Codon-specific Serine Transfer Ribonucleic Acid Synthesis in Avian Liver during Vitellogenin Induction *

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The relative rates of synthesis of two major tRNA^{Ser} species in rooster liver were simultaneously assessed during induction by estradiol-17β of the synthesis of a serine-rich phosphoprotein, vitellogenin. The relative rates of tRNA synthesis were determined by a double-label method in which nonspecific effects of the hormone were avoided. Isotope ratios of highly purified tRNA^{Ser} species were measured following an *in vivo* labeling procedure which included a 7-day labeling period with [5-³H]orotic acid prior to, and a 6 h labeling with [6-¹⁴C]orotic acid from 42 h after the hormone injection. tRNA^{Ser} (AGU,C) and tRNA^{Ser} (UCU,C,A) were extensively purified by chromatography on benzoylated DEAE-cellulose in the presence and absence of Mg²⁺. In three separate labeling experiments the rate of tRNA^{Ser}(UCU,C,A) synthesis was slightly but not significantly increased relative to the rate of tRNA^{Ser}(AGU,C) synthesis during the period when vitellogenin was synthesized at a constant rate and the level of tRNA^{Ser} continued to rise. The results suggest that mechanisms other than a differential rate of transcription are involved in the regulation of tRNA^{Ser} levels in avian liver during vitellogenin induction.

Induction of the hepatic synthesis of vitellogenin, a serine-rich yolk phosphoprotein precursor, in adult rooster is accompanied by a 25 to 50 % increase in the serine acceptance of unfractionated liver tRNA.[1] Similar estrogen-induced changes in serine acceptance are seen when liver tRNA from laying hens and immature chicks is compared.[2,3] Chromatographic studies on the four tRNA^{Ser} species from rooster liver have revealed major quantitative

changes in two species of tRNA^{Ser}. These changes accompany hormonally induced vitellogenin synthesis.[4] A shift in the subcellular distribution of hepatic tRNA^{Ser} species has also been demonstrated.[1] Of the four isoacceptors, the amount of tRNA^{Ser}(AGU,C) is specifically increased in membrane-bound polyribosomes, while tRNA^{Ser}(UCU,C,A) becomes preferentially a non-ribosomal tRNA^{Ser} species.

The present experiments were performed to directly assess the relative synthetic rates of tRNA^{Ser}(AGU,C) and tRNA^{Ser}(UCU,C,A) during hormonally induced vitellogenin synthesis in roosters. The method was an *in vivo* double-labeling procedure with radioactive orotic acid and purification of the tRNA^{Ser} species free of other tRNA species by chromatography on benzoylated DEAE-cellulose in the presence and absence of Mg²⁺.[5] The isotope ratios of the purified tRNA^{Ser} species were found to be almost identical, which suggests that they are synthesized at closely similar rates during vitellogenin synthesis.

EXPERIMENTAL

Animals and chemicals. White Leghorn roosters weighing about 1.5 kg were obtained from a local hatchery and fed *ad libitum*. Estradiol-17β benzoate was dissolved in sesame oil and injected in several portions into leg muscles. Heparinized blood samples were collected from the wing vein and analyzed for alkali-labile protein phosphate as previously described.[4]

[5-³H]Orotic acid (19, 25 and 26 Ci/mmol) and [6-¹⁴C]orotic acid (57 and 59 mCi/mmol) were purchased from The Radiochemical Centre, Amersham. L-[³H]serine (15 Ci/mmol) was from New England Nuclear Chemicals. NCS-

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solubilizer was purchased from Amersham/ Searle and Aquasol from New England Nuclear Chemicals. Estradiol-17β benzoate (estra-1,3,5(10)-triene-3,17β-diol 3-benzoate) was from Nutritional Biochemicals Corp. Ribonuclease T$_1$ (EC 3.1.4.8) was from Sigma Chemical Co. and benzoylated DEAE-cellulose from Schwarz/Mann. Sodium pentobarbital (Nembutal ®) was from Abbott.

Radioactive labeling of the tRNA in vivo. Roosters weighing 1.4 to 1.7 kg were injected intraperitoneally with 2.5 mCi of [5-^{3}H]orotic acid in 0.9 % NaCl. Exactly 7 days after the injection, the animals received intramuscularly 20 mg of estradiol benzoate in sesame oil. A pulse of [6-^{14}C]orotic acid (0.5 mCi) in 0.9 % NaCl was given intraperitoneally at 42 h after the hormone injection. At 48 h, the roosters were anesthetized with 60 mg/kg of sodium pentobarbital, a blood sample was collected from the vein, and liver tRNA was prepared as described below.

