TRANSFER RNA CHANGES IN RAT GRANULATION TISSUE POSSIBLY RELATED TO COLLAGEN SYNTHESIS

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SUMMARY

Transfer RNAs for glycine, proline, lysine, serine and leucine were compared in developing rat granulation tissue 6 and 15 days after sterile subcutaneous implantation of pieces of cellulose sponge. The acceptance of glycine, proline and lysine by unfractionated tRNAs were ca. 30 per cent greater in tRNA derived from 15-day granulation tissue, whereas those of serine and leucine were unaltered. Cochromatography on benzoylated DEAE-cellulose of the ^{3}H- and ^{14}C-labeled aminoacyl-tRNAs from the two sources revealed a significant increase in the relative amount of one of the three glycyl-tRNA fractions in the 15-day granulation tissue, whereas the elution profiles for prolyl-, lysyl-, seryl-, and leucyl-tRNAs were unaltered. The changes observed suggest a causal relation to the enhanced synthesis of collagen in the late-stage granulation tissue.

INTRODUCTION

The amount and nature of isoaccepting transfer RNAs have been shown to vary among specialized cells and tissues (for a review, see 1). The cause of the observed variation is probably different in different cases, since several regulatory processes may be involved, including alteration in the rate of tRNA synthesis and degradation, and possibly in the intracellular processing and modification. In some cases the amounts of specific tRNA species have been shown to reflect the amino acid pattern in the major protein produced by the cell, for example in the case of silk fibroin (2), hemoglobin (3), and phosvitin, an avian yolk protein (4,5). Collagen is a potential candidate for studies of this type since it contains 33 per cent glycine and 22 per cent proline plus hydroxyproline (see 6). The hydroxylation of some of the proline and lysine residues occurs postribosomally (7).

A well-described experimental approach to collagen synthesis is the granulation tissue produced in rats by sterile subcutaneous implantation of pieces of

cellulose sponge (8). The synthesis of collagen starts after the first week of implantation, is maximal during the third week, and then decreases rapidly (8). Lanks and Weinstein (9) have previously reported that the quantity of proline-specific tRNA is significantly greater in rat granulation tissue than in the liver, and that the amount of prolyl-tRNA is greater in 10-day than in 5-day granulation tissue. We have re-examined and extended these findings and shown that the acceptances of glycine, proline and lysine by unfractionated tRNA from 15-day granulation tissue are ca. 30 per cent greater than those from 6-day tissue. In addition, the relative amount of one of the three glycyl-tRNA species is simultaneously increased.

MATERIAL AND METHODS

Male Wistar rats weighing 150 to 180 g were used. Pieces of sterile cellulose sponge were implanted subcutaneously with techniques described in detail elsewhere (8). Transfer RNA and aminoacyl-tRNA synthetases were prepared from pooled granulation tissues 6 and 15 days after the implantation as described previously (4). The content of tRNAs accepting each of the five selected amino acids was determined by measuring the aminoacylation of limiting amounts of tRNA in 0.05 ml reaction mixtures containing the following components: 0.1 M Tris-HCl, pH 7.4, 0.01 M $MgCl_2$, 0.01 M KCl, 0.4 mM dithiothreitol, 5 mM ATP, pH 7.4, 1 mM CTP, 0.1 mM NaEDTA, 0.1 mM 19 nonradioactive amino acids, 0.02 mM radioactive amino acid, tRNA and enzyme. The duration of the acylation incubation at 37° and the amount of tRNA and synthetase were varied in each case to obtain a maximal acylation. Isotopes were from New England Nuclear Corporation and the specific radioactivities were as follows: glycine-2-^{3}H 10700 mCi/mmole, glycine-^{14}C 91 mCi/mmole, L-proline-3,4-^{3}H 5000 mCi/mmole, L-proline-^{14}C 214 mCi/mmole, L-lysine-^{3}H 3000 mCi/mmole, L-lysine-^{14}C 255 mCi/mmole, L-serine-^{3}H 2230 mCi/mmole, L-serine-^{14}C 128 mCi/mmole, L-leucine--4,5-^{3}H 5000 mCi/mmole, and L-leucine-^{14}C 291 mCi/mmole. For analysis aminoacyl-tRNA was precipitated after incubation with cold 5 per cent trichloroacetic acid, collected on glass fiber filters (Gelman Type A), dried, and counted with a toluene-based scintillation cocktail (4) in a Packard liquid scintillation counter.

