of specific DNA in plasma from patients with cancer or with autoimmune or inflammatory diseases serves as a prognostic characteristic to predict disease outcome.[4,5] Circulating NA derived from tumor and virus transformed cells is proposed to be a factor contributing to disease propagation.[6] Apart from cancer, fetal RNA has also been detected in the plasma of pregnant women.[7]

Human milk nucleic acids have important physiological roles in breast-fed infants. Concerning their biological role they not only act as metabolites but are also involved as bioactive substances in the regulation of body functions.[8] However little is known about nucleic acids secreted into mother's milk during lactation. In order to investigate the composition and abundance of human milk NA we adapted the conventional NA isolation method to achieve high yields of bulk nucleic material from milk samples. Estimation of NA concentration, comparison of chain length and determination of primary structures of milk NA are the aims of the study.

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METHODS

Samples of donor milk were centrifugated 300 × g for 20 min and milk plasmas were stored in portions at −70°C. Total milk nucleic acids (NA) were isolated by the standard method.[9] Estimation of NA concentration was carried out by spectrophotometry.

Total milk NA were labeled using methods of 3′-OH ligation with [5′-^{32}P]pCp or 5′-OH phosphorylation with [gamma ^{32}P] ATP and polynucleotide kinase T4.[9]

Isolation of individual forms of milk RNA was carried out by two dimensional PAGE in native followed by denaturating (8 M urea) conditions. Primary structures of

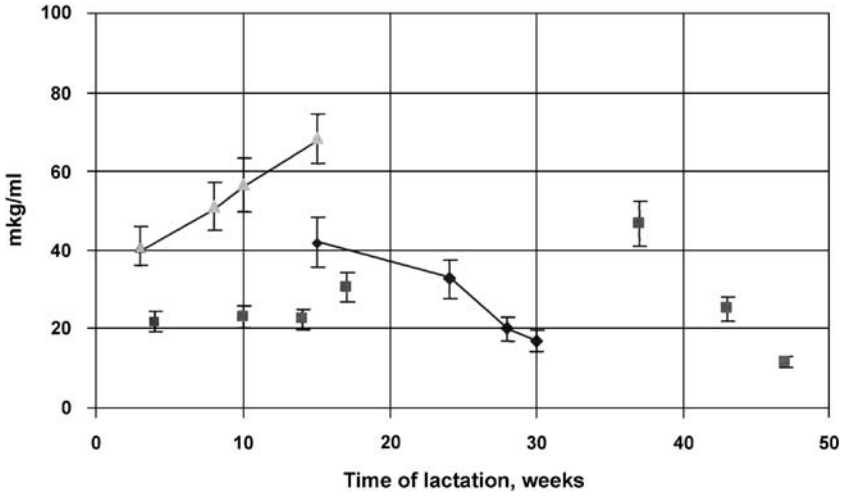

Figure 1. Concentration of total NA in human milk. Connected points correspond to milk samples from individual donor. Set of data from 7 different donors.

several long-chain milk RNAs were determined by limited digestion with a set of base-specific RNAses: T1, U2, PhyM, B.c. and A.[10]

RESULTS

The concentration of total NA in milk samples of different donors varies from 20 to 68 mkg/ml at early stages of lactation (average 44 mkg/ml). The average concentration tends to fall down with lactation development and achieves minimum value ($\sim$ 27 mkg/ml) at the end of barest feeding (Fig. 1).

Low MW oligonucleotides are the most abundant forms of human milk NA. The chain length of major forms of NA varies from mononucleotides up to approximately 100 bases. Compositions of milk oligonucleotides are similar in samples of different

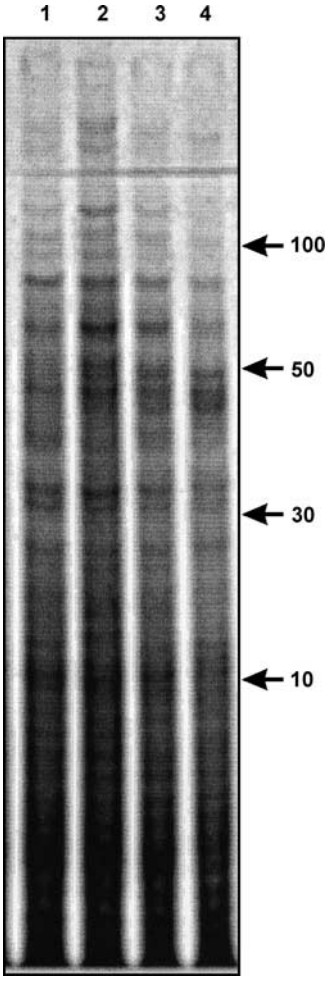

Figure 2. Electrophoretic analysis of total [5′-^{32}P]-labelled NA from milks collected at different periods of lactation from 4 donors. Radioautograph of 20% denaturating PAG.

