SERYL TRANSFER RNA ALTERATIONS DURING ESTROGEN-INDUCED PHOSVITIN SYNTHESIS. QUANTITATIVE ASSAY OF THE HORMONE-RESPONDING SPECIES BY RIBOSOMAL BINDING

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SUMMARY

The poly(U,C)-stimulated binding of unfractionated rooster liver ^{3}H-seryl--tRNA to E.coli ribosomes closely correlated with the relative amount of the UC(U,C,A)-specific species separated from the other seryl-tRNA's by chromatography on benzoylated DEAE-cellulose. The poly(U,C)-stimulated binding of unfractionated seryl-tRNA provides a useful method to follow the course and to examine the mechanism of alteration of the hormone-responding seryl-tRNA.

INTRODUCTION

In recent years the early events in action of steroid hormones on target tissues have been clarified to a large extent (1). However, the mechanism of activation and coordination of specific genes or gene groups at transcription by steroid hormones is for the most part unknown. We have previously shown that in avian liver the level of two of the four seryl-tRNA's is correlated with the level of phosvitin synthesis (2). Since this protein contains greater than 50% serine residues, the observed positive correlation suggests that the increments in specific seryl-tRNA's are connected with the activation of phosvitin structural gene(s). The coding properties of the four seryl-tRNA's have been determined (3). It revealed that the major fraction undergoing alterations after estrogen-treatment is the only species responding strongly to poly(U,C). In this study, a detailed comparison of the poly(U,C)-stimulated binding with the chromatographic method has been made.

MATERIALS AND METHODS

White Leghorn roosters weighing 1.8 to 2.0 kg were used. Estradiol-17β

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benzoate, 10 mg/kg in sesame oil, was injected intramuscularly as previously described (2). Preparation of tRNA, aminoacyl-tRNA synthetases, and aminoacyl-tRNA from rooster liver was carried out as described elsewhere (2). Optimal conditions for seryl-tRNA formation were determined for all tRNA preparations. Chromatography on benzoylated DEAE--cellulose, prepared according to Gillam et al. (4) or purchased from Schwarz BioResearch, was performed as previously described (2). The NaCl gradients were linear from 0.6 to 1.0 M in 0.01 M $MgCl_2$ and 0.005 M sodium acetate, pH 4.44. 14% ethanol in 1.5 M NaCl and 0.005 M sodium acetate, pH 4.44, was used to elute the last seryl-tRNA fraction.

Ribosomal binding of ^{3}H-seryl-tRNA was measured according to Leder (5). Poly(U,C) (1:1) and E.coli K 12 were from Miles Laboratories. E.coli ribosomes were prepared by the method of Nirenberg (6). Binding was for 20 minutes at 24° in 50 µl mixtures containing 0.1 M Tris-HCl, pH 7.2, 0.05 M NH_4Cl, 0.02 M $MgCl_2$, 0 to 5.2 A_{260} units of ribosomes and 0.17 A_{260} unit of poly(U,C). The amounts of different ^{3}H-seryl--tRNA's used in assays are indicated in Figure 1. ^{3}H-serine (specific activity 2230 mCi/mmole) was from New England Nuclear Corp.

RESULTS AND DISCUSSION

The codon responses of the four seryl-tRNA's have been previously determined (3). The peak eluting first from the benzoylated DEAE-cellulose column binds with AGU and AGC, the second peak with UCG, the third and most prominent peak with UCU, UCA and weakly with UCC (see also 7). The last peak eluting in the ethanol-salt buffer does not bind 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 (see also 7). A response to UGA of a similar chicken liver seryl-tRNA fraction has been reported (7). We have not been able, however, to confirm the response to UGA (unpublished data). Since the only peak responding strongly to poly(U,C) was also the major fraction altered after estrogen administration, determination of its relative content by direct binding of unfractionated ^{3}H-seryl-tRNA to E.coli ribosomes in the presence of poly(U,C) was possible.