Preparation of tRNA. Transfer RNA was prepared from the livers using a modification of a previously published method.[4] Animals were decapitated, bled and the livers were quickly removed, weighed, and chilled in cold saline. Three volumes of cold buffer solution (0.25 M sucrose, 50 mM Tris-HCl (pH 7.5), 25 mM NaCl, 5 mM MgCl$_2$, and 0.1 mg/ml of sodium heparin) were added and the tissue minced with scissors. The tissue was homogenized in a glass-Teflon homogenizer with 15 strokes using a tight pestle. After centrifugation at 12 500 g_{max} for 10 min the lipid layer was removed from the top of the tube, and the supernatant decanted into another vessel and subsequently centrifuged at 106 500 g_{av} for 60 min. The supernatant was decanted and shaken with an equal volume of water-saturated phenol at 4 °C for 60 min. The phases were separated by a low-speed centrifugation, the phenol phase re-extracted with homogenization buffer and the aqueous layer with fresh phenol at 4 °C for 30 min. The RNA was precipitated from the combined aqueous phase overnight at −20 °C by adding 0.1 volume of 2 M potassium acetate (pH 5) and 2.5 volumes of 99.5 % ethanol. The precipitate was collected by centrifugation, dried, and deacylated in a solution containing 0.1 M Tris-HCl (pH 9) and 10 mM MgCl$_2$ at 37 °C for 90 min.[6] RNA was precipitated overnight as described above. tRNA was purified by passing the RNA through a column of Sephadex G-100 (2.5×100 cm) in a buffer containing 0.5 M NaCl, 10 mM MgCl$_2$, and 5 mM sodium acetate (pH 4.5) at room temperature. The absorbancy at 254 nm was monitored and the major A_{254}-absorbing peak corresponding to tRNA was pooled, concentrated by passing through a 0.9×2 cm DEAE cellulose column,[4] precipitated with ethanol, collected by centrifugation, dried, redissolved in 5 mM sodium acetate solution (pH 4.43), and stored at −20 °C. A portion of this material was used

for determination of the internal radioactivities of unfractionated tRNA.

Preparation of aminoacyl-tRNA synthetase. Preparation of synthetases from rooster liver was carried out as previously described.[4] Synthetase preparations from untreated animals were used in all acylation experiments since previous results indicated that the extent of serine acceptance and the chromatographic profile are not influenced by the source of the synthetase.[4]

Acylation of tRNA. The acceptance of radioactive serine by various tRNA preparations was determined in reaction mixtures (0.05 ml) in which the amount of tRNA was limiting.[4] The other components of the acylation reaction mixture were: 0.1 M Tris-HCl (pH 7.4), 10 mM MgCl$_2$, 10 mM KCl, 0.5 mM dithiothreitol, 5 mM ATP (pH 7.4), 1 mM CTP, 0.2 mM EDTA, 0.1 mM 19 nonradioactive amino acids, 0.02 mM [^{3}H]serine, and 0.24 to 0.48 mg/ml of synthetase protein. The duration of the acylation reaction at 37 °C and the amount of the synthetase were varied in each case to obtain maximal acylation. After incubation, [^{3}H]Ser-tRNA was precipitated with cold 10 % trichloroacetic acid, collected on glass fiber filters (Whatman, Type GF/A), dried, and counted in a toluene-based scintillant containing 4 g of 2,5-diphenyloxazole and 50 mg of p-bis[2-(5-phenyloxazolyl)]benzene in 1 liter of toluene.

Preparation of [^{3}H]Ser-tRNA for benzoylated DEAE-cellulose chromatography was performed in 0.5 ml reaction mixtures containing optimal amounts of tRNA and synthetase protein. After incubation at 37 °C, the reaction mixture was chilled in ice and passed through a 0.9×2 cm DEAE-cellulose column.[4] After extensive washing with buffer containing 0.25 M NaCl, 10 mM MgCl$_2$, and 5 mM sodium acetate (pH 4.43), [^{3}H]Ser-tRNA was eluted by raising the salt concentration in the buffer to 1.1 M NaCl. Fractions containing [^{3}H]Ser-tRNA were pooled and diluted to a concentration of 0.45 M with respect to NaCl with a solution containing 10 mM MgCl$_2$ and 5 mM sodium acetate (pH 4.43). The preparations were either used immediately for chromatography or stored at −20 °C.

