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SHORT COMMUNICATIONS

Decrease of ribonuclease activity of isolated rat liver cytoplasmic ribosomes after phenobarbital administration

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ADMINISTRATION of phenobarbital (PB) brings about in the rat liver an induction of microsomal enzymes,[1,2] proliferation of smooth endoplasmic reticulum[3] and hypertrophy of the liver.[4] At the same time, changes in the synthesis and degradation of ribonucleic acids in the liver take place. It was further observed that administration of PB prolongs the half-life of cytoplasmic ribosomes,[5] increases the stability of ribosomal RNA,[6] the heavier polysome fraction aggregating,[7] inhibits the synthesis of RNA present in the microsomal membranes[8] and decreases the activity of liver ribonucleases.[9–13]

As indicated by the present information on protein synthesis regulation it may take place in higher organisms not only at the level of transcription but also at the level of translation, i.e. directly at the ribosome.[14] The latent RNase activities associated with the ribosomes[15] may play a role not only during degradation of ribosomes themselves[16,17] but may also participate in the reactions linked with inactivation of messenger RNA. A study of RNase activities of isolated liver ribosomes following administration of PB is presented here.

Material and Methods

Male rats of the Wistar strain weighing 180–210 g were used. PB a sodium salt (Merck) was administered to them in drinking water (1 g/l.) for 7 days. Sixteen hr before decapitation the rats were deprived of food. From the excised liver, ribosomes were prepared according to Munro[18] with some modifications. The ribosomal pellet was suspended in 50 mM Tris–HCl buffer of pH 8·0 (2·0–3·0 mg RNA per ml of ribosomal suspension). The incubation mixture contained 0·3 ml ribosomal suspension and 0·2 ml of the same buffer. Incubation took place at 37°. The reaction was terminated by adding 1·0 ml, 0·2 N $HClO_4$ with 0·1% uranyl acetate. After separation of precipitated protein and of RNA, extinction at 260 nm was determined in the acid soluble residue. The acid soluble material showed a typical u.v. spectrum of nucleotides. The main products of the RNase action are the uridine nucleo-

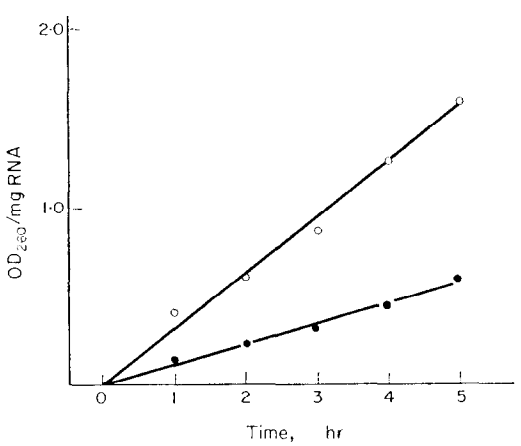

FIG. 1. Time dependence of ribonuclease activity of ribosomes prepared with 1% DOC. Control = ○——○; PB treated = ●——●.

tides as in the case of reticulocyte ribosomes autodegradation.[19] The activity of ribonuclease is expressed as OD $_{260nm}$/mg ribosomal RNA/hr.

Results and discussion

The time course of ribonuclease activity of ribosomes isolated with 1% deoxycholate (DOC), Na salt (Fig. 1) as well as with Triton X-100, is linear for 5 hr with both experimental groups. Administration of PB decreases the ribonuclease activity of the ribosomal particles. The intensity of RNA degradation is pronouncedly affected by the method of ribosome isolation (Fig. 2). Ribosomes isolated with the aid of 1% DOC exhibit lower absolute values of ribonuclease activity than ribosomes prepared with 2% Triton X-100.

The drop of ribonuclease activity is the same in both cases. It was found that addition of PB *in vitro* does not affect the activity of liver RNases.[9–11] In experiments where the isolated ribosomes were incubated in the presence of crystalline pancreatic ribonuclease (Worthington, 10^{-6} g per ml of incubation medium) no differences in the intensity of ribosomal RNA degradation was observed. It thus appears that differences are not due to quality of ribosomal RNA.

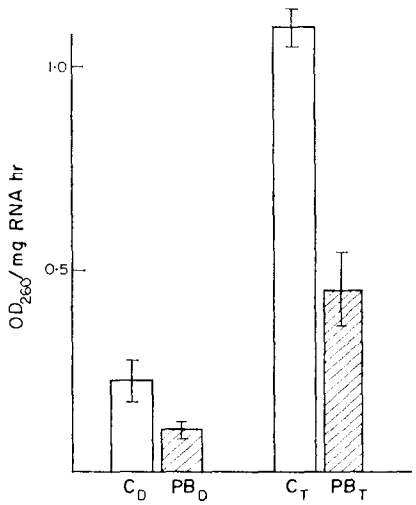

FIG. 2. Ribonuclease activity of ribosomes prepared with 1% DOC and those prepared with 2% Triton X-100. C_D = Control ribosomes prepared with DOC; PB_D = ribosomes isolated from liver following administration of PB, with 1% DOC; C_T = control ribosomes prepared with Triton X-100; PB_T = ribosomes from rat liver following administration of PB isolated with the aid of 2% Triton X-100. The values represent averages from eight estimations, the vertical intercepts indicate standard deviation.

