PREFERENTIAL DEGRADATION OF NEWLY SYNTHESIZED RIBOSOMAL PROTEINS IN RAT LIVER TREATED WITH A LOW DOSE OF ACTINOMYCIN D

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SUMMARY: The administration of a low dose of actinomycin D to partially hepatectomized rats, which selectively inhibited rRNA synthesis, caused the preferential degradation of newly synthesized ribosomal proteins in regenerating rat liver with an apparent half-life of about 20 to 40 min.

Our previous experiments (1) showed that when rRNA synthesis was selectively inhibited in regenerating rat liver by the administration of a low dose of actinomycin D to partially hepatectomized rats, the radioactivity of ribosomal proteins extracted from the total liver homogenate labeled *in vivo* for 1 h with isotopic amino acids was selectively decreased even at 5 min after actinomycin D treatment. We indicated the following possibilities for the decrease: (A) the inhibition of synthesis of ribosomal proteins by actinomycin D treatment and/or (B) the rapid degradation of newly synthesized ribosomal proteins in the livers of actinomycin D-treated rats. The results of our subsequent experiments supported the former possibility, since actinomycin D treatment resulted in the decrease of *in vivo* incorporation of labeled leucine into the newly synthesized ribosomal proteins on liver ribosomes (1) as well as the decrease in its incorporation into ribosomal proteins by the postmitochondrial supernatant from regenerating rat liver (2). However, the finding that the extent of the decrease observed in these experiments was rather

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low, especially in the case of the cell-free system, suggested the second possibility (B) described above. To test the possibility the experiments reported here were carried out.

MATERIALS AND METHODS: L-[^{3}H]leucine (46.9 Ci/mmol) was obtained from the Radioactive Centre, Amersham, England. Partially hepatectomized rats 18 h after the operation (1) were injected intraperitoneally with 550 μg of actinomycin D per kg body weight (3) and 1 h later were used for the labeling experiments. As the control partially hepatectomized rats without actinomycin D treatment were used. Purification procedures of ribosomal proteins from the homogenate of rat liver were as described previously (1, 4). Briefly, 10 volumes of acetone were added to the homogenate and the mixture stood at -20° overnight. From the precipitate after low speed centrifugation ribosomal proteins were extracted with acetic acid and the acetic acid-soluble proteins were subjected to CM-cellulose column chromatography. Fraction I, eluted with a linear NaCl gradient from 0.25 to 0.35 M, contained the bulk of ribosomal proteins. Fraction P, eluted by lower concentrations of NaCl contain the bulk of cell sap proteins (4). In some experiments Fraction I was further purified by Sephadex G-200 column chromatography which yielded Component II, more than 85% of which was shown to consist of ribosomal proteins although it also contained newly synthesized histones and traces of cell sap proteins (4). The radioactivity of protein fractions was measured as described previously (1, 4).

RESULTS AND DISCUSSION: For measuring the degradation rate of proteins we used cycloheximide to inhibit protein synthesis after pulse-labeling of rat liver with [^{3}H]leucine. As a preliminary experiment the inhibitory effects of cycloheximide (5 mg per 100 g of body weight) on the incorporation of [^{3}H]leucine into the total protein and ribosomal proteins of regenerating rat liver were examined. As shown in Fig. 1, the administration of cycloheximide resulted in the rapid and complete inhibition of synthesis of the total and ribosomal proteins, although the complete inhibition of synthesis of ribosomal proteins was somewhat delayed. The results indicate that cycloheximide may be used for the measurement of the rate of degradation of ribosomal proteins in regenerating rat liver. Therefore, the following experiments were carried out.