Preparation of aminoacyl-tRNA for benzoylated DEAE-cellulose chromatography was performed in 0.5 ml reaction mixtures containing optimal amounts of tRNA and synthetase protein. After incubation the reaction mixture was treated with an equal volume of cold, water-saturated phenol, and

after centrifugation, the aqueous phase was passed through a Sephadex G-25 column. The fractions containing radioactive tRNA were collected, pooled, lyophilized, and stored at 4° until used in chromatography. The conditions for chromatography of aminoacyl-tRNA have been described previously (4). Benzoylated DEAE-cellulose was from Schwartz BioResearch (lot number W 2049). For double labeling counting, appropriate corrections were made for ^{3}H and ^{14}C radioactivity appearing in the other channel.

RESULTS AND DISCUSSION

Acceptances of glycine, proline, lysine, serine, and leucine by unfractionated tRNA derived from 6- and 15-day granulation tissues are shown in Table 1. The synthetase used for the assays was from the 15-day granulation tissue, since testing of the corresponding synthetase from 6-day tissue invariably revealed RNase activity, which made interpretation difficult. Therefore, only 15-day synthetase was used in subsequent acylations. Calculations of the optimal aminoacylations showed that the acceptances of glycine, proline and lysine were increased in the tRNA derived from the 15-day granulation tissue by ca. 30 per cent as compared to the 6-day tRNA preparation. The serine and leucine acceptances were unaltered. Glycine and proline are the major amino acids in collagen, whereas lysine, serine and leucine are represented in quantities of less than 4 to 5 per cent each in collagen (6). These results confirm the previous findings by Lanks and Weinstein of the increase in proline-specific tRNA in developing granulation tissue (9). However, their results indicated that glycine was represented by equal amounts of tRNA in 5- and 10-day granulation tissues. The reason for this discrepancy is unknown but it may be due to the different enzymes used (liver vs. homologous synthetase) or to the different stage of granulation tissue selected for examination.

Chromatographic comparisons of the double-labeled aminoacyl-tRNAs on benzoylated DEAE-cellulose revealed essentially identical elution profiles for prolyl-, lysyl-, seryl-, and leucyl-tRNAs from the two sources (Figure 1). A significant difference was seen, however, in the glycyl-tRNA (Figure 2), since the amount of one of the three fractions was selectively increased in tRNA derived from the 15-day granulation tissue. This difference was independent of the isotope used, since interchanging the label yielded an identical result (data not shown). It is noteworthy that the chromatographic method used in this study did not give a good separation for the isoaccepting prolyl-tRNA species. Therefore, no firm conclusion

