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Modulation of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine induced inhibition of cell-free protein synthesis by sulfur compounds*

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N-Methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG†), which is a potent mutagen and carcinogen [1–4] has been demonstrated to inhibit protein synthesis *in vitro* and *in vivo* [5, 6]. Since proteins are important biomacromolecules involved in many regulatory processes of the cell, an inhibition of protein synthesis may severely perturbate normal cellular activities and may also be connected to carcinogenesis. An attempt was made to find out whether thiocompounds which were proven to reduce toxic and carcinogenic effects of *N*-nitrosamines [7–9] may affect the inhibitory action of MNNG on protein synthesis. We report here the influence of PDTC, DDTC, GSH and GS-DDTC on [^{14}C]amino acid incorporation into protein using rat liver postmitochondrial supernatant (S30 fraction) in an *in vitro* protein synthesizing system.

Materials and Methods

ATP, GTP, creatine phosphate, creatine phosphokinase (2.7.3.2), and dithiothreitol were purchased from Serva Feinbiochemikalien (Heidelberg, Germany). MNNG was purchased from Fluka AG (Buchs SG, Switzerland). L-[U-^{14}C]Leucine (sp. act. 308 mCi/mmol), was obtained from the Radiochemical Centre (Amersham, U.K.). The dithiocarbamates used were synthesized in this laboratory [9]. All other chemicals used were commercially available with the highest degree of purity. Male Sprague–Dawley rats (Wiga) weighing 100–120 g were used. They were killed by cervical dislocation and the liver homogenate (25% w/v) was prepared in STKM buffer. Postmitochondrial supernatant (S30 fraction), containing all the components required for *in vitro* incorporation of amino acids into protein, was prepared from the homogenate by centrifugation at 30,000 *g* for 15 min. Amino acid incorporation studies were carried out following the procedure of Richardson *et al.* [10] with slight modifications as described [5]. Each assay mixture (250 μL) contained 10 mM HCl (pH 7.2), 80 mM KCl, 5 mM magnesium acetate, 2 mM ATP, 1 mM GTP, 20 mM creatine phosphate, 2.5 μg creatine phosphokinase, 1 μCi [^{14}C]leucine (sp. act. 308 mCi/mmol) and S30 fraction (2 mg protein). After incubation at 37° for 15 min, a 50 μL aliquot was spotted on Whatman No. 3 MM filter paper circles (2.5 cm diameter) and processed before counting [11]. Protein concentration in S30 fraction was measured by the method of Lowry *et al.* [12].

Results

Each incorporation assay was conducted in duplicate or triplicate, and the result given as the average value. Each experiment was then repeated with S30 fraction from another rat. Variation was found to be within ±10%. Reproducible results were obtained with different preparations.

The inhibitory activity of various amounts of MNNG for [^{14}C]leucine incorporation was increased by the addition of PDTC into the assay system. At lower concentrations of MNNG (0.08 and 0.2 mM) no inhibition was observed (Fig. 1). However, the addition of PDTC (2.0 mM) along with MNNG showed significant inhibition. This increase in inhibition depended on the concentration of MNNG as well as of PDTC (PDTC data not shown). In contrast to PDTC, the addition of DDTC to the assay system stimulated the cell-free protein synthesis in a dose-dependent way up to 143% of the control value (Fig. 2, empty columns). The stimulation of amino acid incorporation by DDTC is blocked by MNNG, in contrast to PDTC where the inhibition is increased. Two other compounds were tested for their effect on the MNNG-induced inhibition of leucine incorporation into protein, namely GSH and the mixed disulfide GS-DDTC. They increase the inhibitory activity of MNNG upon leucine incorporation in a dose-dependent way (Table 1). Both these compounds show effects which are comparable to those produced by PDTC. However, these compounds also inhibit cell-free protein synthesis without MNNG.

Discussion

The thiocompounds showed a different behavior with respect to cell-free protein synthesis. DDTC stimulated the incorporation of amino acids whereas GSH or GS-DDTC showed an inhibition of it. These alterations depend on the concentration of the dithiocarbamates. However, PDTC did not show any significant alteration. The effects observed with PDTC and DDTC on the MNNG-induced inhibition of amino acid incorporation are opposite in action. Since both compounds, PDTC and DDTC, are dithiocarbamates, a qualitatively different effect was not to be expected. There have been few experiments up to

* Dedicated to the late Professor Dr. Dietrich Schmähl.
† Abbreviations: MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; PDTC, prolinedithiocarbamate; DDTC, diethyldithiocarbamate; GSH, glutathione; GS-DDTC, glutathionyldiethyldithiocarbamate.