Purification of tRNA^{Ser}(AGU,C) and tRNA^{Ser}(UCU,C,A) by benzoylated DEAE-cellulose chromatography in the presence and absence of Mg^{2+}. Double-labeled, unfractionated liver tRNA was chromatographed in 0.9×18 cm columns of benzoylated DEAE-cellulose in the presence of Mg^{2+}.[4] After adsorption of the sample, the column was eluted at a rate of 20 ml/h using 400 ml of a linear gradient of 0.6 to 1.1 M NaCl, containing 10 mM MgCl$_2$ and 5 mM sodium acetate (pH 4.43). Fractions (4 ml) were collected at 24 °C and the absorbance at 254 nm was monitored with an LKB Uvicord III absorbance monitor. Elution was continued with 80 ml of a second gradient of 1.1 to 1.5 M

NaCl, containing 10 mM MgCl$_2$ and 5 mM sodium acetate (pH 4.43). The 1.5 M NaCl-buffer also contained 14 % ethanol. Small aliquots of each fraction were mixed with 40 μg of carrier RNA (Yeast RNA, Type XI, Sigma) and RNA was precipitated with 10 % trichloroacetic acid, collected on glass fiber filters and counted for radioactivity as described above. A sample of [^{3}H]Ser-tRNA was chromatographed under identical conditions to facilitate the localization of the individual iso-accepting serine tRNA species. The fractions corresponding to tRNA^{Ser}(AGU,C), tRNA^{Ser}(UCU,C,A), and tRNA$_4$^{Ser} (which is a minor tRNA^{Ser} species eluting in the ethanol-NaCl gradient [7]) were separately pooled, concentrated in small DEAE-cellulose columns and precipitated with ethanol.

The two major tRNA^{Ser} species were further purified on benzoylated DEAE-cellulose in the absence of Mg^{2+}. Prior to chromatography, tRNA^{Ser}(AGU,C) and tRNA^{Ser}(UCU,C,A) were separately acylated with nonradioactive serine in the absence of other amino acids. A column of benzoylated DEAE-cellulose (0.9 $\times$ 18 cm) was eluted with 400 ml of a linear gradient of 0.2 to 2.0 M NaCl, containing 10 mM sodium acetate (pH 4.5), and 1 mM mercaptoethanol.[5] Fractions (4 ml) were collected at 4 °C at a rate of 20 ml/h. After 240 ml of the eluate had been collected, ethanol was added to the 2 M gradient solution to a final concentration of 7 %. An aliquot of each fraction was precipitated with 10 % trichloroacetic acid and counted for radioactivity as described above. Under these conditions Ser-tRNA elutes in the ethanol-gradient while most other tRNAs elute before ethanol.[5] Both tRNA^{Ser} species from the previous chromatography were purified separately according to this scheme, pooled, concentrated in small DEAE-cellulose columns, and the total absorbancy at 260 nm as well as the isotope ratio [^{14}C]/[^{3}H] were determined.

The purity of each tRNA^{Ser} was checked by deacylation and reacylation with [^{3}H]serine and a subsequent chromatography on benzoylated DEAE-cellulose in the presence of Mg^{2+}. Twenty-four A_{260} units * of unfractionated, deacylated rooster liver tRNA were added as a carrier. Radioactivity of each fraction was determined by precipitating the fractions with trichloroacetic acid, collecting the precipitates on glass fiber filters and counting for radioactivity as described above.

Isotope ratios of serine tRNAs. [^{14}C]/[^{3}H]-ratios of different tRNA preparations were determined in triplicate. Small aliquots of the samples were mixed with 40 μg of carrier RNA and precipitated with 10 % trichloroacetic

* *Definition.* An A_{260} unit of RNA is defined as that quantity which, when dissolved in 1 ml of distilled water, gives a solution having an absorbance of 1 at a path length of 1 cm and a wavelength of 260 nm.

acid. The precipitates were collected on glass fiber filters (three filters stacked together), washed with 5 % trichloroacetic acid, and dissolved in 0.5 ml of NCS-solubilizer by incubating overnight in closed vials at 37 °C. After addition of 10 ml of toluene scintillant the radioactivities were determined in a Wallac-LKB liquid scintillation spectrometer with an efficiency of 32 % for [^{3}H] and 68 % for [^{14}C]. Appropriate corrections were made for [^{3}H]- and [^{14}C]-radioactivity appearing in the other channel.

