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# "IN VIVO" ($^{35}$S)-METHIONINE INTERACTION WITH RAT-LIVER DNA AND THE EFFECT OF CHEMICAL CARCINOGENS

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1. After intraperitoneal administration of ($^{35}$S)methionine (25 mg, 1.6 mCi/kg), detectable amount of radioactivity resulted associated to rat-liver DNA: the interaction reached the maximum value (about 18 pmol/μmol DNA P) by 2 h after administration of radioactive aminoacid. 2. The ($^{35}$S)-binding was inhibited by the hepatocarcinogenic ethionine and dimethylnitrosamine, and was stimulated by the non-hepatocarcinogenic methylnitrosourea. 3. Hplc analysis of ($^{35}$S)DNA enzymic digest evidenced two radioactive compounds, the UV behaviour of which is reported. 

It is now established that methionine metabolism has a crucial role in oncogenesis and the possible mechanisms herein involved (1). Through the proximate agent S-adenosylmethionine, this aminoacid represents the precursor for polyamine biosynthesis as well as the methyl donor for DNA biomethylation. Both these reactions have been intensively investigate because of their possible importance in carcinogenic growth processes (2-5). However the factors and the reactions which link methionine to neoplastic processes remain to be elucidated.

This paper reports the characterization of a new type of interaction between methionine and DNA. More precisely, we find that: i) the sulfur of methionine interacts with and binds to rat-liver DNA "in vivo", ii) this interaction is differentially affected by chemical carcinogens, iii) two radioactive compounds can be isolated from ($^{35}$S)DNA enzymatic hydrolysate. The possible significance of these data is discussed.

## MATERIALS AND METHODS

Wistar male albino rats (180-200 g) were injected i.p. with ($^{35}$S)methionine (1072 or 1119 Ci/mmol, New England Nuclear, Boston, MA.) plus unlabelled

Abbreviations: methylnitrosourea, MNU; dimethylnitrosamine, DMNA; deoxyCytidine, dCyd; deoxyGuanosine, dGuo; deoxyAdenosine, dAdo; thymidine, Thd.

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aminoacid to give the final dose of 25 mg, 1.6 mCi/kg b.w.. The animals were given free access to water and food. At the times reported in the legends, rats were sacrificed and livers homogenized in 0.075 M NaCl/0.025 M EDTA, pH 7.6/0.1% Triton. After centrifugation at 3500 rpm for 15 min, the nuclear pellet was washed twice with 0.05 M Tris-HCl, pH 7.6/0.1% Triton, followed by three times with 0.05 M Tris-HCl, pH 7.6. Rat liver nuclei were treated with 0.2 mg/ml Proteinase K in 1 M NaCl/0.01 M Tris-HCl, pH 7.8/0.05 % SDS, 37°C, 4 h, and DNA was extracted (6). Then DNA was treated with RNases (pancreatic and T1, pretreated at 80°C) in 0.05 M Tris, pH 7.8/0.01 M NaCl/0.01 M EDTA, 4 h, 37°C. The purified DNA was dissolved in 0.01 M NaCl, ethanol precipitated (3 times) and assayed for ($^{35}$S)-radioactivity in Maxifluor by liquid scintillation counting in a LS 7800 Beckman.

Enzymic digestion of ($^{35}$S)-DNA to deoxynucleosides was performed with DNase I (Sigma Chem. Co.), Nuclease P1, acid phosphatase (Boehringer, Mannheim) according to Kuo et al. (7). To obtain maximal digestion nuclease S1 (Boehringer, Mannheim) was also added to a final concentration of 400 U/ml. The deoxynucleosides were separated on a Perkin Elmer liquid chromatograph equipped with an LC-100 column oven, an LC-55B spectrophotometric detector with a 254 nm filter and a Shimadzu C-R64 Chromatopac. The column was an 5 μm RP-18 Lichrosorb prefitted with a 7 μm guard column (both from Merck) and isocratically eluted with 0.005 M ammonium formate, pH 4.8/0.5% methanol, at 37°C and at a flow rate of 1 ml/min. The hplc fractions were collected and assayed for radioactivity as described before. UV spectra of radioactive fractions were obtained with a Beckman DU-8 spectrophotometer.

