RELATIVE UTILIZATION OF MAMMALIAN LYS-tRNA ISOACCEPTORS IN PROTEIN SYNTHESIS*

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1. Introduction

Ortwerth and collaborators [1,2] observed a correlation between the relative abundance of a specific lysine isoacceptor (designated Lys-tRNA$_4$) in the tRNA populations of a variety of mammalian cells and the rate of cell division. Although this isoacceptor was virtually absent in non-dividing cells, its level was increased relative to the other lysine isoacceptors in more actively dividing cells. Kleiman et al. [3], however, found that Lys-tRNA$_4$ was enriched in non-dividing erythroid Friend cells. Raba et al. [4] recently sequenced this and other mammalian lysine isoacceptors and demonstrated that Lys-tRNA$_4$ is an undermodified form of one of the other Lys-tRNAs. Although Ortwerth et al. [5] showed that lysine can be incorporated into protein from Lys-tRNA$_4$, neither they nor Raba et al. [4] compared the utilization of this isoacceptor in protein synthesis relative to other lysine isoacceptors which have the same codon recognition properties [4–6]. In the present study, the relative utilization of these lysine isoacceptors during globin synthesis in a wheat-germ extract was determined. Lys-tRNA$_4$ was utilized less extensively for globin synthesis than other Lys-tRNAs which recognize the same codon.

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2. Materials and methods

[^{3}H]Lysine, 63.1 Ci/mmol, was a commercial product. tRNA was prepared from rabbit liver and [^{3}H]Lys-tRNA prepared using rabbit reticulocyte synthetases [6]. A portion of the [^{3}H]Lys-tRNA was resolved over a RPC-5 column [7], the resulting [^{3}H]Lys-tRNA$_4$ isolated, resolved a second time over the RPC-5 column, isolated and added to a total [^{3}H]Lys-tRNA preparation in order to enrich for [^{3}H]Lys-tRNA$_4$.

Wheat-germ extracts were prepared [8] or were the gift of Dr B. Paterson. Protein synthesis was carried out in the presence or absence of rabbit globin mRNA [8]. Wheat germ was the gift of Dr H. Aviv. Globin mRNA was prepared from rabbit reticulocytes [9]. Pilot studies demonstrated that the transfer of lysine into protein in wheat-germ extracts programmed with globin mRNA ceased at 45 min. Deacylation and transfer of lysine into protein were determined as follows: Reaction mixtures with globin mRNA (total volume, 200 μl) and without globin mRNA (total volume, 300 μl) were prepared [8], each containing 15.83 pmol of [^{3}H]Lys-tRNA (1.75 A_{260} units) per 100 μl and excess unlabeled lysine (2.8×10^{-3} M). Two 5 μl aliquots were removed from each reaction mixture at the time intervals shown in the inset to fig.1. One of the 5 μl aliquots was added directly to 0.5 ml of water followed by the immediate addition of 7 ml of 5% trichloroacetic acid (TCA); and the second 5 μl aliquot was added to 0.5 ml of 0.1 M KOH, allowed to stand 3–4 min at 20°C, followed by the addition of 7 ml of 5% TCA. Precipitates were collected on nitrocellulose filters, the filters dried and then counted. The first assay measured the rate of [^{3}H]Lys-tRNA deacylation and the second, the

amount of [^{3}H]lysine incorporated into protein. The relative utilization of lysine isoacceptors was determined from the distribution of the peaks of [^{3}H]Lys-tRNA resolved by chromatography as follows: 100 μl were removed from the above reaction mixtures at 45 min and added immediately to 1.0 ml of 0.45 M NaCl, 0.01 M NaAc, 0.01 M $MgAc_2$ and 0.001 M EDTA, pH 4.5, followed by 1 vol. of water-saturated phenol. [^{3}H]Lys-tRNA was then prepared for column chromatography [6], chromatographed on a RPC-5 column [7], and the column developed in a linear gradient of 0.475 to 0.625 M NaCl.

3. Results

Rabbit-liver [^{3}H]Lys-tRNA which had been specifically enriched with [^{3}H]Lys-tRNA$_4$ was incubated in wheat-germ extracts in the presence and absence of globin mRNA. Aliquots were removed and assayed at the time intervals shown in the inset of fig.1. The rate of deacylation of Lys-tRNA was similar in both reactions as shown by the closed symbols. The transfer of lysine into protein was much greater in the presence than in the absence of globin mRNA (open symbols).

[^{3}H]Lys-tRNA was recovered from incubation mixtures at 45 min and chromatographed on a RPC-5 column in order to determine the relative utilization of the lysine isoacceptors in protein synthesis (fig.1). These samplings were made at the time globin synthesis ceased (see inset to fig.1). The large peak which eluted first from the column (designated Peak I) recognizes AAG [4–6]. This peak contains two isoacceptors which are not separated by RPC-5 chromatography [4]. The large, second eluting peak (designated Peak II) is Lys-tRNA$_4$ and, like Peak I, it recognizes AAG [4–6]. Although Lys-tRNA$_4$ represents less than 10% of the total Lys-tRNA from normal rabbit liver, its amount was increased in [^{3}H]Lys-tRNA preparations used in the present study to determine accurately the relative utilization of this isoacceptor in protein synthesis. The large, third eluting peak (designated Peak III) recognizes AAA [4–6]. The relative utilization of the minor peak which eluted first from the column was not considered.