Partial RNase T$_1$ digest of tRNA^{Ser}(AGU,C). Fractions from benzoylated DEAE-cellulose chromatography of double-labeled liver tRNA corresponding to tRNA^{Ser}(AGU,C) (cf. Fig. 1) were pooled and precipitated with ethanol. The precipitate was collected by centrifugation, dried, and dissolved in distilled water. The sample was adjusted to 15 mM Tris-HCl (pH 7.8), 15 mM EDTA, and 1000 units of RNase T$_1$ were added. The mixture was incubated at 37 °C for 90 min, and the reaction was terminated by addition of an equal volume of cold, water-saturated phenol. After shaking at 24 °C for 10 min, the phases were separated by a low-speed centrifugation. The aqueous phase was re-extracted with fresh phenol and the combined phenol phases were re-extracted three times with water. Traces of phenol were removed from the combined aqueous phase with ether. After addition of solid urea to a final concentration of 7 M, the digest was applied to a DEAE-cellulose column (0.9 $\times$ 18 cm) and eluted with 400 ml of a linear gradient of 0 to 0.3 M NaCl, containing 20 mM sodium acetate (pH 6) and 7 M urea.[5] Fractions (1.6 ml) were collected directly into scintillation vials and the radioactivity determined after addition of 20 ml of Aquasol. The absorbancy of the eluate was continuously monitored at 254 nm with an ISCO UA-5 absorbance monitor.

RESULTS

Estrogen responsiveness of the roosters. The estrogen responsiveness was evaluated on the basis of protein-bound phosphate in plasma, liver weight and appearance, and liver polyribosome profiles. Forty-eight hours after the hormone injection plasma phosphoprotein levels were 3.1 mmol/l (Exp. 1), 2.5 mmol/l (Exp. 2) and 1.6 mmol/l (Exp. 3) When compared to control animals the liver weight increased about 2.8-fold and the color changed from red-brown to yellow. The polysome profiles analyzed by sucrose gradient centrifugation [8] indicated a shift towards larger size polyribosomes (not shown).

Purification of tRNA^Ser^(AGU,C) and tRNA^Ser^(UCU,C,A). Four distinct peaks are observed when rooster liver [^{3}H]Ser-tRNA is chromatographed on benzoylated DEAE-cellulose in the presence of Mg^{2+}.[4] Three peaks are eluted during the NaCl gradient and the fourth after addition of an ethanol-NaCl gradient to the column. Binding studies with *E. coli* ribosomes and the six trinucleotide codons assigned to serine have indicated that the first [^{3}H]Ser-tRNA species binds with AGU and AGC, the second with UCG, the third with UCU, UCC and UCA, whereas the fourth peak does not bind well with any of the six serine codons.[7] This species can be phosphorylated to phosphoseryl-tRNA^Ser^ by an enzyme present in the cytoplasm.[7,9]

The assessment of the relative synthetic rates of individual tRNA^Ser^ species is dependent on the purity of the tRNA^Ser^ preparations. Liver tRNA was prelabeled with [5-^{3}H]orotic acid and [6-^{14}C]orotic acid and purified through the Sephadex G-100 step. It was subsequently chromatographed on benzoylated DEAE-cellulose (Fig. 1) in the presence of Mg^{2+} and the fractions corresponding to tRNA^Ser^(AGU,C), tRNA^Ser^(UCU,C,A), and tRNA$_4$^Ser^ were separately pooled, desalted and concentrated in small DEAE-cellulose columns. A sample of [^{3}H]Ser-tRNA was chromatographed under identical conditions to identify the individual tRNA^Ser^ species. The two major tRNA^Ser^ species were acylated with nonradioactive serine and further purified on benzoylated DEAE-cellulose in the absence of Mg^{2+} (Fig. 2). Under these conditions serine tRNAs are strongly attached to the ion exchange material and can only be eluted with an ethanol-NaCl gradient.[5] Most other tRNAs are eluted with the NaCl gradient alone. The radioactive peaks eluting in the ethanol-NaCl gradients (Fig. 2A and B) were pooled, concentrated and deacylated at pH 9. After acylation with [^{3}H]serine, the purity of a sample of each tRNA^Ser^ was assessed by rechromatography on benzoylated DEAE-cellulose in the presence of Mg^{2+} (Fig. 3). [^{3}H]Ser-tRNA^Ser^(AGU,C) was eluted entirely in its previous position (see Fig. 1) and was free of any other tRNA^Ser^ species. [^{3}H]Ser-tRNA^Ser^(UCU,C,A) contained less than 7 % of tRNA^Ser^(AGU,C) as a contaminant. A part
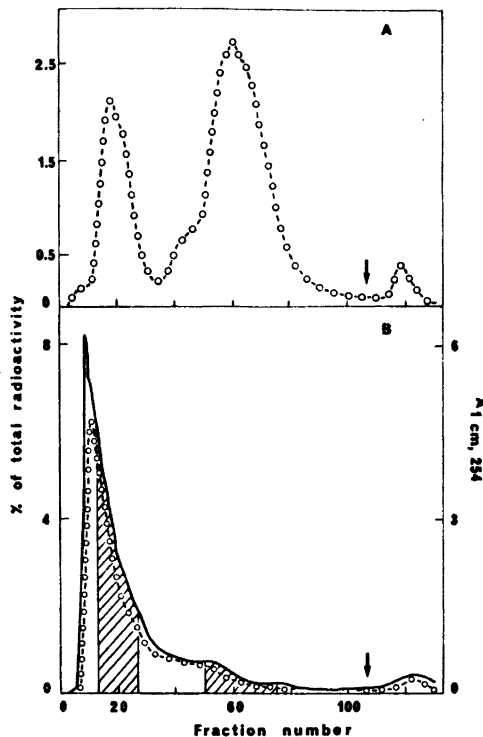

