RNA THIOLATION LEVEL (INCORPORATION OF METHIONINE-S^{35}) IN PERIPHERAL LYMPHOCYTES OF DONORS AND PATIENTS WITH CHRONIC LYMPHATIC LEUKEMIA

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A comparative study of RNA thiolation in short-term cultures of human lymphocytes incubated with methionine-S^{35} showed that preparations of total RNA from donors' lymphocytes had radioactivity on the average twice as high as RNA from lymphocytes of patients with chronic lymphatic leukemia. The specific radioactivity of the 4S fraction of RNA isolated by centrifugation in a sucrose gradient was 3 times as high in donors' lymphocytes as in lymphocytes of patients with chronic lymphatic leukemia. Deacylation of the tRNA lowered the radioactivity by 10-20%.

Specific differences in the modification of tRNA by methylation have been found in tumor and leukemic cells. However, recent investigations have demonstrated modification of tRNA by enzymic thiolation of uridine residues at the polynucleotide level [4, 5]. A connection has also been demonstrated between the presence of 2-thiouridine in tRNA and its ability to recognize the anticodon [6].

The object of this investigation was to determine whether thiolation of RNA takes place in human cells and whether there is any difference between the levels of this process in the lymphocytes of healthy donors and in those of patients with chronic lymphatic leukemia (CLL).

EXPERIMENTAL METHOD

Leukocytes were separated from erythrocytes by allowing the blood to stand after the addition of gelatin. A suspension of leukocytes from patients with CLL was not further fractionated because its lymphocyte content was 80-98%. Subsequent separation of lymphocytes from the leukocyte suspension of the donors was carried out on a column packed with cotton-wool [1].

The separated cells were suspended in Eagle's incubation medium with the addition of inactivated group AB (IV) blood serum, antibiotics, and glutamine. The cell concentration was 2×10^7/ml incubation medium. The lymphocytes were incubated with methionine-S^{35} for 18 h at 37°C. After incubation the lymphocytes were washed 3 times with physiological saline and their total RNA was isolated by Cline's method [3], treated with deoxyribonuclease and pronase, and reprecipitated with ethanol.

The RNA was fractionated in a 10-20% sucrose density gradient containing 0.01 M tris -HCl buffer, pH 7.4, 0.003 M $MgCl_2$, and 0.01 M NaCl. The sample was ultracentrifuged (Spinco L-2; rotor SW-25) for 18 h at 40,000-57,000 g.

Deacylation was carried out by treating the preparations of tRNA with 3 N hydroxylamine [2]. The radioactivity of the preparations was measured on a Nuclear Chicago Mark I counter.

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TABLE 1. Incorporation of Methionine-S^{35} into Preparation of Total RNA of Lymphocytes from Donors and Patients with CLL

Number of cells in 1 ml incubation medium	Activity of methionine-S^{35} in 1 ml incubation medium (in μCi)	Activity of DL-methionine-S^{35} (in μCi/g)	Specific activity of RNA preparation (pulses/min/mg RNA)	
			donors	patients
1.10^7	11,0	280	615	—
2.10^7	14,5	280	635	309
2.10^7	16,5	280	674	326
2.10^7	9,2	90	346	163
2.10^7	9,2	90	237	65
2.10^7	9,2	90	196	81
2.10^7	9,2	90	240	100
2.10^7	9,2	90	213	—

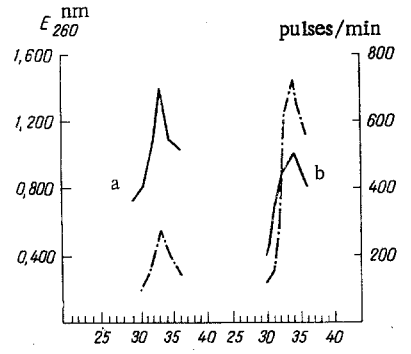

Fig. 1. Incorporation of methionine-S^{35} into 4S RNA of lymphocytes of donors and patients with CLL: a) from lymphocytes of patients with CLL; b) from donors' lymphocytes; continuous line represents optical density (E_{260}, nm); broken line represents radioactivity (in pulses/min). Abscissa, No. of fractions; ordinate: on the left – optical density; on the right – radioactivity.

EXPERIMENTAL RESULTS

As Table 1 shows, the preparations of total RNA from the lymphocytes of 8 donors had radioactivity on the average twice as high as that from the lymphocytes of 6 patients with CLL. Since derivatives of 2-thiouridine have so far been found only in tRNA [4-6], the 4S RNA was isolated by centrifugation in a sucrose density gradient (Fig. 1). The specific activity of the 4S fraction of RNA in the donors' lymphocytes was 3 times higher than in the lymphocytes of patients with CLL. To rule out any possibility that the difference thus discovered in the incorporation of methionine-S^{35} could be due to acylation of $tRNA^{met}$, deacylation of the fraction of soluble RNA was carried out in four experiments. The mean decrease in radioactivity of the soluble RNA preparations after treatment with hydroxylamine was 10-20%; it can accordingly be postulated that the incorporation of methionine-S^{35} observed was due chiefly to thiolation of the soluble RNA.

The presence of derivatives of 2-thiouridine in $tRNA^{glu}$ from yeast and bacteria is related to accuracy of recognition of the codon [6], and for that reason thiolation can be regarded as a definite stage in the regulation of protein synthesis in cells. The considerable decrease in thiolation of tRNA in the lymphocytes of patients with CLL could be the reason for the disturbance of regulation of protein synthesis which may be responsible for their reduced ability to undergo blast transformation.

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