CHANGES IN INTRACELLULAR RIBONUCLEASE ACTIVITY IN THE RAT LIVER DURING INHIBITION OF PROTEIN SYNTHESIS BY CYCLOHEXIMIDE

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Injection of cycloheximide into rats was shown not to change the acid and alkaline ribonuclease (RNase) activity in the cytoplasm of liver cells. Meanwhile inhibition of protein synthesis by cycloheximide led to a decrease in RNase activity of microsomes and membrane-bound polysomes. RNase activity of free polysomes in liver cells was considerably increased after administration of cycloheximide.

KEY WORDS: cycloheximide; protein synthesis; RNA.

It was shown previously that inhibition of protein synthesis by cycloheximide prevents destruction of total mRNA, including that containing polyadenylic acid (polyA), in rat liver cells [1, 3]. Under these circumstances stabilization of the mRNA of membrane-bound polysomes is observed, although the mRNA of free polysomes evidently continues to be destroyed [2, 4]. The question of the reasons for the difference in stability of the mRNA of the two classes of polysomes in liver cells during the action of cycloheximide has not been explained. One approach to its solution is to determine ribonuclease (RNase) activity in liver cells during inhibition of protein synthesis. There have been only isolated publications on this subject. It is known [9], for instance, that administration of various inhibitors of protein synthesis to rats does not change acid or alkaline RNase activity in the liver cell cytoplasm. Other workers have shown that injection of cycloheximide into rats reduces RNase activity in the microsomal fraction of liver cells [11].

The object of this investigation was to study the possible role of RNases in stabilization of mRNA in rat liver cells during inhibition of protein synthesis. Activity of cytoplasmic acid and alkaline RNases, RNase of the microsomal fraction, and also RNases of membrane-bound and free polysomes was determined. These investigations were carried out after administration of the transcription inhibitor actinomycin D, cycloheximide, and of both antibiotics together.

EXPERIMENTAL METHOD

Male Wistar rats weighing 180-200 g were used. Actinomycin D (Reanal) and cycloheximide (Serva) were injected intraperitoneally in doses of 2.5 and 100 mg/kg body weight respectively; the animals were killed 3 h later. The isolated liver was cut into small pieces and homogenized in three volumes of 0.5 M Tris-HCl, pH 7.6, containing 0.025 M KCl, 0.005 M $MgCl_2$ (TKM buffer) and 0.25 M sucrose, in a continuous-flow homogenizer with Teflon pestle. The homogenate was centrifuged at 600g for 10 min to remove the nuclei, and the supernatant (postnuclear fraction) was centrifuged at 15,000g for 10 min to remove mitochondria. The supernatant (the S-15 fraction) was used for determination of activity of acid and alkaline RNases and also to obtain the microsomal fraction, for which purpose the S-15 fraction was centrifuged at 80,000g for 1 h. The supernatant (postmicrosomal) fraction was subsequently used to isolate free polysomes. The residue (microsomes) was suspended either in 0.05 M Tris-HCl, pH 8.0, containing 0.1 M NaCl (to determine microsomal RNase activity), or in TKM buffer containing 0.25 M sucrose (to obtain membrane-bound polysomes). To obtain membrane-bound and free polysomes respectively, the microsomes and postmicrosomal fraction, after treatment with sodium deoxycholate (final concentration 1%), were layered above a two-step (2 ml of each, 2 and 1.35 M) concentration gradient of sucrose made up in TKM buffer, and centrifuged at 105,000g for 18 h. The residues of

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TABLE 1. RNase Activity in Rat Liver under the Influence of Inhibitor of Protein Synthesis

Experimental condition	Specific RNase activity, units/mg protein					
	S-15 fraction			microsomes	polysomes	
	pH 5,6	pH 7,6 without PCMB	pH 7,6 with PCMB		membrane bound	free
Control	1,8	0,34	0,53	1,66	8,85	1,7
Actinomycin D	1,9	0,34	0,62	0,95	1,6	5,3
Cycloheximide	1,76	0,36	0,5	0,63	0,29	6,0
Actinomycin D + cycloheximide	2,2	0,45	0,88	0,7	0,2	5,3

Legend. PCMB) p-chloromercuribenzoate.

membrane-bound and free polysomes were suspended in 0.05 M Tris-HCl, pH 8.1, containing 0.1 M NaCl, and were used to determine RNase activity. This was done by the method described in [5], using RNA-^{14}C as substrate.

RNase activity was judged from the degree of increase of radioactivity in acid-soluble material. The unit of RNase activity was taken to be the quantity of enzyme which catalyzed liberation of 1 nCi acid-soluble material from RNA-^{14}C at 37°C in 1 h. Specific RNase activity was expressed in units/mg protein. The protein concentration was determined by Lowry's method [12].