During solubilization of microsomal membrane nonspecific adsorption of enzymes on ribosomal surface may occur. The first to be absorbed are particularly basic proteins to which cell RNases belongs.[20] These protein molecule may be bound by the phosphate groups of ribosomal RNA. Removal of adsorbed proteins from the ribosome by increasing the concentration of KCl in the isolation medium will pronouncedly affect only the activity of ribonuclease of ribosomes prepared with 2% Triton X-100. The ribonuclease activity of ribosomes isolated with 1% DOC is practically independent of the concentration of KCl in the isolation medium. The difference in ribonuclease activity between the two groups is preserved within the range of KCl concentration studied (Fig. 3).

Activity of liver RNases may be regulated by the changes in the concentrations of the natural inhibitor.[21] Addition of *p*-chloromercuribenzoate (*p*-CMB) to the incubation medium increases the ribosomal ribonuclease activity in both groups of DOC ribosomes (Table 1). Still, the values of the experimental group in the presence of *p*-CMB are lower than in the control group. An increased amount of inhibitor of rat liver RNase following administration of PB was described by Louis-Ferdinand and Fuller. At the same time, it was established that an inhibitor which is not inactivated by *p*-CMB plays a role in the depression of RNase activities.[22] In addition, *p*-CMB caused dis-

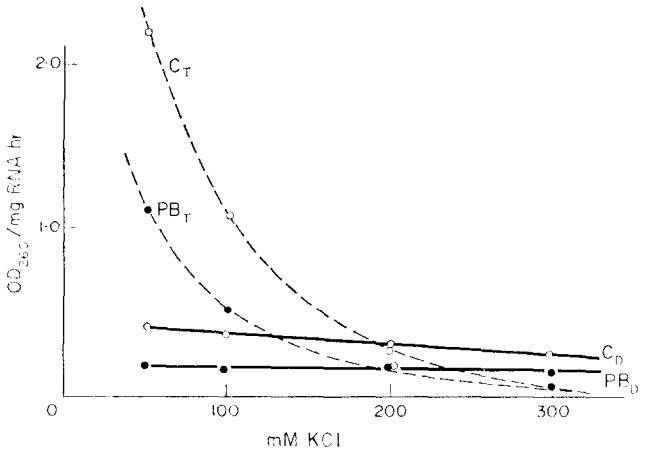

FIG. 3. Dependence of ribonuclease activity of ribosomes on the concentration of KCl in the isolation medium. (For details see the text to the Fig. 2).

aggregation of the rat liver polyribosomes to monosomes and subsequently the dissociation of monosomes to their subunits.[23] This might lead to the activation of the ribosomal latent ribonuclease. The mechanism of affecting RNase activities of ribosomes by *p*-CMB is not clear. With ribosomes prepared with the aid of Triton X-100, *p*-CMB actually brings about a decrease of RNase activity of ribosomes. The effect of Mg^{2+} and EDTA was practically the same in both ribosomal preparations compared and in view of the effect of Mg^{2+} on RNase activity and ribosome dissociation quite predictable.

TABLE 1. RIBONUCLEASE ACTIVITY OF ISOLATED RIBOSOMES IN THE PRESENCE OF Mg^{2+}, EDETA, *p*-CMP AND SPERMIDINE

	DOC		Triton X-100	
	Controls	PB	Controls	PB
	0·26 ± 0·04	0·14 ± 0·02	1·56 ± 0·20	1·04 ± 0·10
$+Mg^{2+}$	0·16 ± 0·02	0·10 ± 0·02	0·80 ± 0·10	0·44 ± 0·06
+EDTA	0·50 ± 0·06	0·26 ± 0·04	2·52 ± 0·30	1·62 ± 0·16
+*p*-CMB	0·44 ± 0·06	0·26 ± 0·04	0·62 ± 0·08	0·62 ± 0·06
+Spermidin	0·15 ± 0·03	0·08 ± 0·02	1·58 ± 0·22	1·08 ± 0·09

Final concentrations in the incubation medium; Mg^{2+} = 10 mM; ethylendiamintetraacetic acid (EDTA) = 1 mM; *p*-CMP = 1 mM; spermidin = 2 mM. The values represent means from eight estimation ± standard deviation.

The presence of spermidine in the incubation medium of DOC ribosomes has an effect similar to that Mg^{2+}. A quantitative determination of spermidine showed that after a one-week administration of PB its amount in the liver of rats is increased 2·5 times (Vácha, Seifert, in preparation). The relationship between the amount of RNA and the concentration of spermidine after administration of PB was observed in mouse liver.[24] It is thus possible that, in addition to depression of RNase activities also the levels of polyamines may play a role in the astabilization of liver ribosomes,[5] and of ribosomal RNA following administration of PB.[7] At the same time, the level of polyamines in the liver may affect the binding of the ribosomes to the membrane of endoplasmic reticulum.[25]

The RNase activity is frequently taken as one of the enzyme activities that may have a functional association with the ribosomal particle.[20] Almost complete loss of ribosomal ribonuclease activity

was reported to occur in ribosomes isolated from regenerating rat liver, which may result in sparing of ribosomes during the period of growth activation.[26] It follows from the above experiments that there is a qualitative difference between the ribosomal preparations under comparison after application of PB. So far it is not possible to decide whether the differences in the ribosomal RNase activity are due to an overall lower activity of RNase which is in functional association with the ribosomes or to other factors. The most important of these might be; (a) nonspecific adsorption of RNases released from microsomal membranes during their solubilization with detergents; (b) different ability of the ribosomes to adsorb microsomal proteins including RNases and their natural inhibitors.

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