

The role of -SH groups in the resistance of *E. coli* cells to *cis*-diamminedichloroplatinum(II) (*cis*-DDP)

Tomasz H. Zastawny and Ryszard Oliński

Department of Clinical Biochemistry, Medical School, M. Skłodowskiej-Curie 9, 85-094 Bydgoszcz, Poland

Resistance to cancer chemotherapeutic drugs is one of the major limitations to their clinical use. Decreased accumulation of the *cis*-DDP-DNA adducts and altered repair of *cis*-DDP induced DNA lesions are the mechanisms which may be involved in the development of resistance to *cis*-DDP [1 - 4]. Another mechanism by which resistant cells might tolerate high doses of *cis*-DDP is through inactivation of the drug by sulfur containing compounds in the cell. Platinum complexes have high affinity towards sulfhydryl groups, elevated intracellular levels of which can block coordination of the drug to DNA [1, 2]. The sulfhydryl groups within the cell are present both in protein and nonprotein compounds, in the latter mainly in glutathione.

It is generally believed that *cis*-DDP-DNA lesions are removed by repair processes which recognize UV-like damage [5]. Therefore it is reasonable to expect that experiments with bacterial strains which are deficient in the expression of the enzyme(s) capable to remove the damage may give some insight into the mechanisms by which cells become resistant to the drug. In our study we have used *E. coli* strain AB 1157 *uvr*⁺ and strain AB 1886 which is *uvrA* deficient. (These strains were a kind gift of Prof. I. Pietrzykowska of the Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw).

To characterize the mechanism(s) of resistance to *cis*-DDP we developed a cisplatin resistant sublines from the *E. coli* K12 "wild" strain AB 1157 (*uvr*⁺) and *uvrA* deficient strain AB 1886. In our previous studies we have shown that the reduction in accumulation of DDP may

be only partly responsible for the bacterial cells resistance, and this mechanism was reported only for the cells deficient in the repair system [6].

The aim of the present studies was to examine the role of intracellular sulfhydryl containing compounds in the drug resistance.

Bacteria were grown in Luria Broth (LB) [2] at 37°C with aeration. To induce *cis*-DDP resistant sublines from AB 1157 and AB 1886 strains, the cells were grown at an initially low concentration of *cis*-DDP (10 µM) for 2 h. Then the cells were plated on selective medium containing 10 µM *cis*-DDP. After 24 h incubation at 37°C the surviving colonies were transferred to the LB medium containing 50 µM *cis*-DDP and the whole procedure was repeated. After subsequent five steps of a gradual increase of *cis*-DDP concentration (during 1 month) the cells grew normally at a concentration of 500 µM.

In order to quantify the amount of sulfhydryl groups in cells the bacteria were incubated in LB medium containing *cis*-DDP as described previously [6], then were washed several times with 10 mM Tris/HCl, 25% sucrose and 10 mM EDTA, pH 7.2.

Sulfhydryl groups were determined by the method of Ando *et al.* [7], nonprotein sulfhydryl groups were estimated after deproteinisation by 20% trichloroacetic acid.

As it can be seen in Fig. 1, there is a distinct difference between the two control sublines of *E. coli* in total sulfhydryl group content in the cells. The two control cell lines contain practically the same amounts of nonprotein sulfhydryl residues (Fig. 2). Consequently, the higher content of protein sulfhydryl groups

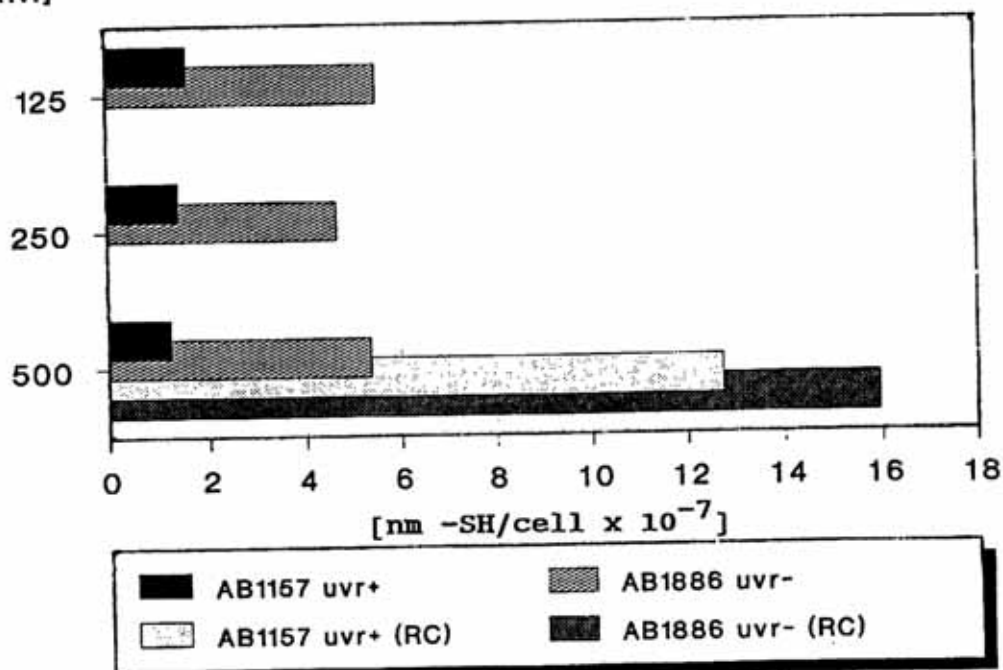
cis-DDP [μM]

Fig. 1. Total level of -SH groups in bacterial cells after cis-DDP treatment.

