THE EFFECT OF DIMETHYLSULFOXIDE ON RIBOSOMAL FRACTIONS FROM RAT LIVER

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1. Introduction

Recently we have been investigating the influence of hepato carcinogenic substances, administrated to rats in vivo, on the properties of the rough endoplasmic reticulum and ribosomes isolated from rat liver [1]. Several workers have used DMSO (dimethyl sulfoxide) as a vehicle for water-insoluble drugs. DMSO seems to facilitate the penetration of these drugs into liver cells [2,3]. In order to be able to investigate the effect of some hydrophobic drugs on subcellular fractions of rat liver we have studied the influence of DMSO on some properties of rat liver, rough endoplasmic reticulum and polyribosomes.

2. Materials and methods

Female, SABRA albino rats, 180–200 g, were obtained from the Hadassah Medical School breeding farm. They were fed a standard laboratory diet adlibitum. The rats were injected interperitoneally with 1.0 ml DMSO and sacrificed 24 h later. They were fasted from the time of injection (with full access to water). Control rats were injected with 1.0 ml saline instead of DMSO. DMSO (analytical grade) was purchased from Fluka. [^{3}H]Leucine, 30.2 Ci/mmol was from the Radiochemical Centre, Amersham. Free polyribosomes and rough endoplasmic reticulum were prepared from rat liver as in [4].

Amino acid incorporation was assayed as follows in final vol. 100 μl. Tris–HCl, pH 7.4, 60 mM; KCl, 70 mM; DTT, 1 mM; $MgCl_2$, 5 mM; ATP, 1 mM; GTP, 0.5 mM; creatine phosphate, 10 mM; creatine phosphokinase, 15 μg protein; non-radioactive amino acid mixture (minus leucine), 5×10^{-2} mM each; [^{3}H]-leucine, 5 μCi; free polyribosomes 1.0 $A_{260\ nm}$ unit or rough membrane fraction, 50 μg protein and aliquots of rat liver pH 5 enzyme (prepared according to [5]).

Samples were withdrawn and the radioactivity of the hot 5% trichloroacetic acid (TCA)-insoluble material was determined as in [6]. RNase activity was measured by following the decrease in 5% TCA insoluble radioactive material upon incubating the different fractions with [^{3}H]phe-$tRNA_{yeast}$ as in [7]. Protein was determined by Lowry's method [8] and RNA by Bloemendal's method [9].

3. Results and discussion

Figures 1A,B show the kinetics of amino acid incorporation catalysed by the free polyribosomal fraction and the rough membrane fraction isolated from the livers of DMSO-treated and control rats. The values given are the average of 5 different experiments. In every separate experiment the fractions were prepared from the combined liver homogenates of 6 animals.

The yield of rough endoplasmic reticulum fraction of the livers of the DMSO treated rats was nearly twice that of the control.

As can be seen from fig.1 the initial rates of amino acid incorporation by the fractions from the DMSO-treated animals and from the control animals are nearly the same. However, the fractions differ considerably in the amount of amino acid incorporated