EXPERIMENTAL RESULTS

As Table 1 shows, specific activity of acid and alkaline RNases in the liver cell cytoplasm (S-15 fraction) was unchanged after administration of actinomycin D, cycloheximide, or a combination of both into rats. Moreover, alkaline RNase activity in all cases was increased equally in the presence of 0.2 mM p-chloromercuribenzoate in the incubation medium. On the other hand, microsomal RNase activity was reduced after administration of cycloheximide alone and together with actinomycin D. Microsomal RNase activity also was reduced by the action of actinomycin D alone, although by a lesser degree than in the presence of cycloheximide or a combination of cycloheximide with actinomycin D. This could be explained by the direct effect of the antibiotics on enzyme activity. However, it was shown previously that actinomycin D [15] and cycloheximide [11] do not affect RNase activity in vitro. Another reason for the decrease in microsomal RNase activity under the influence of cycloheximide could be as follows. After injection of cycloheximide into rats the production of glucocorticoids, which depressed liver microsomal RNase activity in vivo [7], in the adrenal cortex is known to be increased [10]. However, there is evidence [11] of a reduction in RNase activity of the liver microsomes after administration of cycloheximide to adrenalectomized rats also. All this suggests that the decrease in liver microsomal RNase activity under the influence of cycloheximide is most probably due to cessation of synthesis of molecules of this enzyme. Indirect evidence in support of this view is given by the fact that actinomycin D reduces microsomal RNase activity by a lesser degree than cycloheximide (Table 1).

This difference in the action of these antibiotics can be explained as follows. It has recently been shown that inhibition of protein synthesis by actinomycin D in eukaryotes is the result of inhibition of transcription by this antibiotic and not of its direct action on translation [8]. It can accordingly be postulated that under the influence of actinomycin D the synthesis of microsomal RNase will continue for a short time to correspond to the half-life of mRNA of this enzyme. As regards cycloheximide, it ought to inhibit RNase synthesis quickly.

It will be clear from Table 1 that after administration of cycloheximide alone and together with actinomycin D to rats, RNase activity of membrane-bound polysomes of the liver cells was considerably reduced.

Under the influence of actinomycin D alone, RNase activity also was considerably reduced compared with the control, but not to the same degree as by the action of cycloheximide, alone or together with actinomycin D. In that case the reasons for the decrease in RNase activity of the membrane-bound polysomes were evidently the same as in the case of microsomal RNase.

By contrast with RNase of the microsomes and membrane-bound polysomes, RNase activity of free polysomes of the liver cells was increased following administration of actinomycin D, cycloheximide, or a combination of both to rats; in all three cases, moreover, this stimulating effect was the same (Table 1). There are thus differences in the response of RNases of membrane-bound and free polysomes of rat liver cells to

injection of different inhibitors. It may be that there is a specific short-lived (like its mRNA) protein inhibitor of RNase of free polysomes in liver cells. When protein synthesis is inhibited by cycloheximide, the pre-existing molecules of the inhibitor are rapidly destroyed, and synthesis of new molecules is prevented, which ought to lead to an increase in RNase activity of the free polysomes. In the presence of actinomycin D, rapid destruction of the templates (mRNA) of the inhibitor protein evidently takes place and, in turn, leads to cessation of its synthesis and, consequently, to an increase in RNase activity also.

Evidence in support of the suggested model is given by the results of many investigations showing that interaction between labile inhibitor proteins and RNase molecules is the mechanism which lies at the basis of control of RNase activity in eukaryote cells [13]. More direct evidence in support of this hypothesis is that an inhibitor of the endo-RNase which participates in destruction of the poly A segment in the mRNA molecule has been found in free ribosomes of guinea pig adrenals [14]. Finally, two latent endo-RNase activities bound with ribosomes have been found in the skeletal muscles of rats, in which most of the ribosomes are in the free state in the cytoplasm (i.e., not bound with membranes of the endoplasmic reticulum). Under these circumstances the activity of one RNase is considerably increased on the addition of p-chloromercuribenzoate to the incubation mixture, which leads to destruction of mRNA of the polysome. These data also support the existence of an inhibitor (evidently of protein nature) of endo-RNase in the ribosomes [6].

In conclusion, it should be noted that changes in RNase activity of membrane-bound and free polysomes in liver cells during inhibition of protein synthesis by cycloheximide correlate well with the mRNA turnover of these two classes of polysomes under the influence of the antibiotics [2, 4].

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