The elution profiles of [^{3}H]Lys-tRNA show that Peak I disappeared more rapidly than the other peaks in response to globin mRNA. The per cent of lysine incorporation into globin from Peaks I–III and the

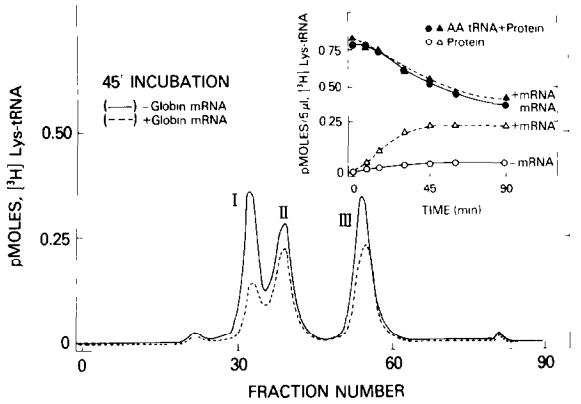

Fig.1. Utilization of [^{3}H]Lys-tRNA in wheat-germ extracts. [^{3}H]Lys-tRNA which was enriched with [^{3}H]Lys-tRNA$_4$ was incubated in wheat-germ extracts. In the inset, two 5 μl aliquots were removed from each reaction at the time intervals shown and (i) precipitated in trichloroacetic acid (closed symbols) which measured at zero time the amount of [^{3}H]Lys-tRNA added to each reaction, and at subsequent times, the rate of deacylation of [^{3}H]Lys-tRNA in presence of globin mRNA (▲) and in absence of globin mRNA (●), or (ii) incubated first in KOH and then precipitated with trichloroacetic acid (open symbols) which measured at zero time the amount of non-specific attachment of [^{3}H]lysine to Millipore filters and, at subsequent times, the rate of transfer of [^{3}H]lysine into protein in presence of globin mRNA (△) and in absence of globin mRNA (○). The amount of [^{3}H]Lys-tRNA was determined by subtraction of the value obtained for labeled protein from the value obtained for the rate of deacylation. The graph shows RPC-5 chromatograms of [^{3}H]Lys-tRNA recovered from reactions with globin mRNA (- - - -) and without globin mRNA (——). For a definition and discussion of peaks I–III, see the text.

per cent utilization of these peaks for globin synthesis are shown in table 1. One-half of the [^{3}H]lysine in globin was donated from Peak I, while 18 and 30% were donated from Peaks II and III, respectively. Peak I was utilized the most for globin synthesis (60%) and Peak II the least (21%).

4. Discussion

The present studies show that Peak II (Lys-tRNA$_4$), which is an undermodified lysine isoacceptor [4], is not utilized as extensively in protein synthesis as Peak I even though both recognize AAG [4–6]. Peak II was also utilized less extensively for globin synthesis when the [^{3}H]Lys-tRNA was prepared from rabbit reticulocyte tRNA or from different prepara-

Table 1
Incorporation of [^{3}H]lysine into globin and utilization of Peaks I–III for globin synthesis

Peak	pmol[a]			% in globin[b]	% Utilized[c]
	–mRNA	+mRNA	△ (Globin)		
I	1.36	0.54	0.82	50	60
II	1.37	1.08	0.29	18	21
III	1.56	1.06	0.50	30	32

[a] pmol in each peak were obtained from RPC-5 chromatograms in fig.1. More than 90% of the [^{3}H]Lys-tRNA which remained in reactions after 45 min incubation was recovered from wheat-germ extracts for chromatography and more than 90% of the [^{3}H]Lys-tRNA added to RPC-5 columns was recovered after chromatography. One half of both [^{3}H]Lys-tRNA samples recovered from wheat-germ extracts after 45 min incubation were added to RPC-5 columns. △, pmol determined the number of pmol donated by each peak to globin and was obtained by subtracting the pmol observed in a given peak in presence of globin mRNA from that observed in absence of globin mRNA

[b] % of pmol in globin which came from each peak was determined by dividing the total pmol of [^{3}H]lysine in globin into that donated by each peak. 2% of the pmol of [^{3}H]lysine in globin came from the minor peak which eluted first from the column. This minor isoacceptor recognizes AAG [6]

[c] % of Peaks I–III utilized for globin synthesis was determined by dividing the pmol observed in a given peak in absence of globin mRNA (column 1) into pmol observed in globin (column 3). This determination does not take into account the amount of deacylation which occurred during the 45 min incubation which was similar for reactions with and without globin mRNA (see inset in fig.1)

tions of rabbit liver tRNA and the protein synthesis system was rabbit reticulocyte lysates or wheat-germ extracts [10 and unpublished data]. Thus, the reduced utilization of Peak II compared to Peak I is not dependent on the source or preparation of tRNA or on the protein synthesis system. It seems likely, then, that the undermodification of Lys-tRNA$_4$ is responsible for its reduced utilization in protein synthesis.

Peak I which recognizes AAG was utilized more frequently for globin synthesis than Peak III which recognizes AAA. Although this observation is consistent with the coding sequences for lysine in rabbit globin mRNA [11,12], it may not reflect an exact ratio of utilization of Peaks I and III for globin synthesis due to competition with endogenous Lys-tRNA.

Carpousis et al. [13,14] have demonstrated preferential usage of an avian alanine and an avian glycine isoacceptor for collagen synthesis by similar assays as described above. Measurements of the relative utilization of aminoacyl-tRNAs may elucidate which isoacceptors are required in greatest abundance for synthesis of specific proteins. Furthermore, such studies may provide information on the effects that specific modifications in the primary structures of isoacceptors have on their utilization in protein synthesis.

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