ACQUISITION OF RESISTANCE TO ALKYLATING AGENTS BY EXPRESSION OF METHYLTRANSFERASE GENE IN REPAIR-DEFICIENT HUMAN CELLS

Mutsuo Sekiguchi¹, Hiroshi Hayakawa¹, Ken-ichi Kodama¹, Kanji Ishizaki² and Mituo Ikenaga²

¹Department of Biochemistry, Faculty of Medicine, Kyushu University, Fukuoka 812, and ²Radiation Biology Center, Kyoto University, Kyoto 606, Japan

INTRODUCTION

Alkylating agents are potent mutagens and carcinogens and sometimes cause cell death. These effects of alkylating agents are mainly attributed to the formation of various alkylated bases in DNA. More than ten kinds of methylated bases are produced by the treatment of cells with a simple methylating agent, such as N-methyl-N-nitrosourea (MNU) and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG).

To counteract such effects, many biological systems possess elaborate mechanisms to repair alkylated lesions in DNA. There are a variety of enzymes that recognize and repair such lesions, and DNA methyltransferase is one of the most notable. It transfers methyl groups from O⁶-methylguanine and/or other methylated moieties of the DNA to its own molecule, thereby repairing DNA lesions by a single step reaction. The enzyme is found in many organisms, from bacteria to human cells.

Some human tumor cell lines show the Mer⁻ (Mex⁻) phenotype characterized by defects in repair of O⁶-methylguanine (Yarosh, et al., 1983). Since the Mer⁻ cells are deficient in O⁶-methylguanine-DNA methyltransferase activity, we examined whether introduction of the cloned bacterial gene encoding methyltransferase would make Mer⁻ cells resistant to alkylating agents. Here we describe the result of these experiments and approaches which can be used for cloning of the human repair genes.

METHYLTRANSFERASE GENES OF E. COLI

The ada gene of Escherichia coli encodes a 39,000-dalton methyltransferase. The gene has been cloned from E. coli strains K12 and B, and the entire nucleotide sequence of the ada coding and control regions has been determined (Demple, et
The Ada protein catalyzes transfer of the methyl groups from alkylated DNA to its own protein molecule. Analysis of the purified Ada protein revealed that it carries two distinct methyltransferase activities, one to transfer a methyl group from methylyphosphotriester and the other to transfer a methyl group from O^6-methylguanine. These two activities reside on the N-terminal and the C-terminal halves of the protein, and can be separated by proteolytic cleavage (Yoshikai, et al., 1988). A specific cysteine residue (Cys^69) close to the amino-terminus of the Ada protein could accept the methyl group from one of the two stereoisomers of methylyphosphotriesters in alkylated DNA while a cysteine residue (Cys^321) which is present near the carboxy-terminus could be an acceptor site from O^6-methylguanine (Teo, et al., 1986). Takano, et al. (1988), recently constructed mutant forms of the ada gene in which each one of the codons for cysteine is replaced by that for alanine. The results obtained with such mutant genes support the notion that Cys^69 and Cys^321 are the methyl acceptor sites from methylyphosphotriester and O^6-methylguanine, respectively, and further revealed that Cys^321 accepts a methyl group also from a minor methylated base, O^5-methylthymine.

Evidence for the presence of a second methyltransferase enzyme in E. coli was recently reported (Potter, et al., 1987). This gene, named ogt, codes for a protein with a molecular weight of 19,000, which resembles the C-terminal half of Ada protein. The enzyme repairs O^6-methylguanine in the DNA.

An amount of the Ada protein increases when cells are exposed to a low level of alkylating agents whereas an amount of the Ogt protein is unchanged by such treatments. On induction of the ada gene expression, genes such as alkA, alkB, and aidB which are involved in repair of alklation damage are also induced. Since no such induction occurs in ada^- cells, it was suggested that the Ada protein, which itself is a methyltransferase, acts as a positive regulator both for its own synthesis and for the expression of other genes belonging to the ada regulon. Support for this idea was obtained by findings in in vitro transcription experiments (Teo, et al., 1986; Nakabeppu and Sekiguchi, 1986).

**EXPRESSION OF THE ADA GENE IN HUMAN CELLS**

Yarosh, et al. (1983), reported that about a fifth of human tumor cell lines show the Mer^- (Mex^-) phenotype, which is characterized by the inability to support the growth of MNNG-treated adenovirus and also by defects in the repair of O^6-methylguanine produced in cellular DNA. The Mer^- cells are deficient in methyltransferase activity and are much more sensitive than repair-proficient Mer^+ cells to killing by alkylating agents. Thus, experiments were performed to observe whether introduction of the cloned ada gene would make Mer^- cells resistant to alkylating agents.

A DNA fragment carrying the ada gene was inserted into pSV2neo vector. The ada gene was flanked by an SV40 promoter sequence and a poly(A) site on the 5' and 3' ends, respectively.