Biochemical Pharmacology, Vol. 28, pp. 2521-2524.

0006-2952/79/0815-2521 $02.00/0

STEREOSELECTIVE ACTIVATION OF VICINAL DIHALOGEN COMPOUNDS TO MUTAGENS BY GLUTATHIONE CONJUGATION.

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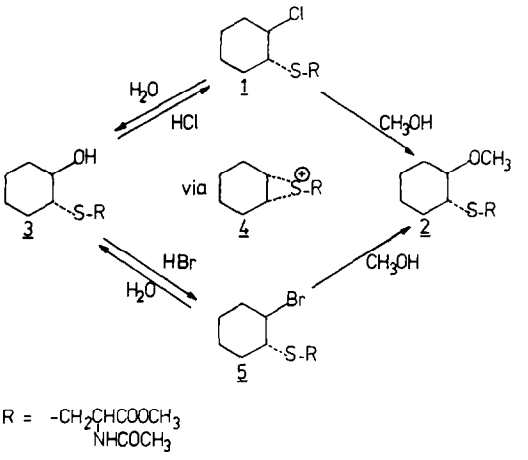
(*Received* 20 *April* 1979; *accepted* 1 *June* 1979)

Recently, the conjugation to glutathione, one of the major detoxification pathways in mammals ultimately leading to the formation of N-acetyl-S-cysteine derivatives (mercapturic acids) has be implied in the activation of foreign compounds to potentially carcinogenic electrophiles: In a re on the mutagenicity of 1,2-dichloro-ethane[1] it was shown that the mutagenic effects of this compc toward bacteria were greatly enhanced by the presence of cytosol (115.000 g supernatant), as a source of glutathione transferases, and added glutathione. In terms of chemical reactivity, this phenomenon is readily explainable[2], because substitution of one of the chlorine atoms by glutathi produces the structure of a β-halogen thioether or sulfur mustard, one of the oldest mutagens knc In fact, S-(2-chloroethyl)-L-cysteine was tested in Drosophila as early as 1960[4], and found to be mutagenic. In this communication we present further evidence for the hypothesis that the mutageni of vicinal dihalogen compounds is the result of their conversion into glutathione conjugates. It shown that the mutagenic activity is strongly dependent upon stereochemical factors. Substituted cyclohexanes were chosen in a study on the relation between chemical structure, metabolism and mutagenicity as model substrates. They combine several favourable characteristics such as synthet availability, well defined stereochemistry and relatively low volatility. In addition, S-cyclohex -N-acetylcysteine methyl ester derivatives can serve as model compounds for the corresponding glutathione conjugates and mercapturic acids.

Firstly the chemical behaviour of a number of methyl S-cyclohexyl-N-acetylcysteine derivatives, substituted at position 2 of the cyclohexylring with different leaving groups, was studied. Methy S-(trans-2'-chlorocyclohexyl)-N-acetylcysteine (1) was prepared via addition of the sulfenylchlor of methyl N-acetylcysteine to cyclohexene. The reactivity of 1 is illustrated by its complete conversion into the corresponding 2-methoxy derivative 2, simply by dissolution in methanol (4 h, r.t. and by its reaction with water (2 h, r.t.) to give methyl S-(trans-2'-hydroxycyclohexyl)-N- -acetylcysteine (3). The observed retention of the trans-configuration provides additional evider that these reactions proceed via the thiiranium ion 4. Conversely, the chloride 1 and the

corresponding bromide 5 can be obtained from 3 by treatment at room temperature with a solution of the appropriate hydrogen halide in methanol.[5]



The apparent reactivity of the thiiranium ion 4 indicates its potential to act as a mutagen by alkylating macromolecular cell constituents. This possibility was verified by assaying the direc mutagenic activity toward the base pair substitution sensitive, histidine-requiring strain TA 10(Salmonella typhimurium in the standard plate test as described by Ames et al.[6] The results,prese in Fig. 1, show a clear dependence of mutagenicity on the leaving group ability of the substitue the 2'-bromo and the 2'-chlorine substituted compounds being more mutagenic than the other derivat

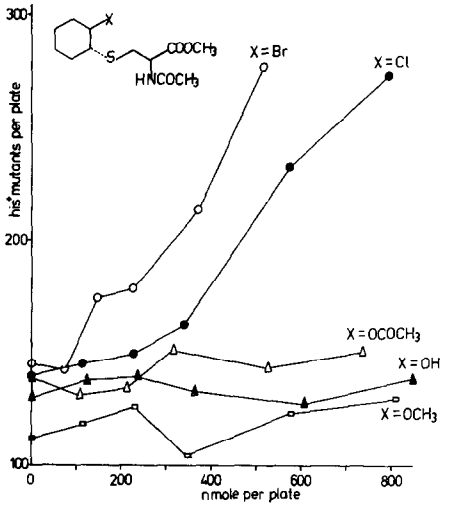
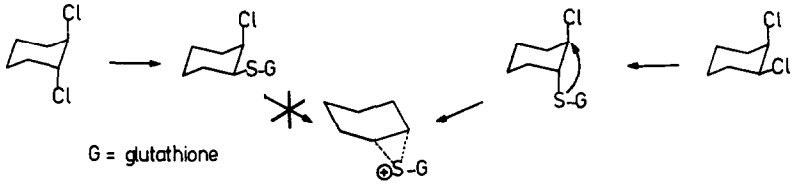