Fig. 1. Chromatography of double-labeled rooster liver tRNA on benzoylated DEAE-cellulose in the presence of Mg^{2+}. 320 A_{260} units of unacylated liver tRNA prelabeled with [5-^{3}H]orotic acid and [6-^{14}C]orotic acid were chromatographed on benzoylated DEAE-cellulose as described in Experimental. (A) Chromatography of marker [^{3}H]Ser-tRNA. A similar column and identical conditions were used as in B. (O) [^{3}H]serine radioactivity. The ethanol gradient was started at arrow. (B) Chromatography of double-labeled tRNA. Shaded areas represent fractions which were pooled for further purification of tRNA^Ser^(AGU,C) and tRNA^Ser^(UCU,C,A). (—) Absorbance at 254 nm; (O) [^{3}H] plus [^{14}C]-radioactivity in 0.15 ml aliquots of each fraction.

of the peak was retained in the ethanol-NaCl gradient in the presence of Mg^{2+} for reasons that are unclear but may relate to an irreversible conformation change in the absence of Mg^{2+}. Measurements at 254 nm indicated that the yields of tRNA^Ser^(AGU,C) and tRNA^Ser^(UCU,C,A) were 2.7 and 3.9 % (Exp. 1), 1.6 and 2.2 % (Exp. 2) and 1.6 and 2.4 % (Exp. 3) of unfractionated tRNA, which was
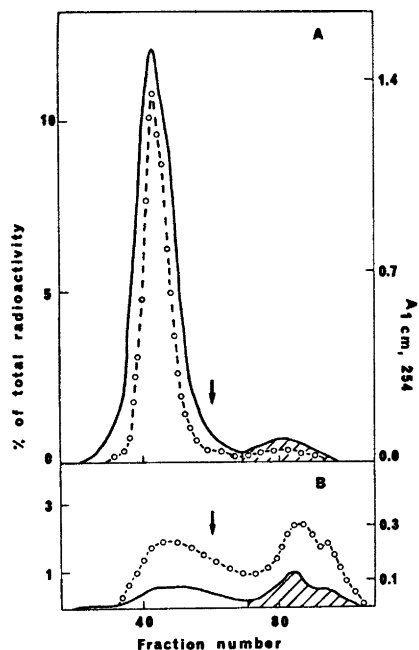
Fig. 2. Further purification of tRNA^{Ser}-(AGU,C) and tRNA^{Ser}(UCU,C,A) on benzoylated DEAE-cellulose chromatography in the absence of Mg^{2+}. (A) Pooled, desalted and concentrated tRNA^{Ser}(AGU,C) fraction from Fig. 1B was acylated with nonradioactive serine and chromatographed on benzoylated DEAE-cellulose in the absence of Mg^{2+} as described in Experimental. A 0.3 ml aliquot of each fraction was precipitated and counted for radioactivity. The hatched area in the ethanol gradient was pooled, desalted and concentrated. (−) Absorbance at 254 nm; (O) [³H]-plus [¹⁴C]-radioactivity. The arrow indicates ethanol addition. (B) A similar chromatogram of tRNA^{Ser}(UCU,C,A) derived from the second tRNA pool in Fig. 1B.

