CHROMATOGRAPHIC STABILITY OF CODON-SPECIFIC SERYL-tRNA'S FROM AVIAN AND MAMMALIAN TISSUES AND TWO CASES OF A SPECIFIC VARIATION

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1. Introduction

Chromatographic differences of isoaccepting transfer RNA's from normal and neoplastic tissues have frequently been described (for recent references, see [1]). In general, transfer RNA's for amino acids with multiple codons show the most variation. We have previously reported that in avian liver the level of two of the four seryl-tRNA species is coordinately altered with the estrogen-induced synthesis of phosvitin, a phosphoprotein containing greater than 50% serine residues [2]. To investigate the biological stability of seryl-tRNA elution profiles and, consequently, the significance of the observed alterations, a survey of seryl-tRNA's from different animal sources was carried out using the same chromatographic procedure as previously. Different stages of chick embryogenesis and livers of 1-month-old and adult roosters, and of rats and rabbits were studied. Rabbit reticulocytes were also studied as an example of a cell synthesizing mainly one type of protein. Very similar elution profiles were observed in all cases, except in rooster liver during phosvitin synthesis and in rabbit reticulocytes, where the direction of the alteration was the opposite to that found in avian liver. These results suggest that the synthesis of specific seryl-tRNA species may be independently regulated and coordinated with the protein structural gene(s) at transcription. Consequently, the variable patterns of isoaccepting tRNA's may reflect the specific requirement of the different messengers to be translated.

2. Materials and methods

Leghorn chicks and roosters, male adult albino rats and rabbits were used. The methods for the preparation of tRNA, crude aminoacyl-tRNA synthetases, and aminoacyl-tRNA and for the chromatographic separation of the radioactive seryl-tRNA's on benzoylated DEAE-cellulose have been previously described in detail [2]. Benzoylated DEAE-cellulose was from Schwartz BioResearch (lot numbers 6029 and W2049 with slightly different elution characteristics were used). Transfer RNA was also prepared from reticulocytes of phenylhydrazine-treated rabbits [3].

3. Results and discussion

The codon responses of the four chromatographically separated rooster liver seryl-tRNA's have been determined [4]. The peak eluting first from the benzoylated DEAE-cellulose column binds with AGU and AGC, the second peak with UCG, the third large peak with UCU and UCA (and weakly with UCC, see also [5]). The last seryl-tRNA fraction, eluting in the ethanol–salt buffer, does not respond appreciably with any of the six serine triplets. It binds to some extent with poly(U,C), but this may represent a chromatographic contamination from the previous peak. A response to UGA of a chromatographically similar chicken liver seryl-tRNA fraction has been reported [5]. We have not been able, however, to confirm the binding with UGA.