After actinomycin D-treated rats were labeled with [^{3}H]-leucine for 15 min, one group of rats was sacrificed as 0 time

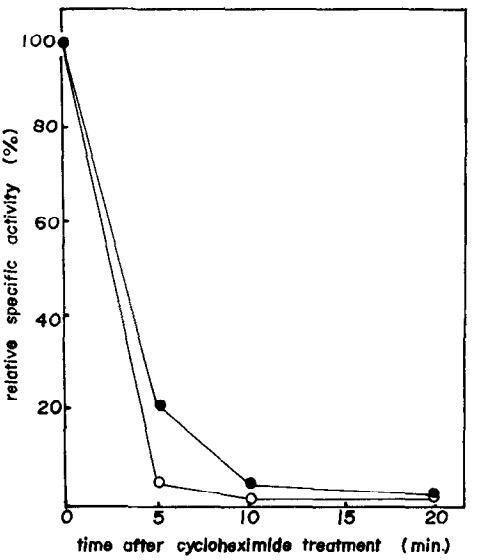

Fig. 1. Effects of cycloheximide treatment on the in vivo labeling of the total and ribosomal proteins of regenerating rat liver. Rats at 18 h after partial hepatectomy received an intraperitoneal injection of 5 mg of cycloheximide per 100 g of body weight and after various periods 10 μCi of [^{3}H]leucine per rat was administered. One hour later rats were sacrified and total ribosomal protein was prepared from EDTA-treated ribosomes and its specific activity was measured as described previously (1). The relative specific activity was expressed as % of the value of rats without cycloheximide treatment. ●———●, total ribosomal protein; o———o, total liver protein.

control and others were treated with cycloheximide. Cycloheximide-treated rats were sacrificed at 40 min and 60 min after the administration of [^{3}H]leucine. From the liver homogenate ribosomal proteins were purified as described previously, finally by Sephadex G-200 gel filtration which yielded Component II.

The specific activities of Component II and the total liver protein at each time point are shown in Fig. 2. While the specific activity of the total protein remained almost constant at least for 40 min after cycloheximide treatement, that of Component II decreased with a half-life of about 20 to 40 min. The actual half-life may be somewhat shorter, since Component II is contami-

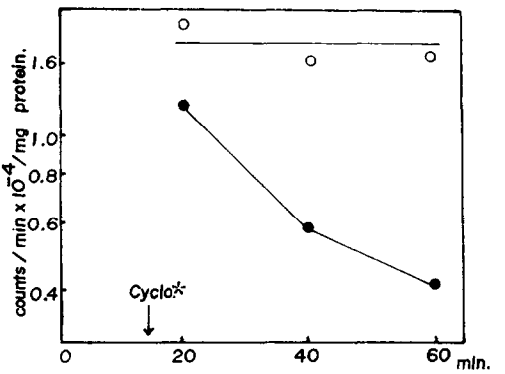

Fig. 2. Time course of specific radioactivities of total and ribosomal proteins of actinomycin D-treated rat liver after administration of cycloheximide. Actinomycin D-pretreated rats received an intraperitoneal injection of 50 µCi of [^{3}H]leucine per 100 g of body weight. After 15 min rats were injected with cycloheximide as described in Fig. 1. At the indicated times rats were sacrificed and Component II was prepared from the livers as described previously (4). The proteins of Component II were precipitated with trichloroacetic acid. The specific activities of the total liver protein and Component II were measured as described previously (4). *cycloheximide; ●———●, ribosomal proteins (component II); o———o total liver protein.

nated with histones and traces of cell sap proteins the degradation of which may not be affected by actinomycin D treatment.

To obtain further evidence for preferential degradation of ribosomal proteins in actinomycin D-treated rat liver, the following double labeling experiments were carried out according to the time schedule shown in Fig. 3a. Partially hepatectomized rats without actinomycin D treatment were labeled with either [^{14}C] or [^{3}H]leucine and sacrificed as 0 time controls. The other group of these rats and rats pretreated with actinomycin D were administered with cycloheximide after labeling with [^{14}C]-leucine for 15 min and then sacrificed at specified time points. The ^{14}C-labeled liver homogenate at each time point was mixed with ^{3}H-labeled liver homogenate from 0 time control rats at the ratio indicated. Fraction P and Fraction I which contained the