Fig. 3. Rechromatography of purified [³H]Ser-tRNA^{Ser}(AGU,C) and [³H]Ser-tRNA^{Ser}-(UCU,C,A) on benzoylated DEAE-cellulose in the presence of Mg^{2+}. (A) 1.4 A_{260} units from the shaded area of the tRNA in Fig. 2A were deacylated, reacylated with [³H]serine, and chromatographed on benzoylated DEAE-cellulose in the presence of Mg^{2+} as in Fig. 1. Twenty-four A_{260} units of unfractionated, deacylated rooster liver tRNA were used as a carrier in the chromatography. The arrow indicates ethanol addition. (O) [³H]serine radioactivity. (B) 2.1 A_{260} units from the shaded area of the tRNA in Fig. 2B were similarly chromatographed and analyzed for radioactivity.

the starting material for tRNA^{Ser} purification. Purity estimations based on [³H]serine acceptance of the highly purified tRNA^{Ser} species were not possible because of interference from the radioactivity of the tRNA and the small amounts of purified tRNA^{Ser}.

RNase T_1 *digest of* tRNA^{Ser}*(AGU,C)*. The RNase T_1 digest of tRNA fractions enriched in tRNA^{Ser}(AGU,C) was studied to ascertain that radioactive labeling with orotic acid had taken place throughout the tRNA molecule and not just at the 3'CCA terminus, which turns over independently of transcription. Double-labeled
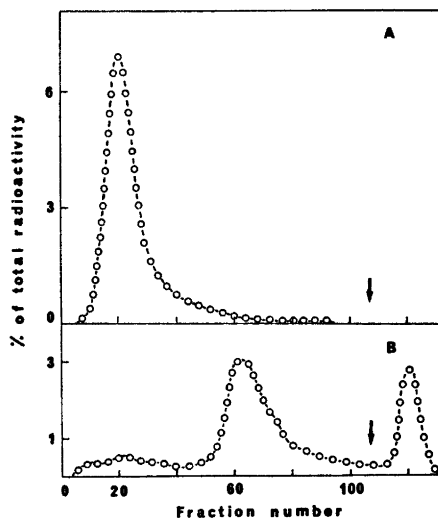
tRNA^{Ser}(AGU,C) was isolated and digested with RNase T_1 as described in Experimental and chromatographed on DEAE-cellulose in the presence of 7 M urea (Fig. 4). The digestion fragments were not completely separated probably due to the presence in the preparation of several other tRNA species in addition to tRNA^{Ser}(AGU,C). Nevertheless, the absorbance and radioactivity profiles indicated that labeling had occurred in various internal positions of tRNA. The [³H]-plus [¹⁴C]-radioactivity profile is shown in Fig. 4 for clarity. Both [³H]- and [¹⁴C]-radioactivities were separately measured and the profiles were essentially identical with the combined radioactivity profile. These results indicate that CCA turnover does not contribute to differences observed in the radioactive labeling pattern of tRNA.
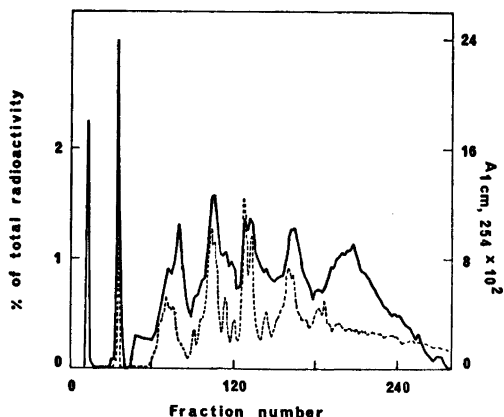
Fig. 4. Chromatography of the RNase T_1 digest of double-labeled tRNA^{Ser}(AGU,C) on DEAE-cellulose. Twenty-eight A_{260} units of tRNA enriched in tRNA^{Ser}(AGU,C) and prelabeled with [5-^{3}H]orotic acid and [6-^{14}C]orotic acid were digested with 1000 units of RNase T_1 and chromatographed on DEAE-cellulose in the presence of 7 M urea as described in Experimental. Fractions were monitored for absorbancy at 254 nm (—) and precipitated and counted for radioactivity (– – –).