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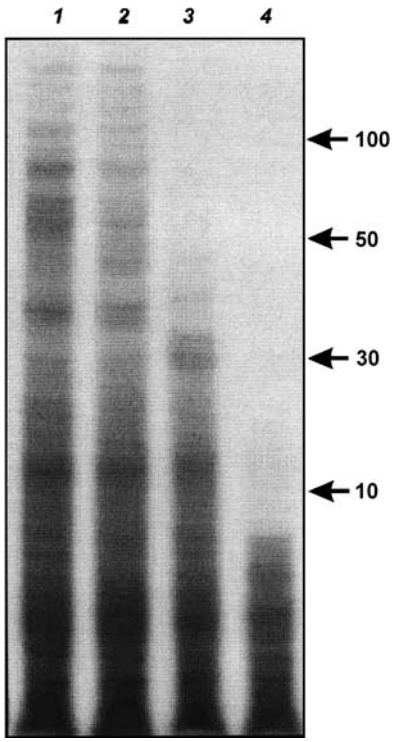

Figure 3. Electrophoretic analysis of total [5′-^{32}P]-labelled milk NAs (1) treated with DNAase I (2), RNAse A (3) or 50 mM KOH (4). Radioautograph of 20 % denaturating PAG.

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donors. The array of milk oligonucleotides undergo only minor changes as lactation proceeds (Fig. 2).

Major milk oligonucleotides are formed of RNA, as judged from the lability of internucleotides phosphodiester bonds under the RNAses and mild alkali treatment (Fig. 3). These and most of subsequent results allow us to estimate the DNA level as not exceeding 1 mkg per ml of human milk.

Human milk contains a set of long-chain oligonucleotides with a developed secondary structure. As is seen from their behavior under the denaturing-after-native two dimensional gel-electrophoresis, they are composed both of hairpins-like and double stranded oligo-RNAs fragments (Fig. 4).

Primary structures of several long-chain milk RNAs were determined using methods of 3′-OH ligation with [5′-^{32}P]pCp or 5′-OH phosphorylation with [gamma ^{32}P] ATP and polynucleotide kinase T4 and limited digestion with a set of base-specific RNAses.

Sequences of three milk oligo-RNAs

5′-NNUCCCGGGGCUACGCCUGUCUGAGCGUCGCUU-3′
5′-NNGGGGCUACGCCUGUCUGAGCGUCGCUU-3′
5′-NGGGGCUACGCCUGUCUGAGCGUCGCUU-3′

correspond to 3′-part of 5.8 S human ribosomal RNA.

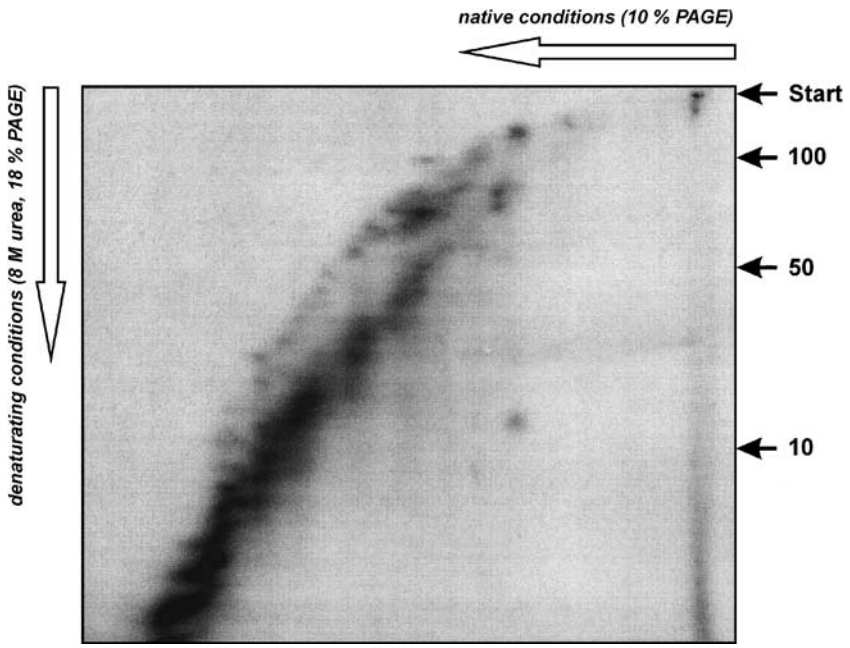

Figure 4. Separation of [5′-^{32}P]-milk RNAs by two-dimensional PAGE under native in first direction followed by denaturating conditions in second direction. Radioautograph of PAG.

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The sequences of others oligo-RNAs correspond to 3′-parts of tRNA-Val and tRNA-Tyr:

5′-CGGUUCGAAACCGGGCGGAAACA-3′
5′-UUCGAUUCCGGCUCGAAGGA-3′

Both milk derived fragments of tRNAs lacked the constant feature of mature tRNAs–aCCA-acceptor tail. Primary structures of some others oligo-RNAs were related to the fragments of human 18S and 28S rRNAs.

Thus human milk oligoribonucleotides are the fragments of the most abundant cellular RNAs.

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