STUDIES ON THE HALF LIFE TIME OF RAT LIVER TRANSFER RNA SPECIES

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Received 23 July 1970

The tRNA fraction, extracted from very high speed supernatant fluid, from livers of rats injected with ^{3}H-orotic acid, attained maximum specific activity after a little over 24 hr and, thereafter, decayed with an apparent half life of 5 days. This behaviour of tRNA was indistinguishable from that of liver rRNA and the acid soluble pool. Chromatography of tRNA, doubly labelled during a period of short synthesis and of prolonged decay, on a BD cellulose column, indicated that individual tRNA species turn over at a constant rate with respect to one another.

1. Introduction

The half life time of ribosomal RNA in a variety of mammalian species has been established to be 4 to 5 days [1–4]. The turn over rate of soluble RNA, on the other hand, is clearly a controversial issue. Thus, depending upon the approach utilized, 2 to 3 [5–6], 3 to 4 [7] and 4 to 5 [3, 8] days have been proposed as the half life time of sRNA in mammalian liver. Moreover, no information is available on the turn over rate of individual transfer RNA species. Due to the pivotal role of tRNA in translation of genetic message, and due to the changes in the abundance and specificity of tRNAs under a variety of pathophysiological alterations [8–12], it was considered relevant to obtain definitive information on this subject. Studies reported here were concerned, first, with the half life time of total rat liver tRNA and, second, the relative turn over rates of individual tRNA species.

2. Materials and methods

6-^{14}C-Orotic acid (35 mCi/mmole), and 5-^{3}H-orotic acid (16 Ci/mmole) were obtained from Schwarz Bioresearch, Inc., Orangeburg, N.Y. All chemicals were reagent grade. At appropriate times after injection of radioactive orotic acid, male, Holtzman, albino rats, 200–250 g body weight, were sacrified and liver RNAs extracted from the 150,000 *g* pellet and the supernatant fractions. Details for the processing of liver, extraction of RNAs and calculation of radioactivity have been previously described [9–10].

3. Results and discussion

Data in fig. 1 show that tRNA attained maximum specific activity a little after 24 hr. Thereafter, radioactivity decayed with an apparent half life time of 5 days. In fact, this behaviour of tRNA was identical to that of microsomal RNA. Furthermore, after an initial high specific activity, the remainder of radioactivity in the acid soluble pool decayed parallel to that of the RNAs. The random first order kinetics of decay eliminates the possibility that rat liver possesses tRNA populations with profoundly different turn over rates. Data in this figure, however, do not give any indication of the relative turn over rate of individual tRNA species, and it is to this aspect of the problem that attention was next directed.

A double isotope labelling experiment was performed for this purpose. After a 7 day incorporation period of ^{3}H-orotic acid (50 μ Ci/100 g body weight)

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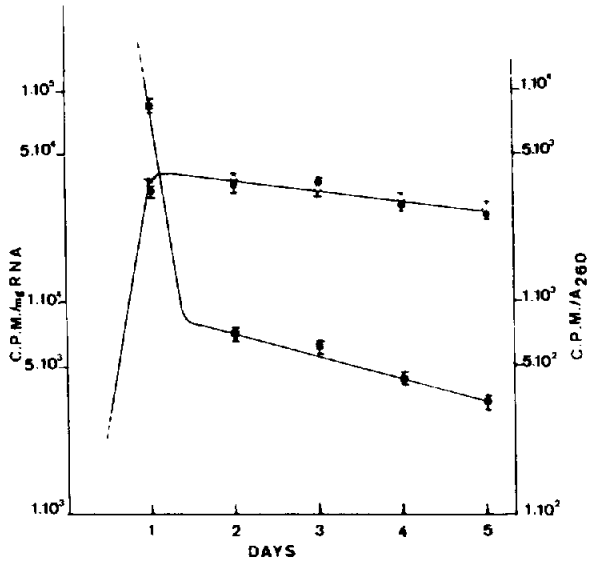

Fig. 1. Semilogarithmic plot showing decay of radioactivity in rat liver RNA and the acid soluble pool. Twenty animals were injected with ^{3}H-orotic acid (5 μ Ci/100 g body weight) and sacrificed in groups of 4 everyday, for 5 days thereafter. The microsomal RNA (•) and the transfer RNA (△) refer to the nucleic acids extracted from the 150,000 g sediment and the supernatant fluid, respectively. The specific activity of the acid soluble pool (■) is expressed as cpm/A_{260} unit. Each value represents the mean for 4 separate determinations ± the standard error.