## RESULTS

DNA isolated from livers of rats that had received ($^{35}$S)methionine (25mg, 1.6 mCi/kg) was found to contain detectable amount of radioactivity. The time course and the extent of the "in vivo" DNA labelling were studied at different time intervals up to 96 h. As reported in Fig.1, maximal binding was about 18

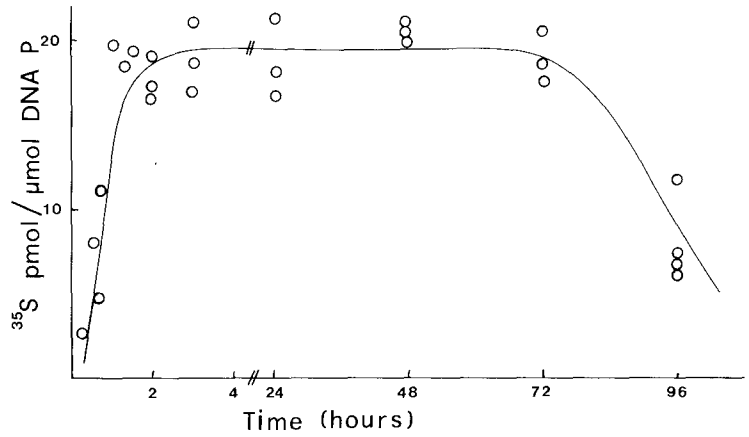


Fig.1 - Time-course of "in vivo" rat-liver DNA labeling after i.p. injection of ($^{35}$S)methionine (25 mg, 1.6 mCi/kg). Points correspond to single separate experiments.

Table 1 - Effect of chemical carcinogens on rat-liver DNA labeling by ($^{35}$S)-methionine. MNU (104 or 240 mg/kg) and DMNA (100 mg/kg) were administered by a single i.p. injection. Ethionine was given by 2 (or 3) separate i.p. injections at 24 h interval each, to give the final dose of 260 mg (or 390 mg) per kg. Three hours later (or 24 h after the last injection in the case of ethionine), animals received a 2 h pulse of ($^{35}$S)methionine i.p. Data are reported as mean value ± S.D. Number of experiments in parentheses.

| Carcinogen | Dose (mg/kg) | pmol $^{35}$S/μmol DNA P |
|---|---|---|
| None | | 17.10 ± 0.35 (3) |
| DMNA | 100 | 11.28 ± 2.33 (3) |
| Ethionine | 260 | 13.05 ± 4.61 (4) |
| | 390 | 10.61 ± 1.90 (4) |
| MNU | 104 | 28.54 ± 6.64 (3) |
| | 240 | 23.78 ± 4.19 (3) |

pmol sulfur / μmol DNA P and the interaction was almost complete by 2 h after administration of the aminoacid isotopically labeled in the sulfur. From 2 to 72 h the level of $^{35}$S-binding did not vary significantly. After 72 h, the level of radioactivity rapidly decreased.

The effect of chemical carcinogens on this interaction is shown in Table 1. Interestingly, the hepatocarcinogenic compounds ethionine (260 mg/kg) and DMNA (100 mg/kg) exerted an inhibitory effect on DNA-($^{35}$S)methionine interaction (24 and 34% inhibition respectively). At the dose of 390 mg/kg, the ethionine inhibition raised to 38%. On the other hand administration of the non-hepatocarcinogenic MNU (104 mg or 240 mg/kg, single i.p. injection) resulted rather in a stimulatory effect.

Representative hplc analysis of enzymic digest of ($^{35}$S)DNA is reported in fig.2. Reversed-phase separation of the enzymic hydrolysate of radioactive DNA isolated from rat liver 2 h after injection, showed only two radioactive peaks. One peak was in correspondence to the first fractions (Rt=3.5 min) and the other was eluted from the column by changing the eluting buffer into 60% methanol. No radioactivity was found in the four deoxynucleosides. The radioactivity ratio of the methanol-eluted peak to the buffer-eluted peak was 0.23 and did not change in enzymatic digest of ($^{35}$S)DNA isolated from rat liver 3 days after administration of the labeled aminoacid.

The UV-spectra of the two radioactive peaks are reported in fig.3. It can be seen that the buffer-eluted compound (Fig. 3A) has a far UV absorbance, which can be indicative of a ring-saturation reaction (8), while the methanol-