Comparison of the relative synthetic rates of tRNA^{Ser} species during vitellogenin synthesis. Table 1 shows the isotope ratios of the two major tRNA^{Ser} species isolated and extensively purified as described above. The ratios of tRNA^{Ser}(AGU,C) and tRNA^{Ser}(UCU,C,A) were very similar in the three labeling experiments. In each case, however, the ratio of tRNA^{Ser}(UCU,C,A) was slightly higher than

the ratio of tRNA^{Ser}(AGU,C), but the difference (6.5 ± 4.1 %, n = 3) was not significant. The isotope ratio of tRNA$_4$^{Ser} was also similar, but not quite identical with the ratio of tRNA^{Ser}(AGU,C) (1.039, 1.105 and 1.091 in experiments 1, 2 and 3). The difference (7.8 ± 2.0 %, n = 3) resembled that of tRNA^{Ser}(UCU,C,A). tRNA$_4$^{Ser}, although purified through benzoylated DEAE-cellulose chromatography, is not as pure as the other tRNA^{Ser} species.[10]

DISCUSSION

Previous studies on quantitative alterations in tRNA^{Ser} levels during hormonally induced vitellogenin synthesis in avian liver have suggested that the amounts of the two major tRNA^{Ser} species recognizing the code triplets AGU,AGC and UCU,UCC,UCA may be independently regulated.[1] This suggestion has been based on the finding that [^{3}H]serine acceptance of unfractionated liver tRNA is increased up to 50 % during vitellogenin induction and that there is a concomitant shift in the chromatographic profile and the intracellular distribution of the hepatic tRNA^{Ser} species.[1,4] Of the four isoacceptors, the amount of tRNA^{Ser}(AGU,C) is specifically increased in membrane-bound polyribosomes while tRNA^{Ser}(UCU,C,A) is found preferentially as a non-ribosomal tRNA^{Ser}.[1,4] However, evidence against a specific increase in tRNA^{Ser} synthesis relative to total tRNA synthesis has been obtained by determination of the rate of unfractionated tRNA^{Ser} synthesis in estrogenized

Table 1. Relative synthetic rates of purified tRNA^{Ser} species during vitellogenin induction. The labeling procedure included a 7-day labeling period with [5-^{3}H]orotic acid prior to, and a 6-h labeling with [6-^{14}C]orotic acid from 42 to 48 h after estrogen injection. The tRNA^{Ser} species were purified by chromatography on benzoylated DEAE-cellulose as shown in Figs. 1 and 2. The isotope ratios were determined as described in Experimental.

Experiment	tRNA radioactivity (dpm) ^{14}C	^{3}H	Isotope ratio ^{14}C/^{3}H	Relative ratio [a] ^{14}C/^{3}H
(1) tRNA^{Ser}(AGU,C)	1330	2260	0.5885	1.000
tRNA^{Ser}(UCU,C,A)	2950	4780	0.6172	1.049
(2) tRNA^{Ser}(AGU,C)	850	2440	0.3484	1.000
tRNA^{Ser}(UCU,C,A)	4670	13360	0.3496	1.003
(3) tRNA^{Ser}(AGU,C)	1561	5979	0.2611	1.000
tRNA^{Ser}(UCU,C,A)	1634	5478	0.2983	1.142

[a] Relative ratio is the isotope ratio of tRNA^{Ser}(UCU,C,A) relative to the isotope ratio of tRNA^{Ser}(AGU,C).

chicks during the period of vitellogenin induction where the serine acceptance of tRNA is increased and the amount of total tRNA is almost doubled.[3] In the present experiments the synthetic rates of tRNA^Ser(AGU,C) and tRNA^Ser(UCU,C,A) were separately determined during vitellogenin induction using a radioactive double-label method and a newly developed purification method for tRNA^Ser isoacceptors.[5]

The assessment of the estrogen responsiveness (plasma phosphoprotein, alterations in liver weight and appearance, shift in liver polyribosome profiles) indicated that the animals used in evaluation of the synthetic rates of tRNA^Ser(AGU,C) and tRNA^Ser(UCU,C,A) responded to estrogen to the same extent as the birds in previous work.[1,8]

The assessment of the relative synthetic rates of individual tRNA^Ser species is dependent on the purity of the tRNA^Ser preparations. The chromatographic method with benzoylated DEAE-cellulose offers a relatively rapid two-step procedure for purification of rooster liver tRNA^Ser species. Rogg et al.[5] have used this method for purification of rat liver tRNA^Ser(AGU,C) for sequence studies and noted that the method can be used for complete purification of all rat liver tRNA^Ser species. A purity of more than 90 % for tRNA^Ser(AGU,C) was reported.[5] We found that this method can be used for purification of rooster liver tRNA^Ser(AGU,C) and also for purification of the other major tRNA^Ser species recognizing the code triplets UCU, UCC and UCA. Purity determinations on the basis of [3H]serine acceptance were not possible here because of the interfering radioactivity of the tRNA and the small amounts of tRNA^Ser obtained. We found, however, that when unfractionated rooster liver tRNA is chromatographed on benzoylated DEAE-cellulose in the absence of Mg^{2+}, about 6 % of the tRNA elutes as a distinct peak in the ethanol-NaCl gradient. This amount corresponds reasonably well with the yields of highly purified tRNA^Ser species (total amounts 6.6, 3.8 and 4.0 %). tRNA^Ser(AGU,C) was found to be free of other tRNA^Ser species by rechromatography on benzoylated DEAE-cellulose in the presence of Mg^{2+}, whereas tRNA^Ser(UCU,C,A) contained less than 7 % of tRNA^Ser(AGU,C) as a