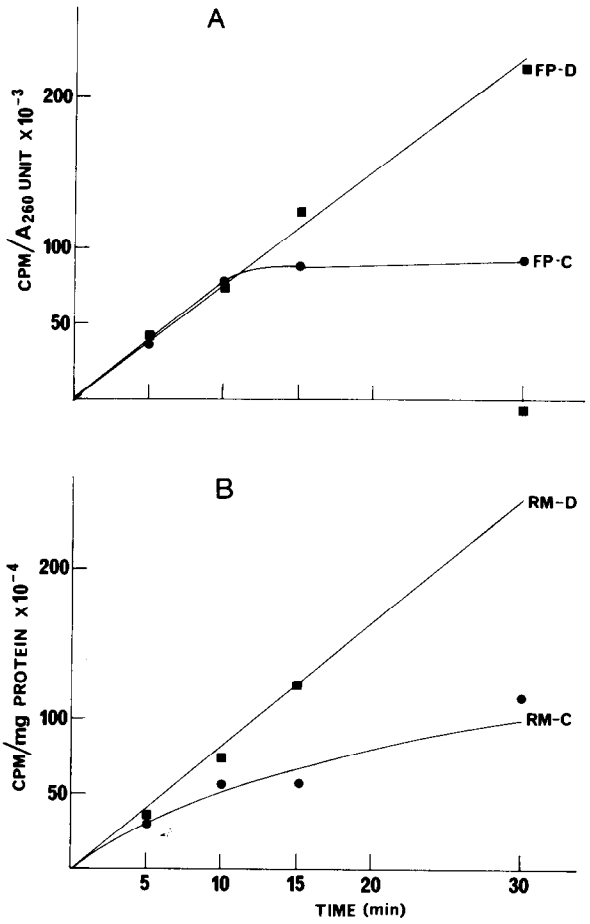

Fig.1. The rate of amino acid incorporation by free polyribosomes (A) and rough endoplasmic reticulum (B). For experimental details see section 2. FP-C, Free polyribosomes; RM-C, rough endoplasmic reticulum, from control rats. FP-D, Free polyribosomes and RM-D rough endoplasmic reticulum, from DMSO-treated rats.

after 30 min. In the case of the fractions of the control animals the amino acid incorporation reaches a plateau level after 15–20 min but the amino acid incorporation by the fractions from DMSO treated animals continued linearly during all the time of the incubation (30 min). The enzyme fraction used in all the experiments described in fig.1A,B is the same (pH 5 fraction prepared from the livers of normal untreated rats), therefore the results described can be due only to differences in the fractions containing the ribosomes.

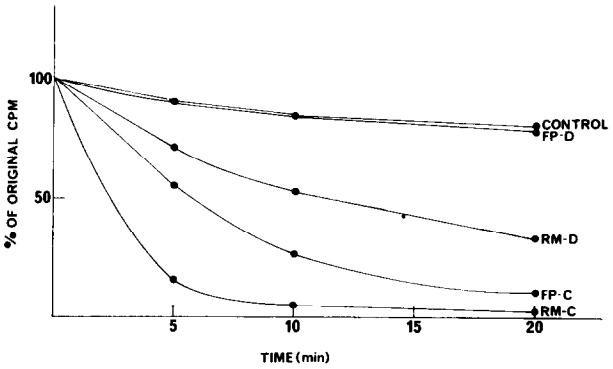

Fig.2. The RNase activity of free polyribosomes and rough endoplasmic reticulum. The incubation mixtures contained: 12 000 cpm [^{3}H]phe-tRNA$_{yeast}$ spec. act. 6 Ci/mmol and 1.0 A_{260} nm units of polyribosomes or rough endoplasmic reticulum (50 μg protein).

DMSO has no direct effect on the amino acid incorporation activity. Amino acid incorporation assays were carried out with $\leqslant$ 10% DMSO (final concentration). In no case could a change in the in vitro amino acid incorporation activity be detected.

It was shown that the in vitro amino acid incorporation activity is influenced by the RNase activity of the ribosome containing fractions [10]. Therefore we measured the RNase activity of the fractions used in experiments 1A and 1B. As can be seen from fig.2, the RNase activity of the fractions obtained from DMSO treated animals is much lower than that of the corresponding fractions obtained from control animals. These results may explain the results described in fig.1A,B. The mRNA in the free and bound ribosomes from control rats may be more damaged than that of the fractions of the DMSO treated aninals. If the average length of the ribosomal bound mRNA in the fractions isolated from the DMSO-treated animals is greater than that in the fractions of the control animals the amino acid incorporation may continue for a longer period. Addition of tRNA to the incubation mixture did not lead to an increase in the amino acid incorporation level therefore depletion of the tRNA content is not the cause of the results shown in fig.1A,B.

DMSO can be metabolized in vivo and the main metabolic product is dimethylsulfone ($DMSO_2$) [11]. $DMSO_2$ was injected into rats and the experiments

described above were repeated. The results obtained were nearly identical to those described above. Therefore it seems likely that the effects observed are caused by dimethylsulfone or by a common metabolite of dimethylsulfone and dimethyl sulfoxide.

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