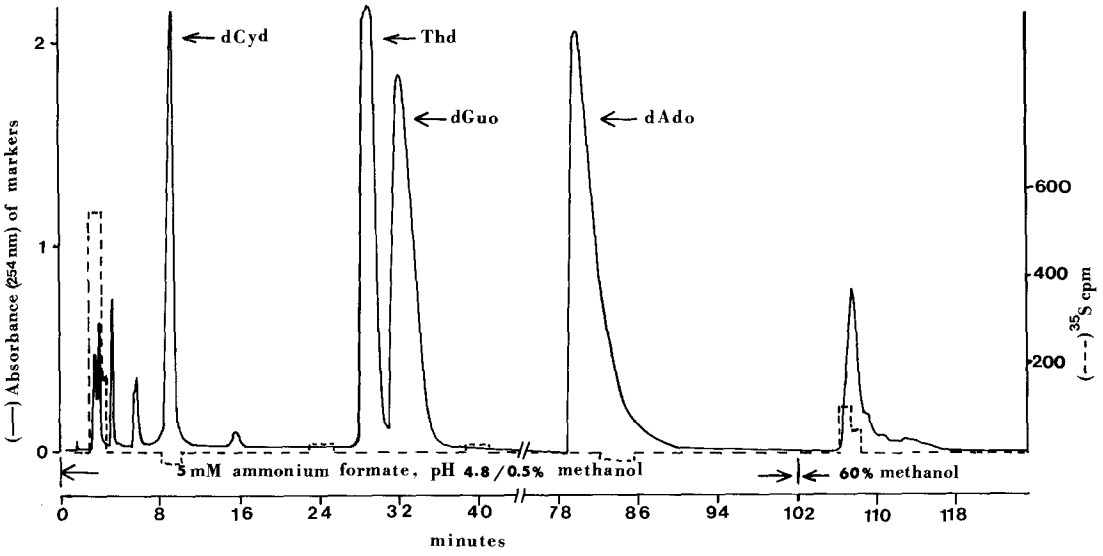


Fig.2 - RP-hplc chromatography of ($^{35}$S)DNA enzymic digest.

eluted compound (Fig. 3B) has a maximal absorbance at about 260nm and resulted stable under acid and basic conditions. Anyway storage of the methanol-eluted compound at 0°C led to changes in the UV spectra in a few weeks. At present studies are under way in this laboratory to clarify the chemical nature of the

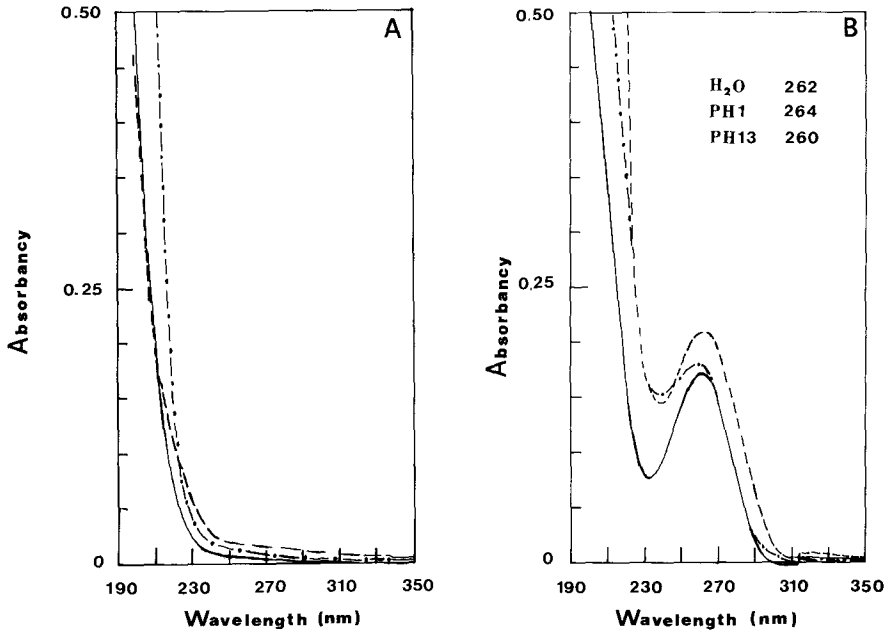


Fig.3 - Ultraviolet absorbance spectra of buffer-eluted (A) and methanol-eluted (B) radioactive compound isolated from ($^{35}$S)DNA enzymic digest.

two radioactive compounds isolated from enzymatic DNA hydrolysate as well as the exact mechanism by which methionine sulfur can interact with the nucleic acid.

## DISCUSSION

It is evident from the results of this study that the sulfur of methionine interacts with DNA "in vivo". The reaction has never been reported till now: it is possible that the low extent of reaction (around 20 pmol sulfur/$\mu$mol DNA P at the methionine dose here used, i.e. 25 mg/kg, and presumably much lower with a low supplementation of methionine as normally employed) has prevented its discovery and study.

Moreover, albeit this study does not establish the precise chemical nature of this interaction, it clearly shows that $^{35}$S-radioactivity specifically binds to components of DNA (see figs.2 and 3). The fact that $^{35}$S-radioactivity eluted in association to two products leads, as logical consequence, to hypothetize the possibility that 5-methylcytosine might not represent the only possible physiological DNA modification.

The hepatocarcinogenic methionine-analogue partially inhibited this interaction. This inhibition appears a significant datum, being still unknown the mechanism of ethionine carcinogenesis notwithstanding the number of studies and approaches in various laboratories (9-12). The two electrophilic carcinogens MNU and DMNA affected differently the $^{35}$S-binding. Whether there is a relationship between the differential effect by these carcinogens on $^{35}$S-binding and their ability to induce hepatic tumors remains an interesting hypothesis to verify.

Thus, whatever the mechanism of interaction between DNA and ($^{35}$S)methionine, it appears noteworthy and indicate new metabolic positions of this key aminoacid, so heavily involved in the molecular control of gene expression and cell proliferation (13). More generally, while the important contributions made by cysteine, methionine and, in general, S compounds to growth and cell division are well established (1, 13), the exact role and function of these compounds in molecular terms remain obscure. In this larger context the interaction here reported offers an attractive new avenue to explore.

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