L-RIBONUCLEOSIDES DO NOT PENETRATE BACTERIAL CELL WALLS

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1. Introduction

Recently, the L-ribonucleosides (see I, fig. 1), i.e. enantiomers of naturally occurring ribonucleosides (see II, fig. 1) and their nucleotide derivatives have been prepared by various synthetic routes [1, 2]. The *in vitro* experiments performed with some nucleolytic enzymes demonstrated resistance of internucleotidic linkages containing L-ribonucleotides towards the enzymes mentioned [2, 3]. Moreover, the triplet codons containing these compounds, on examination by means of binding experiments, do not possess any significant template activity for aminoacyl-tRNA binding to *E. coli* ribosomes [2]. It was therefore of interest to investigate the biochemical activity of L-ribonucleosides (I) *in vivo*. In this paper, we report the results obtained with L-enantiomers of uridine, cytidine and adenosine and two groups of bacteria – *E. coli* and *B. subtilis*. In addition to the above compounds we studied the behaviour of 9-(α-L-lyxofuranosyl)adenine (see III, fig. 1) [4] which differs from adenosine by configuration at the C-4'-atom only.

2. Materials and methods

E. coli B was cultivated in a mineral medium [5], *E. coli* K 12 Hfr was grown in the same medium containing 0.2% casamino acids (Difco) and *B. subtilis* SMYW in a medium of Spizizen [6] enriched by 0.2% casamino acids.

Labelled L-8-^{3}H-adenosine (35 μCi/μmole) and 9-(α-L-lyxofuranosyl)-8-^{3}H-adenine (III) (22 μCi/μmole) were prepared by an exchange reaction with tritium oxide [7]; L-5-^{3}H-uridine (24 μCi/μmole) and L-5-^{3}H-cytidine (24 μCi/μmole) were obtained by catalytic reductive dehalogenation of the corresponding 5-bromo derivatives [7]. The compounds were chromatographically homogeneous and contained $< 0.5\%$ of the heterocyclic base. L-^{3}H-Ribonucleosides (I) were added to an exponentially growing bacterial culture at the beginning of the exponential phase, at the concentration 20 μg/ml of bacterial suspension. Aliquot samples were taken at 10 min intervals.

The penetration of labelled compounds into bacterial cells was determined by the filtration technique on nitrocellulose membrane filters Synpor 6, Vchz-Synthesia, Czechoslovakia. In time intervals, the radioactivity uptake was estimated with the whole cells, the 5% TCA (cold) precipitate (nucleic acids), and 5% TCA (cold) soluble fraction (pool). Simultaneously, the aliquots of medium and pool were analyzed by paper chromatography on Whatman No 3 MM paper in systems: 1-butanol–acetic acid–water (10:1:3) (pyrimidine nucleosides) and 1-butanol–2.5% ammonia (86:14) (purine nucleosides [8].

The nucleoside kinase activity (*in vitro*) assay was performed with *E. coli* B cell-free system in 0.02 M Tris-HCl pH 7.4, 1 mM $MgCl_2$, 5 mM ATP and 0.1 mM ^{3}H-nucleoside with 500 μg protein of the cell-free extract in 100 μl incubation mixture. Incubation, 90 min at 37°.

3. Results and discussion

Stationary experiments performed with compounds

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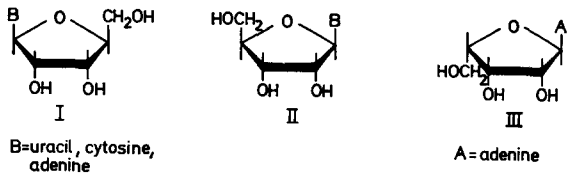

Fig. 1.

I and III did not reveal any bacteriostatical activity up to 1000 μg/ml of bacterial suspension. The distribution of radioactivity in whole cells, 3% TCA soluble and insoluble fractions, showed that there is essentially no penetration of L-ribonucleosides into bacterial cells throughout the whole time period followed (70 min). The total radioactivity of all three fractions did not exceed the minor value corresponding to radiochemical impurities of materials used (< 1%). On chromatographic analysis, medium samples were found to contain unchanged starting materials only, even after 70 min incubation.

Similar results were obtained with the lyxofuranosyl derivative III which does not enter into bacterial cells under the experimental conditions.

L-Uridine was found to be a substrate for some non-specific phosphotransferases [9]; L-ribonucleosides can also serve as acceptors for 3′-nucleotide transferases (cyclising, ribonucleases) [1, 2]. Therefore, we were interested in a possible acceptor activity of L-ribonucleosides for bacterial nucleoside kinases *in vitro*. The experiments performed with a cell-free extract of *E. coli* B in a presence of 50 fold excess ATP [10] did not reveal any phosphorylation with all compounds tested (L-uridine, L-cytidine, L-adenosine, lyxofuranosyl derivative III), within the range of experimental error. At the same time, there was observed neither a splitting of a nucleosidic linkage, nor a deamination of the heterocyclic base (with L-adenosine and L-cytidine). The deamination occurs only with the compound III (37%). The comparative experiments with uridine, cytidine and adenosine led to a significant phosphorylation (up to 12%), nucleoside linkage splitting and deamination reaction. From the experiments described it follows that the regulation mechanism of ribonucleoside transport into bacterial cells of both types of bacteria mentioned is unequivocally capable of distinguishing between enantiomers of ribonucleosides, the steric relations and electron distribution of which do not differ. Therefore, the factor implicated in the transport should also possess a chiral character, on grounds of which its interaction with the chiral molecule of nucleoside could play a decisive role in the regulation. Such a factor – a permease – is assumed to interact with the nucleoside molecule in at least three different points including both the heterocyclic base and the sugar moiety. The arabinofuranosyl analogues of ribonucleosides do enter into bacterial cells [11]; therefore the configuration at the 2′-carbon atom cannot possibly play any decisive role in the transport through the cell wall. However, no penetration of the lyxofuranosyl derivative III indicates that the steric arrangement around the C-4′-atom is decisive for the nucleoside recognition during the transport.

Bacterial nucleoside kinase (*E. coli*) which evidently cannot phosphorylate L-ribonucleosides belongs to the group of enzymes interacting with the nucleoside molecule at three points including both the base and the sugar residue [2, 3]. The lack of activity towards the compound III indicates the significance of the orientation of the hydroxylic group to be phosphorylated towards the other parts of molecule.

Finally, the deaminases responsible for cytidine and adenosine deamination do not show any activity towards the corresponding enantiomers of natural substrates. Conclusions following from this fact must be identical with those mentioned above.

It is noteworthy that the results obtained with bacterial cells are quite at variance with the experiments on mammalian cells which are able to accept L-ribonucleoside and subject it to phosphorylation procedures [12]. The bacterial control of cell-wall penetration of nucleosides appears more explicit.

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