Fig. 1

Induction of reverse mutations in S-typhimurium TA 100 by 2'-trans substituted methyl S-cyclohexyl-N-acetyl-cysteines as a function of dose per plate. The experimental values are the mean of three parallel runs of one representative experiment. Experiments were performed according to the procedure described by Ames[6] with slight modifications: 0.3 ml overnight culture of TA 100, 0.5 ml nutrient broth and 0-0.07 ml of a solution of the compound in DMSO were mixed and incubated for 15 min at room temperature. Then 10 ml of soft agar (48°C) was added, and 3.0 ml of this mixture was poured on his⁺ mutation plates. The plates were incubated at 37° C for 72 hours.

It should be pointed out that, because formation of the thiiranium ion takes place via an intramolecular displacement of the leaving group by sulfur, the relative position in space of the two substituents should be a factor of crucial importance in determining the mutagenic properties of vicinally substituted glutathione or cysteine derivatives.

This hypothesis was put to test by comparing the mutagenic behaviour of cis- and trans-1,2-dich cyclohexane with and without metabolic activation. Assuming that glutathione transferases catalyz a S_N2-reaction, cis-1,2-dichlorocyclohexane would be expected to give a trans-substituted conjuga whereas trans-1,2-dichlorocyclohexane should give the corresponding cis-conjugate, in which the sulfur atom is not in a favourable position to activate the halogen substituent.



Four sets of experiments were carried out, again using the Salmonella TA 100 strain. In the fir one, no activating system was added. The second set of experiments was carried out in the usual w with rat 9000 g supernatant added to the incubation mixture. A third set was done in the presence only microsomes, NADPH and glutathione and in the final set 100.000 g supernatant + glutathione w added to the mixture. As can be seen from Fig. 2, the trans-compound is not active under any of t conditions used.

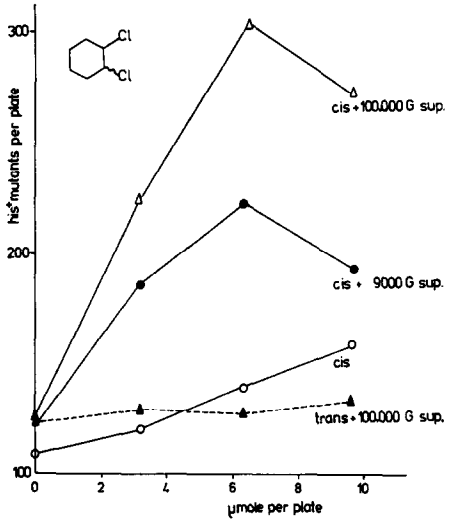


Fig. 2

Induction of reverse mutations in S-typhimurium TA 100 by cis- and trans-1,2-dichloro-cyclohexanes with and without added metabolizing systems. Livers of 6 Wistar-rats (♂, 200 g) were pooled. One part was used to prepare the standard 9000 g sup., another part to prepare 100.000 g sup. (cytosol) and microsomes. Experimental conditions were as described in Fig. 1. 0.2 Ml metabolizing system and 0.6 mmol of glutathione were added to the reaction mixture.

The experiment with cis-substrate + microsomes and glutathione gave the same values as cis alone. For the trans compound only the experiment with 100.000 g sup. + gluth. is shown. Identical values were obtained under the other conditions tested.

The cis-isomer however already appears slightly mutagenic by itself and upon addition of 9000 g supernatant. Addition of only microsomes + NADPH does not markedly increase the yield of his^+ mutations. In the presence of 100.000 g supernatant and of excess glutathione, however, cis-1,2-dichlorocyclohexane becomes a powerful mutagen. These experiments thus provide a clear demonstra of the importance of stereochemical factors in determining mutagenicity caused by glutathione conjugation.[7]

The small but distinct mutagenic activity shown by cis-1,2-dichlorocyclohexane in the absence activating additives is in line with a similar activity displayed by 1,2-dihalogenoethanes[1,8] an may well be due to spontaneous reaction with thiols or amines, giving rise to mustard-type compo similar to the glutathione conjugates. Alternatively, it may indicate that the Salmonella typhim cells used in these experiments do have some intrinsic activating potential.

The appreciably smaller mutagenic activity observed with 9000 g as compared with 100.000 g supernatant can probably be ascribed to the presence of large numbers of protein molecules in th former, which may act as scavengers for the mutagenic electrophiles. This raises the question whether the mutagenicity of some compounds that would give a positive result when tested in the presence of added 100.000 g supernatant, could escape detection when subjected to the standard Ames test. It can be concluded that in principle any compound substituted with good leaving grou on vicinal carbon atoms can be converted into a potential mutagen by glutathione conjugation. Th extent of mutagenic activity, as observed by the (modified) Ames test, is strongly dependent, no only on the nature of these substituents, but also on their spatial relationship.

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