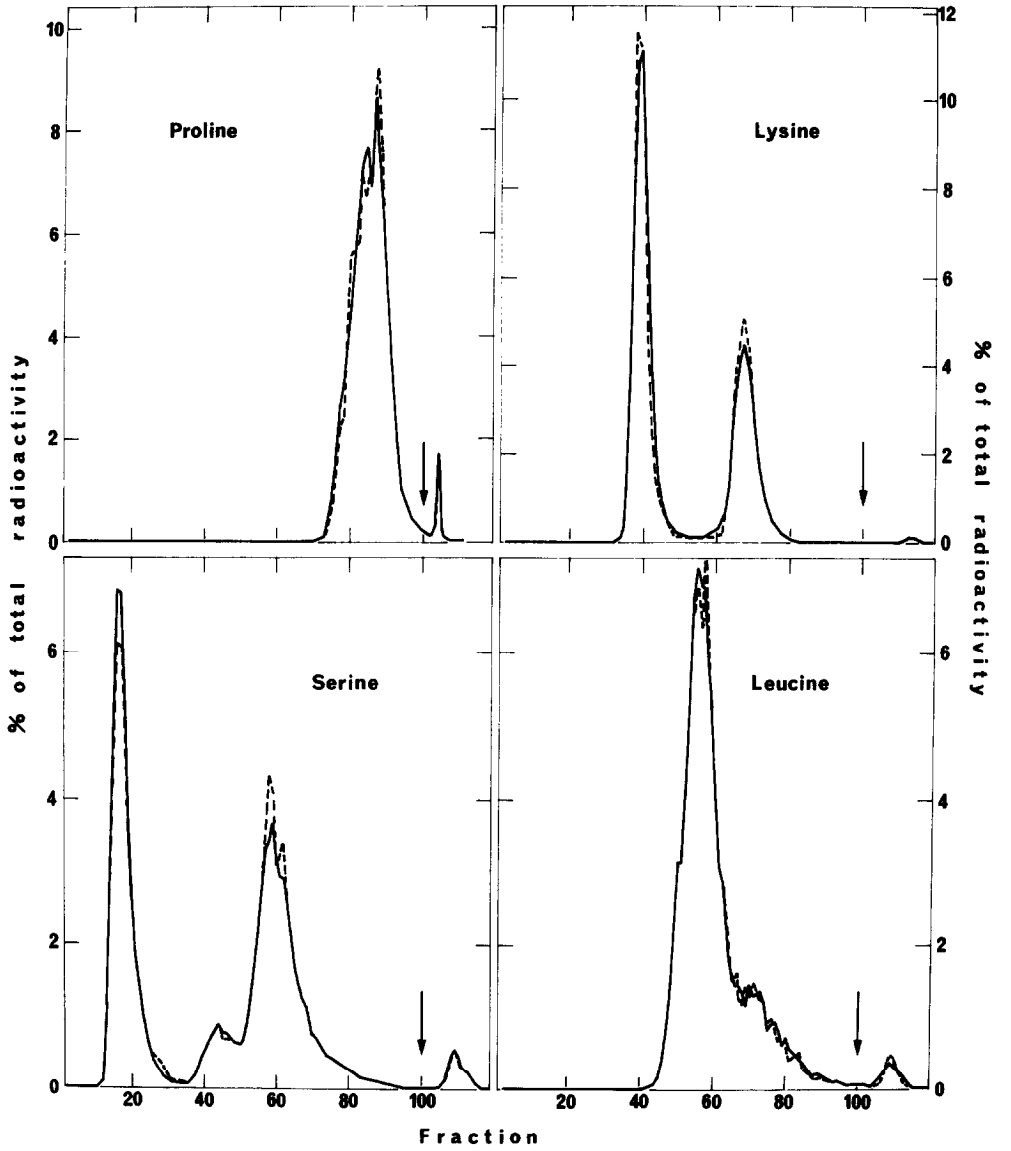

Figure 1. Comparisons of elution profiles of prolyl-, lysyl-, seryl-, and leucyl-tRNAs on benzoylated DEAE-cellulose. ^{3}H-aminoacyl-tRNAs (solid line) derived from 6-day granulation tissue were cochromatographed with corresponding ^{14}C-aminoacyl-tRNAs (interrupted line) from 15-day granulation tissue. Each tRNA was acylated with synthetase from 15-day granulation tissue. Benzoylated DEAE-cellulose columns were prepared as previously described (4), and used at 24°. 400 ml linear NaCl gradients were employed at a flow rate of 0.4 ml/min and 4 ml fractions were collected. Radioactivity was detected in eluates by precipitating aminoacyl-tRNA in 7 per cent trichloroacetic acid, collecting precipitates on glass fiber filters, and counting in a Packard liquid scintillation counter (4). The NaCl gradients (containing 10 mM $MgCl_2$ and 5 mM sodium acetate, pH 4.43) for the individual aminoacyl-tRNAs were as follows: 0.45 to 0.8 M for prolyl-tRNA, 0.45 to 1.0 M for lysyl-tRNA, 0.6 to 1.0 M for seryl-tRNA, and 0.45 to 1.0 M for leucyl-tRNA. At arrow the elution was continued with a 80 ml gradient of 1.0 to 1.5 M NaCl containing 5 mM sodium acetate, pH 4.43 and 15 per cent ethanol.

