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SHORT COMMUNICATION

RIBOSOMAL RIBONUCLEASES IN AVIAN LIVER DURING ESTROGEN-INDUCED VITELLOGENIN SYNTHESIS

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Summary—The ribonuclease activity associated with rooster liver ribosomes decreases and the endogenous ribosomal RNAase inhibitor becomes undetectable during estrogen-induced vitellogenin synthesis. The RNAase-catalyzed autodegradation of ribosomes *in vitro* is inhibited by Mg^{2+} and spermidine, while EDTA in low concentrations has an activating effect. Single-stranded, uridylic acid containing ribonucleotide polymers are the best substrates for the enzyme. Of the four homopolyribonucleotides, ribosomal RNAase catalyzes the degradation of poly(U), poly(A), and poly(C) in decreasing order of reactivity, while poly(G) is not degraded. Ribosomal RNAases from control and estrogen-stimulated roosters show differences in response to Mg^{2+}, spermidine and EDTA. In addition, the reactivities of poly(U) and poly(C) as substrates using RNAases from stimulated roosters are markedly different from those obtained with the enzymes from control roosters.

INTRODUCTION

Alterations in RNAase activities are known to be associated with changes of protein synthesis [1–4] and to be controlled by the binding of a specific RNAase inhibitor protein, which can be inactivated *in vitro* using a sulfhydryl reagent (e.g. *p*CMB) [1, 3, 5–7]. The ratio of the inhibitor to RNAase varies according to the RNA and protein synthetic activity of the cell [2, 3, 6–8]. Polyribosome stability in different growth and physiological states is affected by changes in ribosomal RNAase activity [1, 3, 4], which depends at least in part on the amount of the inhibitor [2, 3, 7].

Synthesis of an egg-yolk protein precursor, vitellogenin, can be induced in livers of immature and male birds by administration of estrogens [9]. Large amounts of vitellogenin mRNA appear in the cytoplasm [8], and the synthesis of ribosomal proteins and rRNA increases [9, 10]. A rapid increase in the quantity of ribosomes and a shift in the polyribosome pattern towards larger polysomes take place coordinately with the synthesis of vitellogenin [9, 10]. In this work, changes in the activity and properties of ribosomal RNAase and its inhibitor were studied during vitellogenin induction in the rooster liver.

EXPERIMENTAL

Three to four month old white Leghorn roosters fed *ad libitum* on a standard diet were used. β—Estradiol-3-benzoate (ICN Pharmaceuticals, U.S.A.) dissolved in warm sesame oil (20 mg/2 ml) was injected by dividing the dose to muscles of both legs. Control animals were not treated.

Total tissue ribosomes were isolated according to the Mg^{2+}-precipitation method [11] without using heparin and diethyl pyrocarbonate in solutions. The final ribosome suspensions in 20 mM Hepes (pH 7.5) were frozen in liquid nitrogen and stored at −80°C.

Assay of ribonuclease activity

The 1.0 ml-incubation mixture contained 100 μl of 1 M Tris–HCl (pH 7.8), and an appropriate volume of 8 mM *p*CMB, 10 mM spermidine (both from Fluka AG), 100 mM $MgCl_2$, or 5 mM EDTA to yield the final concentration and 25 A_{260}-units of ribosome suspension. The incubation was at 37°C. At 0, 15, 30, 60, 120, and 180 min, 100 μl was transferred to 250 μl of precipitation solution (ice-cold 20 mM $La(NO_3)_3$ in 12% (w/v) $HClO_4$). After 10 min in ice and centrifugation in Beckman Microfuge B for 3 min at 4°C, 200 μl of the supernatant was mixed with 800 μl of water and the absorbance of the soluble nucleotides was measured at 260 nm.

Synthetic polyribonucleotides were used as exogenous substrates in addition to RNA present in ribosomes. The 0.5 ml-reaction mixture contained 50 μl of 1 M Tris–HCl, 20 μl of 5 mg/ml polyribonucleotide (Boehringer), and 50 μl of 8 mM *p*CMB when indicated. To gain an equal degradation rate of endogenous RNA, different amounts of ribosomes, depending on their origin, were added to start the reaction at 37°C. 75 μl was transferred to 200 μl of precipitation solution at 0, 20, 40, and 60 min (see above).