The amount of -SH groups was measured in control cells which were treated with the indicated concentrations of cis-DDP and in resistant cells (RC) which grew continuously in 500 μM cis-DDP

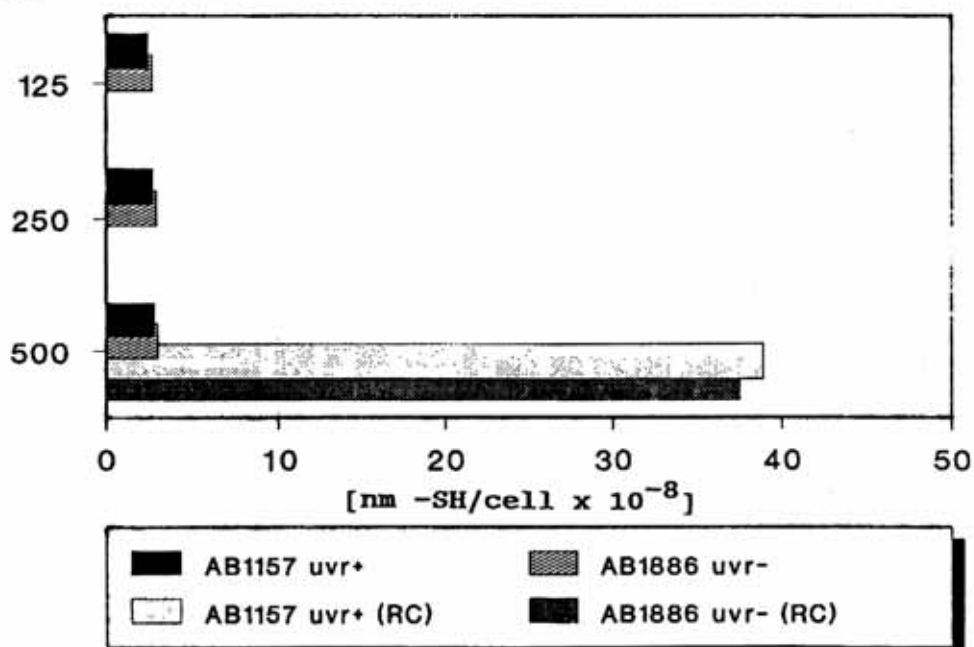
cis-DDP [μM]

Fig. 2. Level of nonprotein -SH groups in bacterial cells after cis-DDP treatment.

The amount of -SH groups was measured in control cells which were treated with the indicated concentrations of cis-DDP and in resistant cells (RC) which grew in 500 μM cis-DDP

should be responsible for the observed higher number of total sulfhydryl groups in the *uvr*⁻ cells.

uvr Genes are believed to play an important role in cell defence against several genotoxic agents [8]. Therefore it seems that the sulfhydryl-group containing proteins especially at higher concentration, may have a protective effect against such agents and thus compensate (at least partly) for the deficiency of repair enzymes within the cell.

The resistant sublines have shown several-fold higher levels of both total and nonprotein sulfhydryl residues (Fig. 1 and Fig. 2), the increase of the latter residues (presumably glutathione) being more pronounced. This suggests that the increased concentration of glutathione may contribute to a major extent to the *cis*-DDP induced resistance.

In recent years evidence has been accumulated concerning the role of endogenous thiol as modulator of responses to various genotoxic compounds [9]. In accordance with these studies we have shown that elevated levels of sulfhydryl residues can constitute the most efficient mechanism leading to development of resistance of the cells against the anticancer drug *cis*-DDP.

REFERENCES

1. Bruhn, S.L., Toney, J.H. & Lippard, S.J. (1990) *Prog. Inorg. Chem.: Bioorg. Chem.* **38**, 477 - 516.
2. Eastman, A. (1987) *Pharmacol. Ther.* **34**, 155 - 166.
3. Eastman, A. (1988) *Biochemistry* **27**, 4730 - 4734.
4. Mann, S.C., Andrews, P.A. & Howell, B. (1990) *Cancer Chemother. Pharmacol.* **25**, 236 - 243.
5. Germanier, M., Defais, N., Johnson, P. & Villani, G. (1984) *Mutation Res.* **145**, 35 - 41.
6. Zastawny, T.H. & Oliński, R. (1991) *Appl. Biol. Commun.* **1**, 127 - 134.
7. Ando, Y. & Steiner, M. (1973) *Biochim. Biophys. Acta* **311**, 26 - 37.
8. Scancar, A. & Ruoo, W.D. (1983) *Cell* **33**, 249 - 260.
9. Andrews, P.A., Murphy, M.P. & Howell, S.B. (1985) *Cancer Res.* **45**, 6250 - 6253.