TABLE 1

mino acid	Acceptance (nmoles/A_{260}) 6-day preparation	15-day preparation	Ratio 15-day/6-day
lycine	10.7	14.3	1.34
roline	5.9	7.6	1.29
ysine	16.2	21.1	1.30
erine	48.4	48.6	1.00
eucine	28.6	29.5	1.03

minoacylation of unfractionated tRNAs derived from 6- and 15-day granulation tissues was tested in reaction mixtures (0.05 ml) containing 0.2 to 2.0 A_{260} units of tRNA, 0.01 to 0.5 mg of enzyme protein, and radioactive amino acids at 0.02 mM concentrations. The remaining components are described in the text.

can be drawn regarding the stability of the isoaccepting prolyl-tRNA species in developing granulation tissue. Also, it should be pointed out that even in the 15-day granulation tissue collagen synthesis may be only a small fraction of total protein synthesis (see 8). Hopefully, development of experimental systems in which collagen synthesis is proportionally even greater will allow a better understanding of the specific tRNA alterations involved. However, we have interpreted the present results as in accord with the general hypothesis that, in the cell, the amounts of tRNAs specific for certain amino acids are regulated to meet the requirements of the different messengers to be translated.

ACKNOWLEDGEMENTS

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REFERENCES

1. Sueoka, N., and Kano-Sueoka, T., Progr. Nucl. Acid Res. and Mol. Biol., 10, 23 (1970).
2. Garel, J.P., Mandel, P., Chavancy, G., and Daillie, J., FEBS Letters 7, 327 (1970).
3. Smith, D.W.E., and McNamara, A.L., Science 171, 577 and 1040 (1971).

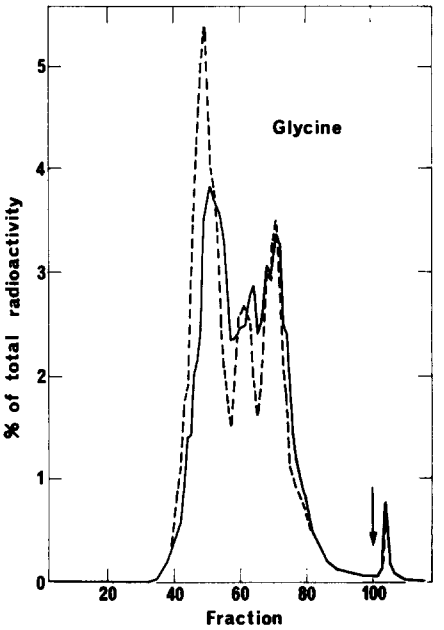

Figure 2. Comparison of elution profiles of glycyl-tRNAs on benzoylated DEAE-cellulose. ^{3}H-glycyl-tRNA (solid line) from 6-day granulation tissue was cochromatographed with ^{14}C-glycyl-tRNA (interrupted line) from 15-day granulation tissue. 15-day synthetase was used in both acylations. The conditions for chromatography are as in Figure 1, except that a concave, four-chamber gradient (300 ml of 0.45 plus 100 ml of 0.8 M NaCl buffer) was used.

4. Mäenpää, P.H., and Bernfield, M.R., Biochemistry 8, 4926 (1969).
5. Mäenpää, P.H., FEBS Letters 23, 171 (1972).
6. Pikkarainen, J., Acta Physiol. Scand., Suppl. 309 (1968).
7. Grant, M.E., and Procop, D.J., New England J. Med., 286, 194 (1972).
8. Ahonen, J., Acta Physiol. Scand., Suppl. 315 (1968).
9. Lanks, K.W., and Weinstein, I.B., Biochem. Biophys. Res. Commun., 40, 708 (1970).