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Comparison of the effects of cysteine upon the decomposition of nitrosoureas and of 1-methyl-3-nitro-1-nitrosoguanidine

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1-METHYL-1-NITROSOUREA (MNU) is active as a mutagen,[1] carcinogen,[2] and anticancer agent,[3] and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) are among a number of nitrosoureas that are active in the treatment of experimental neoplasms in laboratory animals[4–6] and of human neoplasms,[6–8] including brain tumors.[9,10] Therefore it is desirable to know as much as possible about the chemistry and metabolism of these compounds. Chemical evidence[11] is consistent with the decomposition of nitrosoureas to yield progenitors of carbonium ions and isocyanates; the carbonium ions and isocyanates might then react with a variety of components of biological systems.

1-Methyl-3-nitro-1-nitrosoguanidine (MNNG) is also a potent mutagen[12] and carcinogen,[13–15] and it has some activity against murine neoplasms.[16] It decomposes in neutral solution to yield a methylating moiety and a salt of nitrocyanamide.[17] Lawley and Thatcher[17] have recently shown that this decomposition of MNNG is greatly accelerated in the presence of cysteine, and they confirmed the observation of McCalla[18] that incubation of MNNG with DNA resulted in more extensive alkylation of the DNA if cysteine were also present. It was suggested that the quantity of sulfhydryl compounds in cells or in biological systems might therefore significantly influence the rate and extent of alkylation caused by this agent.

Because of the similarities of the chemical properties of nitrosoureas and nitrosoguanidines and because of the desire to understand the mechanisms of action of BCNU and CCNU, it seemed reasonable to compare the effects of cysteine upon the rates of decomposition of several nitrosoureas and of MNNG.

The nitrosoureas and the MNNG were obtained from the Organic Chemistry Department of Southern Research Institute or from the Cancer Chemotherapy National Service Center. The cysteine hydrochloride was purchased from Calbiochem, Los Angeles, Calif.

The MNNG and the nitrosoureas were dissolved in absolute ethanol at a concentration of 100 mM for MNNG and of 5 mM for the nitrosoureas. Cysteine hydrochloride was dissolved in 0·2 M phosphate buffer, pH 7·4, at a concentration of 100 mM (for the experiments with MNNG) or 50 mM (for experiments with the nitrosoureas) immediately before preparing the mixtures for determination of absorbance.

To measure the effect of cysteine upon the rate of decomposition of MNNG, the changes in absorbance at 400 nm of the following solutions were followed with a Gilford automatic spectrophotometer, model 2000: 10·0 mM MNNG in buffer (0·2 M phosphate, pH 7·4); 10·0 mM MNNG and 10·0 mM cysteine hydrochloride in buffer.

In the experiments with the nitrosoureas, the changes in optical densities at 230 nm of the following solutions were followed: 0·083 mM nitrosourea in buffer; 0·083 mM nitrosourea and 0·833 mM cysteine hydrochloride in buffer; and 0·833 mM cysteine hydrochloride in buffer.

The temperature of the cell compartment was maintained at approximately 37° by passing water through thermospacers.

Figure 1a shows that MNNG decomposes steadily in 0·2 M phosphate buffer; the half-life under these conditions is approximately 55 min. In agreement with the data of Lawley and Thatcher,[17] cysteine caused a great acceleration of the decomposition of MNNG. By the time that the mixture of MNNG and cysteine was placed into the spectrophotometer and a measurement made, the absorbance was about one-half that of a solution of MNNG in buffer, and it continued to decrease rapidly. The absorbance of cysteine at 400 nm is negligible.

Figure 1b shows that 1-methyl-1-nitrosourea (MNU) decomposes more rapidly than MNNG in buffer. This part of Fig. 1 also shows that the absorbance of cysteine in buffer at 230 nm under the described conditions decreases with time, perhaps because of oxidation to the disulfide. Therefore the observed change in the absorbance of the solution containing both MNU and cysteine might be due to a combination of factors, including decomposition of MNU, oxidation of cysteine, and reaction of cysteine with MNU or materials derived from MNU. If the cysteine causes no catalysis of the decomposition of MNU and if there is no reaction between cysteine and MNU, then the slope of the curve for the mixture of these compounds should be similar to that for the sums of the absorbances of the individual solutions of the two compounds. The curve for the sums of Fig. 1b is very similar to the curve for the mixture and therefore there is little or no evidence of interaction between cysteine and MNU. The small quantitative differences in the absorbances for the sums and the