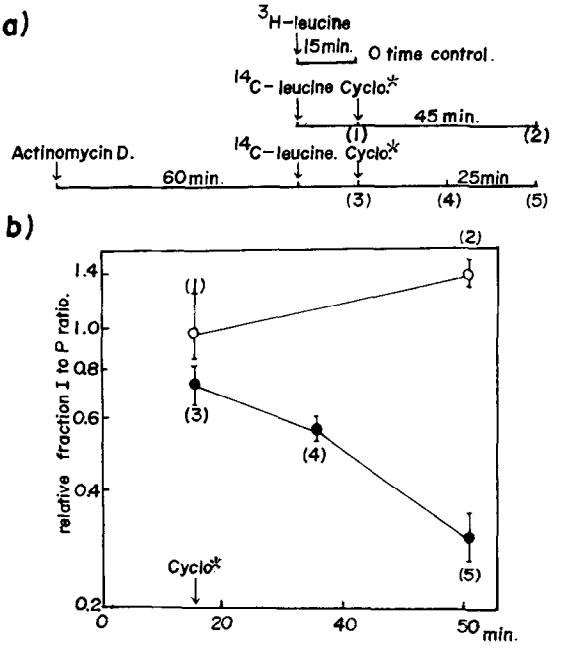

Fig. 3. Time courses of relative radioactivities of ribosomal proteins of rat livers from actinomycin D-treated and control rats after cycloheximide treatment. Actinomycin D-treated (1 h) and control rats received an intraperitoneal injection of 25 μCi of [^{14}C]leucine per rat. After 15 min the rats were injected intraperitoneally with cycloheximide and sacrificed according to the time schedule shown in the upper panel (a). As the 0 time control, two control rats received an intraperitoneal injection of 200 μCi of [^{3}H]leucine and were sacrificed after 15 min. [^{14}C] labeled liver homogenate at each time was combined with 0 time control liver homogenate in the ratio to make ^{3}H to ^{14}C ratio to be 3. Fractions P and I were prepared from the combined homogenate and subjected to one-dimensional acrylamide gel electrophoresis. The ^{14}C to ^{3}H ratios of the individual gel fractions from Fraction I were measured as described previously (1) and the relative Fraction I to P ratio at each time was expressed as described in the text. *cycloheximide; ●———●, actinomycin D-treated rat liver; o———o, control rat liver.

bulk of cell sap proteins and ribosomal proteins, respectively, were prepared from the combined homogenate. Both fractions were then subjected to one-dimensional acrylamide gel electrophoresis at pH 8.6.* ^{14}C to ^{3}H-ratios of individual gel fractions were corrected by multiplying by a suitable factor such that the

* While the bulk of proteins of Fraction P runs to the anode, almost all ribosomal proteins run to the cathode at pH 8.6 (4).

average of the ratios of gel fractions from Fraction P became 1 and designated as corrected ^{14}C to ^{3}H ratio. The average of corrected ^{14}C to ^{3}H ratios of ribosomal proteins on the gel was then determined. This average ratio at each labeling time was further corrected so as to make that of the 0 time control to be 1 and the ratio was designated as the relative Fraction I to P ratio. Since the incorporation of labeled amino acid into cell sap protein was only slightly inhibited by actinomycin D treatment (data not shown) and cell sap proteins were not degraded during such a short time period even in the case of actinomycin D-treated rat liver as indicated in the earlier experiments, the time course of the relative Fraction I to P ratio could show the relative degradation rate of ribosomal proteins.

The results are shown in Fig. 3b. In the case of actinomycin D-treated rats the relative Fraction I to P ratio at 0 time was less than 1, suggesting that the rate of synthesis of ribosomal proteins was inhibited by actinomycin D treatment as described previously (1).

It is noticeable that the relative Fraction I to P ratio decreased almost linearly up to 45 min after cycloheximide treatment preferentially in actinomycin D-treated rat liver, while in the case of control rats this ratio increased slightly at 45 min after cycloheximide treatment probably due to the delayed inhibition of ribosomal protein synthesis by cycloheximide.

From these results it may be concluded that newly synthesized ribosomal proteins in actinomycin D-pretreated rat liver which are unavailable for the assembly of ribosomes owing to the lack of newly synthesized rRNA are rapidly degraded by unknown mechanisms. It must be added that after the completion of this work, Phillips

and McConkey suggested such a degradation of unbound ribosomal proteins in HeLa cells. (5).

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