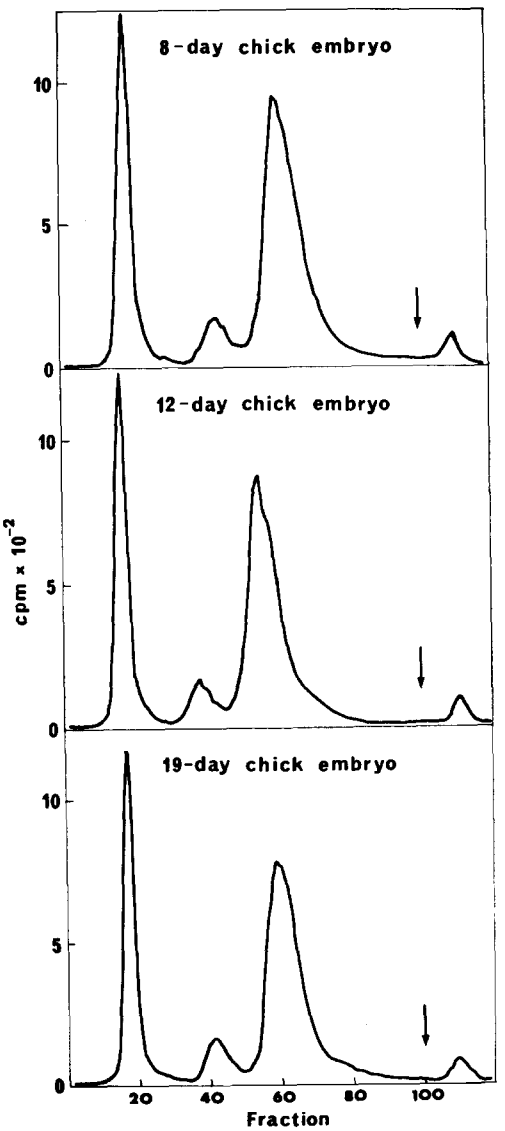

Fig. 1. Elution profiles of ^{3}H-seryl-tRNA of 8-day, 12-day, and 19-day chick embryo. Transfer RNA's were acylated with rooster liver synthetase. Benzoylated DEAE-cellulose columns were prepared as previously described [2] and used at 24°. 400 ml linear NaCl gradients were employed at a flow rate of 0.4 ml/min and 4 ml fractions were collected. Radioactivity was detected in eluates by precipitating seryl-tRNA in 10% trichloroacetic acid, collecting precipitates on glass fiber filters (Gelman, Type A), and counting in a Packard liquid scintillation counter [2]. Specific radioactivity of ^{3}H-serine was 2230 mCi/mmole.

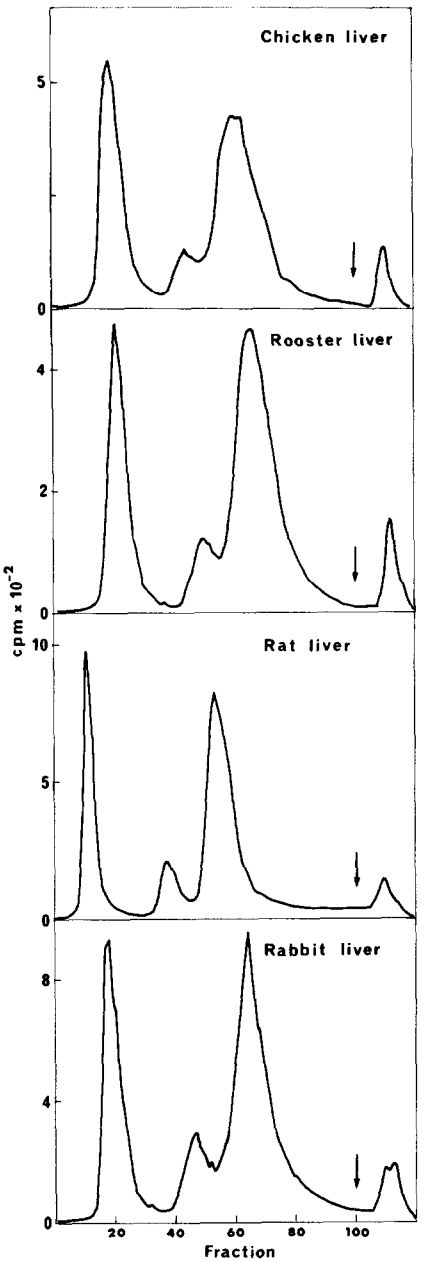

Fig. 2. Elution profiles of liver ^{3}H-seryl-tRNA of 1-month-old and adult rooster, and of rat and rabbit. Transfer RNA's were acylated with homologous synthetases. Column conditions, detection of radioactivity and specific radioactivity of serine were the same as those in fig. 1.

During chick embryogenesis, the elution profile of seryl-tRNA remained very stable (fig. 1 and table 1). The quantitative differences in various seryl-tRNA fractions can, at least partly, be explained by the fact that the first peak responds to two, the second peak to one and the third peak to three codons. Thus, per one serine codon in the messenger the absolute amounts of specific seryl-tRNA's are probably not very dissimilar.