contaminant. In separate experiments, we have studied the elution characteristics of radioactive Ala-, His-, Leu-, Lys-, Met-, Pro-, and Tyr-tRNAs on benzoylated DEAE-cellulose in the presence and absence of Mg^{2+} and found that, with the exception of a minor Leu-tRNA species, no other aminoacyl-tRNA elutes in the ethanol-NaCl gradient in the absence of Mg^{2+} (unpublished). The minor Leu-tRNA species partly overlaps with tRNA^Ser(UCU,C,A) on chromatography in the presence of Mg^{2+} and causes a contamination of less than 2 % in purified tRNA^Ser(UCU,C,A). Taken together, these results indicate that tRNA^Ser(AGU,C) and tRNA^Ser(UCU,C,A) are more than 90 % pure.

The half-life of total tRNA in mammalian tissues varies between 72 and 144 h.[11-15] The labeling period used in this study provided an internal label of [3H] which gives a reference value for estimating the synthetic rates of tRNA^Ser after estrogen treatment. The 6-hour labeling with [14C]orotic acid was long relative to the half-life of pre-tRNA, 5 to 15 min,[16,17] thus minimizing the effect of pre-tRNA maturation. [14C]label was given during the period when the level of vitellogenin in plasma continued to rise and the level of tRNA^Ser increased.[1,4] A possibility of an error due to an estrogen-induced change in the extent of modification of pyrimidine nucleosides at the 5 position such as methylation and pseudouridine formation, which could alter the amount of radioactive label in bases derived from orotic acid, was bypassed by using [6-14C]orotic acid after the hormone injection. [14C]label would not be expected to be lost even if there is a change in the extent of tRNA modification. Single animals were used for the double-labeling procedure to minimize differences in the labeling kinetics due to a possible individual variation in label distribution and metabolism. Also, alterations in radioactive nucleotide pool sizes after estrogen treatment would not have an effect, since labeling of the tRNA^Ser species occurs from the same nucleotide pool.

A concept of functional adaptation of tRNA implies that the amount of each tRNA species is separately controlled in a manner that is dependent upon its frequency of use in translation.[18] In the posterior silkgland of Bombyx mori L. the adaptation seems to occur at the level

of transcription or pre-tRNA maturation.[13] Klyde and Bernfield have, however, found that the synthetic rate of unfractionated tRNA^{Ser} in avian liver is not specifically increased during vitellogenin synthesis.[3] The concentration of tRNA^{Ser} remains elevated even at 7 days after estrogen injection[4] making it unlikely that the elevated tRNA^{Ser} content could be[*] a result of a brief increase in tRNA^{Ser} synthetic rate which occurs prior to 42 h. The quantitative increase in tRNA^{Ser} has been seen during vitellogenin synthesis regardless of acylation conditions, enzyme source, and age or sex of the animals.[1,2,4] The selective change in two major tRNA^{Ser} has been documented by chromatography[1,4] and confirmed by ribosomal binding[19], which are procedures dependent on prior serine acylation. The proportion of tRNA which is aminoacylated has been found to be unaltered after estrogen treatment.[20]

Other explanations for elevated levels of tRNA^{Ser} during vitellogenin synthesis include alterations in precursor processing, transport or storage, or in tRNA modification, degradation or other steps. tRNA^{Ser} modification and degradation or both are, in the absence of a specific change in tRNA^{Ser} synthesis, the most likely mechanisms by which the observed changes in tRNA^{Ser} levels can be explained.[3] An additional consideration may arise from the altered intracellular distribution of individual tRNA^{Ser} species during vitellogenin synthesis, since tRNA^{Ser}(AGU,C) becomes largely bound to membrane-bound polyribosomes, while tRNA^{Ser}(UCU,C,A) is predominantly non-ribosomal.[1] The difference in intracellular localization (and probably function) may be a factor determining the susceptibility of the individual tRNA^{Ser} species toward degradation and thus indirectly lead to changes in the levels of tRNA^{Ser} species.

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