An examination of the proportion of unfractionated ^{3}H-seryl-tRNA bound before and at various times after estrogen-treatment indicates a close

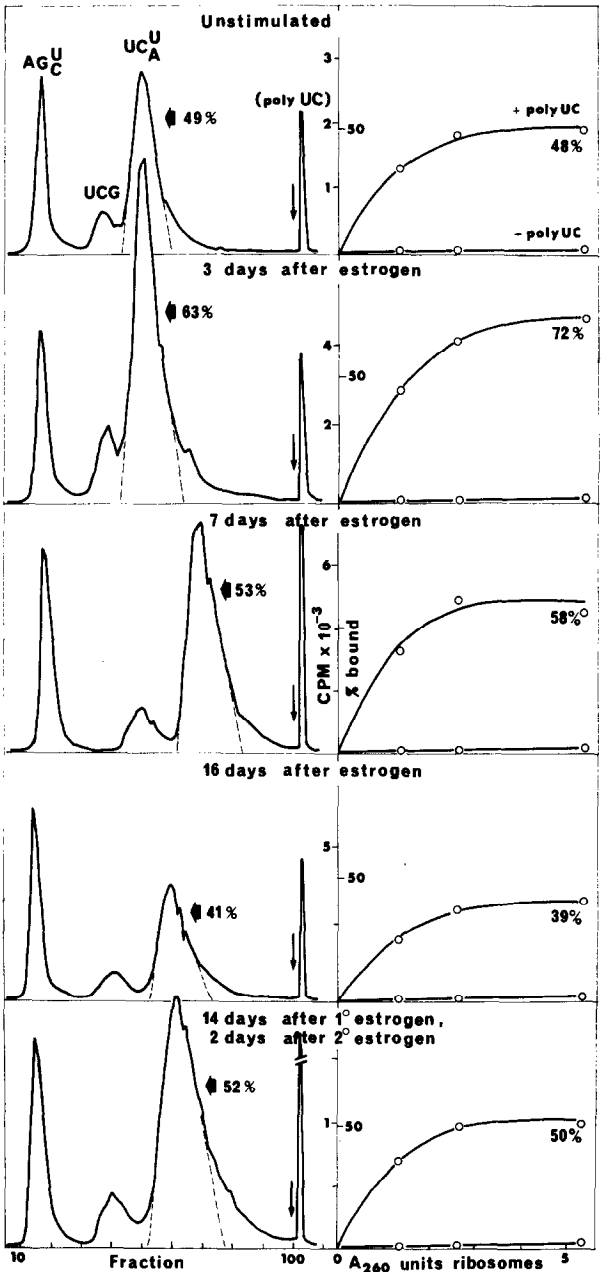

Figure 1. Comparison of the radioactive seryl-tRNA chromatographic profiles (on the left) and the amounts of radioactivity bound to E.coli ribosomes from the corresponding unfractionated ^{3}H-seryl-tRNA's in the presence and absence of poly(U, C) (on the right). Seryl-tRNA's were prepared from control roosters (unstimulated), during the ascending (3-day), and during the descending (7-day) part of the plasma phosvitin curve, after its return to the control level (16-day), and after

a second estradiol-17β benzoate injection at day 14 (2). Radioactive seryl-tRNA's were eluted from the benzoylated DEAE-cellulose column (0.9 x 20 cm) as described in the text. The arrow indicates the start of the ethanol-salt buffer. Four ml fractions were collected, tRNA precipitated and collected on glass fiber filters and the radioactivity determined as described elsewhere (2). Binding of the ^{3}H-seryl-tRNA's to E.coli ribosomes was carried out as described in the text. The amounts of the ^{3}H-seryl-tRNA's used in the binding assays were (from the top to the bottom) 0.87, 0.97, 0.62, 1.08 and 1.03 pmoles respectively.

correlation with the amount of the UC(U,C,A)-specific species separated from the other isoaccepting seryl-tRNA's by chromatography on benzoylated DEAE-cellulose (Figure 1). The only difference was a somewhat larger binding of the 3-day ^{3}H-seryl-tRNA. This is probably due to the fact that, in binding studies, the acylation mixtures contained 1 mM CTP in addition to the usual components (2). This has been found to further increase the relative proportion of the UCX-specific peaks in the 3-day seryl-tRNA elution profile (unpublished data), probably reflecting incomplete -CCA ends in these tRNA species. At 10 mM Mg^{2+}, the binding was not quantitative (data not shown).

The relative proportion of the UC(U,C,A)-specific seryl-tRNA in rooster liver can be determined by chromatographic separation of the four seryl--tRNA's on benzoylated DEAE-cellulose (2). The results presented in this paper indicate that the poly(U,C)-stimulated binding of unfractionated seryl-tRNA gives an identical result. The binding assay would appear to be useful for the detailed investigation of the nature of alteration of the major hormone-responding seryl-tRNA species in avian liver.

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