In livers of 1-month-old and adult rooster and of rat and rabbit, the seryl-tRNA elution profile also remained very stable (fig. 2 and table 1). This probably reflects the multiple types of proteins made and, consequently, the multiple types of code words used in this tissue. In two instances, however, a deviation from this stable seryl-tRNA elution pattern was observed. As reported previously, the induction of the synthesis of phosvitin, a serine-rich phosphoprotein, is accompanied with concomitant alterations in the UCX-specific seryl-tRNA fractions (fig. 3 and table 1). Since the total serine acceptance was increased by more than 25%, the conclusion was drawn that the UCX-specific species are increased as compared to the AGU(C)-specific fraction [2]. These changes are fully reversible and the seryl-tRNA elution profile approaches the control pattern on termination of phosvitin synthesis [2]. In rabbit reticulocytes, the seryl-tRNA elution pattern was also changed. In this case (fig. 3 and table 1), the direction of the change was the opposite to the situation in the estrogen-treated rooster liver. The UCX-specific species were decreased as compared to the AGU(C)-specific fraction. These results indicate a possible coupling of transfer RNA and protein structural genes at transcription. Further

Table 1
Summary of seryl-tRNA chromatography.

Seryl-tRNA preparation	Radioactivity in Peak I	Peaks II+III+IV
8-Day chick embryo	0.28	0.72
12-Day chick embryo	0.30	0.70
19-Day chick embryo	0.30	0.70
Chicken liver (1-month-old)	0.34	0.66
Rooster liver	0.27	0.73
Rat liver	0.25	0.75
Rabbit liver	0.28	0.72
Rabbit reticulocyte	0.44	0.56
Rooster liver, 3 days after estrogen	0.14	0.86

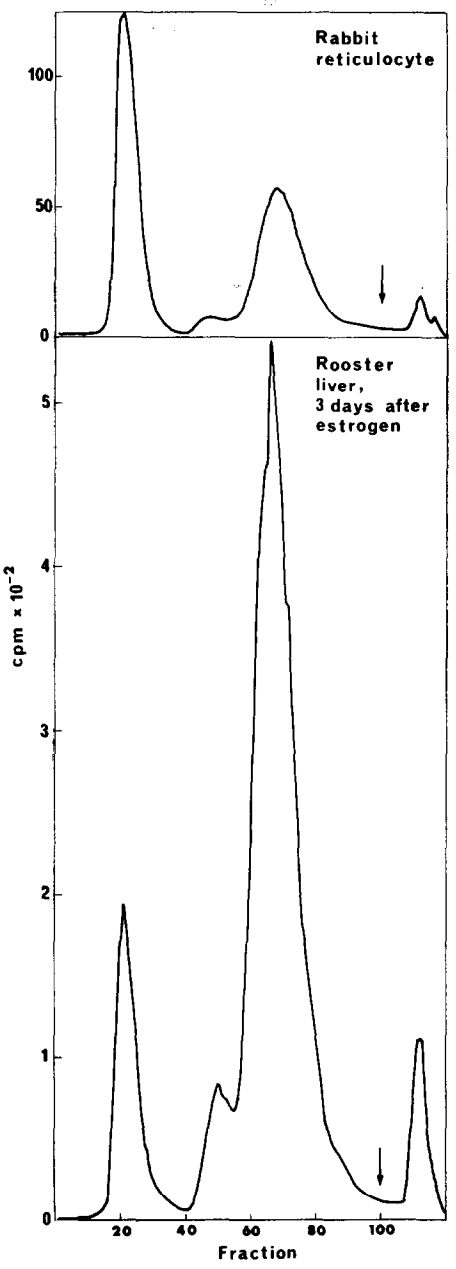

Fig. 3. Elution profiles of ^{3}H-seryl-tRNA of rabbit reticulocytes and estrogen-treated rooster liver. Rabbits were made anemic by subcutaneous injections of phenylhydrazine [3]. Adult roosters were injected with 10 mg/kg of estradiol-17β benzoate intramuscularly three days prior to the preparation of transfer RNA [2]. Transfer RNA's were acylated with homologous synthetases. Column conditions, detection of radioactivity and specific radioactivity of serine were the same as those in fig. 1.

work is in progress to determine the roles of each seryl-tRNA species in cell-free, tRNA-dependent, syntheses of phosvitin and hemoglobin.

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