The RNAase activity was determined as a change of absorbance at 260 nm in 1 h (ΔA_{260}/h), obtained from the linear part of the curve A_{260} vs time. The Mann–Whitney U-test was used for calculation of significances.

RESULTS AND DISCUSSION

The fall of RNAase activity is a phenomenon commonly observed in tissues turning to more active protein synthesis. The RNAase activity of rooster liver ribosomes fell to its minimum 2 days after stimulation of vitellogenin synthesis by estrogen, at a time when the ribosome concentration and the proportion of ribosomes in polysomes have earlier been

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Abbreviations: An A_{260} unit of ribosomes is defined as that quantity which, when dissolved in 1 ml of water, gives a solution having an absorbance of 1 at a path length of 1 cm and a wavelength of 260 nm; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; *p*CMB, *p*-chloromercuribenzoic acid.

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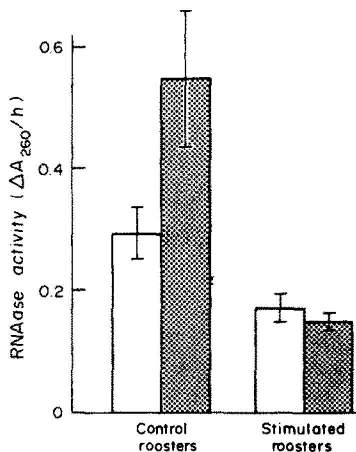

Fig. 1. Free and total RNAase activity of liver ribosomes isolated from control and estrogen-stimulated roosters. The incubation of 25 A_{260}-units of ribosomes and the determination of liberated nucleotides are described in the Experimental section. The column and the bar represent the mean ± SD for separate experiments with ribosomes from 5 control and 4 stimulated roosters. Open column indicates free RNAase activity and shaded column total RNAase activity determined in the presence of 0.8 mM *p*CMB.

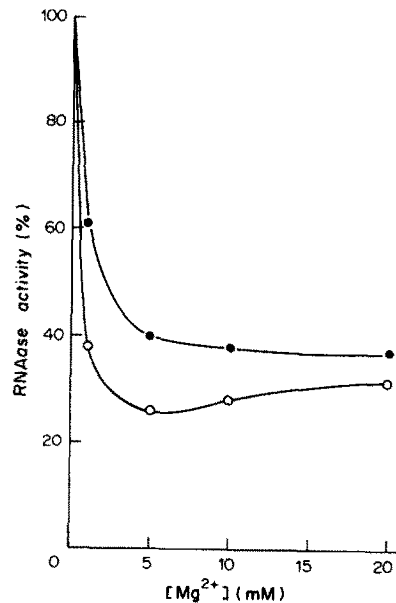

Fig. 2. Effect of Mg^{2+} on free RNAase activity of liver ribosomes from control and stimulated roosters. 25 A_{260}-units of ribosomes were incubated in different $MgCl_2$ concentrations, and the activity was determined as described in the Experimental section. Each point represents the mean of three experiments with ribosomes from separate roosters (SD's less than 3.4%). ○, control roosters; ●, stimulated roosters.

reported to be at their highest [9, 10]. The RNAase activity then was about half ($P < 0.01$) of the activity observed in the ribosomes from control roosters (Fig. 1). The amount of ribosomes calculated per wet weight of the liver increased by 17% ($P < 0.01$) during the same period.

With 0.8 mM *p*CMB, the activity in control ribosomes was nearly twice the activity without *p*CMB ($P < 0.005$), while the activity in ribosomes from stimulated roosters remained virtually unchanged with *p*CMB addition. Therefore, the decrease of RNAase activity is apparently not caused by an increased concentration of the endogenous ribosomal RNAase inhibitor protein. A disappearance of the RNAase inhibitor has also been observed after estrogen stimulation in the uterus of immature rats, accompanied by increased RNAase activity [12]. In tissues of the adult rat [2, 13, 14] and in rooster liver [7], however, an increased amount of the inhibitor has been found to result in a decrease of the enzyme activity in response to the elevated levels of estrogen. The present results together with earlier observations [7, 9, 10] suggest, that the stability of ribosomes and polyribosomes in the rooster liver is at least in part controlled by ribosomal RNAase activity.