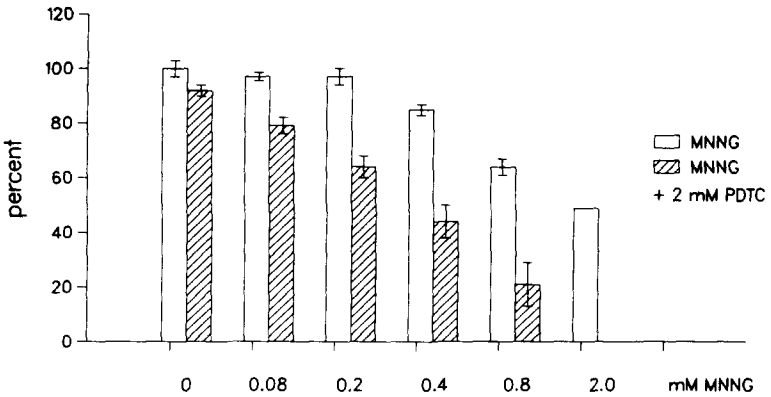

Fig. 1. Influence of 2 mM PDTC on inhibition of [^{14}C]leucine incorporation by various concentrations of MNNG. (Conditions are as described in the text.) The data are given in per cent of control, and are the average of three experiments with standard deviation. Empty and hatched columns represent the incorporation in the presence of MNNG and MNNG plus 2 mM PDTC, respectively.

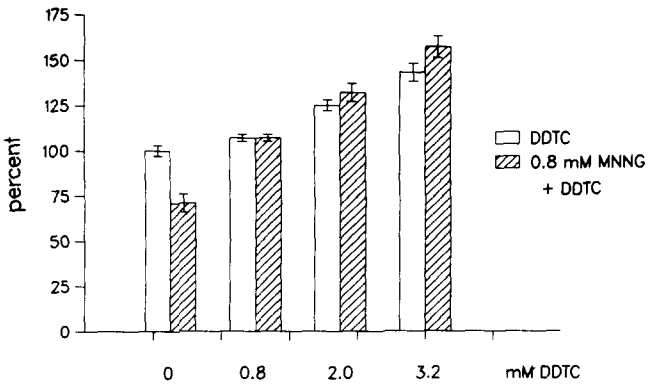

Fig. 2. Influence of various concentrations of DDTC on MNNG-induced inhibition of [^{14}C]leucine incorporation. (Conditions are as described in text.) The data are given in per cent of control, and are the average of three experiments with standard deviation. The empty and hatched columns represent the incorporation after addition of DDTC and combination of DDTC plus MNNG, respectively.

Table 1. Effect of GSH and GS-DDTC on inhibition of [^{14}C]leucine incorporation into polypeptide by MNNG

Thiol added (mM)	dpm × 10^3/mg protein 0	0.4 mM	0.8 mM	4.0 mM
Control	2.2	—	—	—
GSH	—	1.7	1.8	1.04
GS-DDTC	—	1.3	0.6	—
MNNG (2.0 mM)	0.7	—	—	—
MNNG + GSH (2.0 mM)	—	—	1.3	0.5
MNNG + GS-DDTC (2.0 mM)	—	0.4	0.1	—

Values are medians of two experiments, made in duplicate.

Other conditions are as described in the text.

now comparing the biochemical effects of both compounds [7, 8]. We found both compounds to inhibit *N*-nitroso-diethylaminedeethylase and *N*-nitroso-dimethylamine-demethylase to a similar extent. Both compounds have no effect on the activity of glutathione-*S*-transferase, glutathione reductase and the level of reduced GSH in rat liver.

It is known that the SH group of cysteine stimulates the degradation of MNNG [13] and of *N*-nitrosocimetidine, a compound structurally related to MNNG [14]. Similarly the degradation of MNNG may be stimulated by dithiocarbamates forming products which may either be stimulatory or inhibitory for protein synthesis. The chemical stability of the dithiocarbamates are different [15, 16], forming different amines. DDTC forms the lipophilic diethylamine whereas in the case of PDTC the naturally occurring amino acid, proline, will arise. A competitive inhibition of [^{14}C]leucine incorporation by an excess of proline in the assay can be excluded since incubation experiments with proline instead of PDTC showed no

influence on protein synthesis or on its inhibition by MNNG (data not shown).

In contrast to DDTC, GS-DDTC had no stimulatory effect upon [^{14}C]leucine incorporation. The degradation products of GS-DDTC are probably the naturally occurring tripeptide GSH and DDTC. The effect of GS-DDTC is supposed to be specific and not due to its presumptive degradation products. GSH had no effect between 0.2 and 0.6 mM and the onset of the inhibitory activity of GSH was observed above concentrations of 0.7 mM.

The interference of dithiocarbamates with the MNNG-induced inhibition of protein synthesis may be originated by the scavenging of the methyl group, which occurs during degradation of MNNG. After incubation of disulfiram with alkylnitrosoureas in rat liver supernatant fraction the corresponding alkyl-dithiocarbamates were formed [17]. However, it is not known whether the dithiocarbamates exert a different scavenging capacity upon the methyl group leading to a different modulation of MNNG effects. In neutral solution MNNG decomposes to yield a methylating species which can interact with nucleic acids and proteins [4]. It has been shown that MNNG inhibits the synthesis of DNA, RNA and proteins [18, 19]. The inhibitory activity of MNNG on protein synthesis is believed to be due to a modification of tRNA molecules by methylation leading to an alteration of aminoacylation [20] and a modification of ribosomal proteins possibly by nitroguanidination [6]. Experiments on the reaction products formed between MNNG and dithiocarbamates and their possible influence on RNAs and proteins involved at the level of translation will help to elucidate the mechanism of modulation. In summary PDTC, GSH and GS-DDTC increase the toxic effect of MNNG on cell-free protein synthesis in a concentration-dependent manner while DDTC reduces this effect.

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