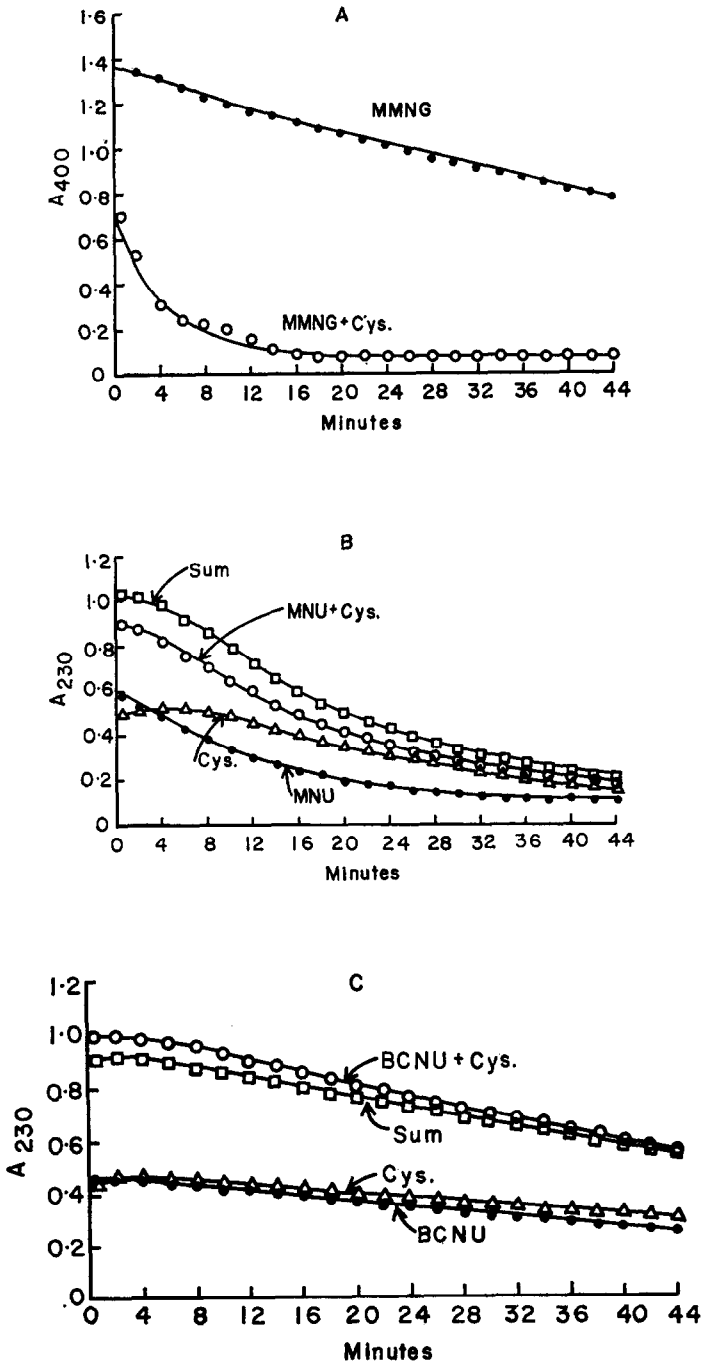

Fig. 1. Effects of cysteine upon the change of absorbance of solutions of the indicated compounds during incubation in 0·2 M phosphate buffer, pH 7·4, at 37°. The points on the curve marked "Sum" were obtained by adding the absorbances of the solution of cysteine (Cys.) and the solution of 1-methyl-1-nitrosourea or of 1,3-bis(2-chloroethyl)-1-nitrosourea.

mixture are probably due to experimental errors involved in measuring and transferring small volumes of solutions. It should be noted that the ratio of cysteine to MNU was 10:1, whereas the ratio of cysteine to MNNG was 1:1.

The curves obtained for BCNU (Fig. 1c) show that the BCNU decomposes more slowly than MNU under the conditions of the experiment and that cysteine did not alter the rate of decomposition. Similar results were obtained with CCNU.

These results indicate that the sulfhydryl compounds occurring in cells and biological fluids probably would have little effect upon the rate of generation of alkylating moieties and of isocyanates from nitrosoureas, whereas sulfhydryl compounds might greatly accelerate the decomposition of 1-methyl-3-nitro-1-nitrosoguanidine with the resulting generation of reactive moieties. These chemical differences might partially account for the differences between the biological effects of these two types of compounds.

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