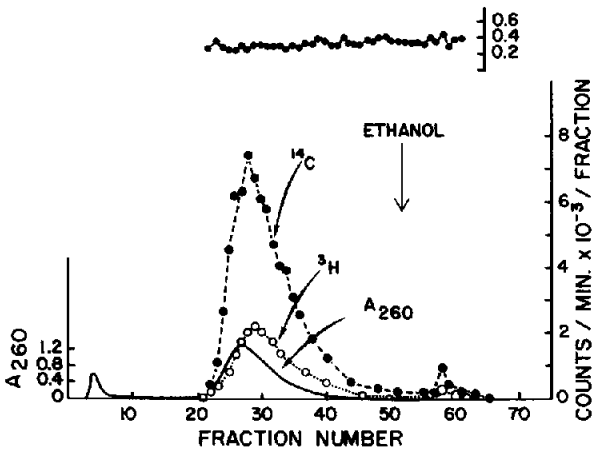

Fig. 2. Chromatography of doubly labelled tRNA on a BD cellulose column. A BD cellulose column (0.8 cm X 12 cm) was equilibrated with 0.01 M Na acetate, pH 4.5, 0.01 M $MgSO_4$ buffer containing 0.3 M NaCl. 4 mg of tRNA in 2 ml buffer, prepared as described in the text, were applied to the column. A linear gradient elution was carried out using 100 ml each of 0.3 and 1.3 M NaCl both buffered as described above. The column was then washed with 200 ml of a linear 5–30% ethanol gradient, in 1.5 M NaCl, buffered as above. Fractions of 4 ml each were collected at a flow rate of 30 ml/hr and processed for determination of radioactivity, as described in the text.

a pulse of ^{14}C-orotic acid (25 μ Ci/100 g body weight) was given 3 hr before sacrifice. The tRNA, extracted as previously described [9–10] was fractionated on a 5–20% sucrose gradient and the fractions corresponding to the 4 S tRNA were pooled for subsequent chromatography on a benzoylated-DEAE-cellulose column, according to the method of Gillam [13] adapted as described in earlier reports [8–11]. All fractions were precipitated on 0.45 μm membrane filters dissolved in Bray's solution and counted in a Packard Tricarb Scintillation Spectrometer [8–11].

Data in fig. 2 reveal that the elution profiles of both isotopes were identical and the ^{3}H/^{14}C ratio was constant in all fractions. Previous evidence [8–11, 13] indicated that each type of tRNA is eluted as a rather sharp peak at a characteristic position in the eluate from the B.D. cellulose column. Since the ^{3}H radioactivity reflects mainly the decay, and the ^{14}C mainly the rate of tRNA synthesis, any variation in the relative rate of turn over of individual tRNA species would probably have been reflected as a concurrent discrepancy in the elution profile of both isotopes. On the other hand, since there is considerable overlap in the elution of individual tRNAs, particularly in the central region of the major A_{260} peak, this experiment does not exclude the possibility of minor variations in the relative turn over rates of one or two molecular species of tRNA. Thus, the turn over rate of individual tRNA species was measured both during the period of short synthesis, and of prolonged decay, and found to be identical relative to one another. A similar procedure has been successfully employed to study the relative turn over rates of rat liver endoplasmic reticulum proteins [14].

It is clear, therefore, that the relative turn over rates of individual tRNA species are likely to be identical and, as shown in fig. 1, this appears to be 5 days. Our data are in agreement with the half life time of

rabbit liver tRNA [3], and our previous studies in rat liver [8–11]. It is likely that the various values for the half life time of rat liver tRNA, as previously reported [5–7, 15], were due to the contamination of soluble RNA with degraded mRNA. We think that the relative centrifugal force of 150,000 *g* or more can be successfully exploited for obtaining a cleaner tRNA preparation. It is not clear whether the discrepancy in the half life time of mammalian tRNA is related to variations in sex, age or diet of the experimental animals. The relative stability in the half life time of individual tRNA species, as described in this report, suggests that under usual physiological conditions, most of the individual tRNAs are synthesized and degraded at similar rates and makes it unlikely that normal physiological homeostasis proceeds via drastic alternations in the turn over rates of individual tRNAs.

Acknowledgments

Part of this work was conducted during tenure of post-doctoral fellowships at the Institute of Cancer Research, Columbia University, College of Physicians and Surgeons , New York, N.Y. We wish to thank Dr. I.B. Weinstein for critical appraisal of the manuscript.

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