The stabilizing effect of Mg^{2+} and spermidine, and the destructive effect of EDTA on the ribosome structure [15] are reflected in the effects of these substances on the ribosomal RNAase activity. Especially in low Mg^{2+} concentrations, the RNAase activity of control rooster ribosomes was inhibited relatively more than with ribosomes from stimulated roosters (Fig. 2). The maximum activation of the autodegradation rate of ribosomes by EDTA was achieved at the concentration of 1.0 mM. However, the activity of control ribosomes was increased by 100% while only a 40% increase was seen with ribosomes from stimulated roosters. Increasing the EDTA concentration above 1.0 mM caused a rapid fall of the activity of ribosomes from stimulated roosters to about 25% of the initial activity remaining at 1.5 mM EDTA. In contrast, the activity of ribosomes from control roosters was still above the initial value at 1.5 mM EDTA.

In addition to the dissimilarities in the responses to Mg^{2+} and EDTA between ribosomes from control and stimulated roosters, the RNAase from stimulated roosters was inhibited almost equally with 1 mM spermidine (63 ± 1.6% of the activity left, $n = 3$) and 1 mM Mg^{2+} (Fig. 2), while with control rooster ribosomes the inhibition with 1 mM Mg^{2+} was more effective than with 1 mM spermidine (54 ± 0.8%,

Table 1. Relative reactivities of polyribonucleotides catalyzed by ribonucleases associated with ribosomes from control and stimulated roosters

	Relative reactivity when catalyzed by RNAse from		
Substrate	Control roosters	Control roosters with 0.8 mM *p*CMB	Stimulated roosters
Poly(C)	0.60 ± 0.14 (5)	0.33 ± 0.08 (4)	0.25 ± 0.05 (4)
Poly(A)	1.44 ± 0.12 (4)	1.03 ± 0.11 (4)	1.43 ± 0.35 (4)
Poly(U)	1.52 ± 0.22 (5)	2.95 ± 0.38 (4)	3.31 ± 0.92 (4)
Poly(C, U)	2.12 ± 0.08 (3)	1.90 ± 0.23 (3)	2.70 ± 1.10 (3)
Poly(A, U)	2.84 ± 0.70 (4)	3.28 ± 0.40 (4)	3.25 ± 0.65 (4)

Each value was obtained by subtracting the blank (degradation of endogenous RNA) from the total absorbance of liberated nucleotides, and by relating the net degradation rate of the exogenous substrate to the amount of RNAase activity used. The values are means ± SD and the number of experiments with ribosomes from separate roosters is given in parenthesis.

$n = 3$). A possible explanation for these differences is a different location of the enzyme in ribosomes of control and stimulated roosters or different properties of the RNAases.

Ribosomal RNAase catalyzed the degradation of poly(U), poly(A), and poly(C) in a decreasing order of reactivity (Table 1), while poly(G) inhibited the degradation of endogenous RNA. Uridylic acid containing copolymers poly(C,U) and poly(A,U) were also good substrates. Double-stranded poly(I)·poly(C) was not hydrolyzed by the enzyme.

The relative reactivity of poly(C) obtained with RNAase from stimulated roosters was significantly smaller and that of poly(U) larger ($P < 0.01$) than when catalyzed by the enzyme from control roosters. This implies differences between the properties of the activities present in ribosomes from control and stimulated animals. The relative reactivity of poly(C) obtained with RNAase from control roosters was smaller in the presence of *p*CMB ($P < 0.01$). This indicates that the latent activity is similar to that present during stimulated protein synthesis. Alterations in RNAase activity are accompanied in yeast by changes in reactivities of synthetic substrates [16], and Swida *et al.* [4] have demonstrated that the relative proportions of two different RNAases change depending on the growth phase of yeast.

These results suggest that there are at least two different RNAases associated with rooster liver ribosomes. In addition to decreasing the total ribosomal RNAase activity, the stimulation of vitellogenin synthesis with estrogen causes a qualitative alteration in ribosomal RNAases. The role of the endogenous RNAase inhibitor in this system remains to be determined.

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