O$_6$-Alkyguanine-DNA Alkytransferase Activity in Normal Human Tissues and Cells

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ABSTRACT

Normal adult human tissues and cultured bronchial epithelial cells and fibroblasts exhibit O$_6$-alkylguanine-DNA alkytransferase activity in vitro by catalyzing the repair of the promutagenic alkylation lesion O$_6$-methylguanine from DNA. The amount repaired by extracts of liver, peripheral lung, and colon extracts was proportional to the amount of extract protein. Repair of O$_6$-methylguanine led to stoichiometric regeneration of guanine in the DNA and stoichiometric formation of S-methylcysteine in protein. Alkytransferase activity varies in the different human tissues tested in the decreasing order of liver > colon > esophagus > peripheral lung > brain. Extracts of lung tissues, cultured tissues tested in the decreasing order of liver > colon > esophagus > peripheral lung > brain. Extracts of lung tissues, cultured tissues tested in the decreasing order of liver > colon > esophagus > peripheral lung > brain. Extracts of lung tissues, cultured tissues tested in the decreasing order of liver > colon > esophagus > peripheral lung > brain. Extracts of lung tissues, cultured tissues tested in the decreasing order of liver > colon > esophagus > peripheral lung > brain.

RESULTS

Extracts from a variety of human tissues were found to contain O$_6$-alkylguanine-DNA alkytransferase activity (Chart 1; Table 1). This activity was characterized and quantitated in 3 ways (Chart 1): (a) measuring the specific loss of labeled O$_6$-mGua from a $^3$H-methylated DNA substrate; (b) measuring the production of protein containing S-[$^3$H]methylcysteine during the reaction with...
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this DNA substrate; and (c) measuring the formation of [8-3H]-guanine in DNA when the extracts were incubated with a synthetic DNA substrate containing O6-mGua labeled in position 8. Increasing amounts of protein of liver, colon, and lung extracts gave proportional increases in transalkylase activity until more than 1.2 pmol of O6-mGua (corresponding to about 65% of the input) were removed. The loss of O6-mGua occurred in parallel with a stoichiometric formation of S-methylcysteine in protein. With the synthetic DNA substrate, the loss of O6-mGua was accompanied by a stoichiometric generation of guanine. Aliquots of selected samples were also assayed by measurement of the methylated purine content after separation by high-performance liquid chromatography. Both methods gave the same results for O6-mGua content, and the high-performance liquid chromatographic analysis indicated that 7-methylguanine was not released from the DNA substrate.

The specific activity of specimens from several human tissues was measured under linear conditions and compared with the activity in corresponding rat tissues (Table 1). Extracts of human colon, esophagus, and lung had lower activities than those found previously in human liver samples but showed somewhat higher activities than in human brain. When compared to the corresponding rat tissue, human tissue samples contained 2- to 10-fold higher levels of alkyltransferase activity.

As can be seen in Chart 2, all 10 human colon specimens and 12 of 13 human lung specimens showed higher alkyltransferase activity than the mean value obtained in the corresponding rat organs (Table 1). The interindividual variation of activity on both the lung and colon samples was about 4- to 5-fold and showed a unimodal distribution.

We also compared O6-alkylguanine-DNA alkyltransferase activity in 2 types of cultured human bronchial cells. The removal of O6-mGua was proportional to the protein content of the extracts in both cell types with up to 2 mg of protein added. When assayed under linear conditions, human fibroblasts and epithelial cells removed 193 ± 45 and 137 ± 28 fmol O6-mGua per mg of protein (S.D.), respectively. No significant difference was observed between either exponentially growing or highly confluent bronchial fibroblasts. When the activity of Chinese hamster V79 cells was assayed under similar conditions as the human cells, the activity of V79 cells was so low that it could only just be detected in extracts obtained from 10⁶ cells and corresponded to 1.6 ± 1.2 fmol of O6-mGua removed per mg of protein.

DISCUSSION

Several types of human tissues and bronchial epithelial cells and fibroblasts exhibited substantial O6-alkylguanine-DNA alkyltransferase activity when crude extracts were isolated and tested in vitro with methylated DNA as a substrate. By using 3 different methods, the removal of the O6-methyl group from DNA was correlated with the formation of S-methylcysteine in protein and the regeneration of guanine in DNA. At all protein concentrations, there was good agreement between the 3 assays, establishing that the reaction does take place by stoichiometric transfer of the methyl group from the O6-position of guanine to a cysteine acceptor site on the protein. Furthermore, assay of the methylated purine content of the DNA substrate indicated that the extracts did not contain significant nonspecific nuclelease activity which could contribute to the loss of O6-mGua.

A comparison of the alkyltransferase activity of the different human tissues with the corresponding rat tissues indicated that human tissues have much higher activities. Human liver was previously shown to be 10-fold more active than rat liver (16) and all of the human colon, lung, and esophagus samples except one showed higher activity than the mean value of the corresponding rat tissues (cf. Table 1; Chart 2). Whereas each tissue from individual inbred rats showed a typical experimental variation that was less than 20%, the human colon (10 cases) or lung samples (13 cases) showed a 400 to 500% interindividual vari-
ation. In a recent study, Krokan et al. (9) have shown a less than 10-fold interindividual variation of alkyltransferase activity in extracts prepared from liver, colon, and stomach. However, a 42-fold interindividual variation was observed in 12 specimens from small intestine. Several human fetal tissues contain similar levels of alkyltransferase activity as adult tissues, except that 5-fold lower levels were observed in fetal than in adult liver (9). The alkyltransferase activity of extracts from human lymphocytes has been shown to vary approximately 8-fold (19, 20), and human lymphocytes vary considerably in their capacity to remove O6-mGua (7). However, the levels of this DNA repair activity in human tissues and cells do not vary among individuals as much as the activities of enzymes responsible for metabolic activation and deactivation of chemical carcinogens (6).

Since the O6-alkylguanine-DNA alkyltransferase is consumed and inactivated by accepting the methyl group from the DNA substrate, the number of acceptor sites present in the tissues or cells can be estimated. Assuming that only one methyl group was bound per molecule, and that calculation was on the basis of DNA content, human liver contains approximately 500,000 alkyltransferase molecules per cell, whereas colon, esophagus, and peripheral lung contain 76,000, 65,000, and 39,000 enzyme molecules, respectively. Isolated cultures of human bronchial fibroblasts and human bronchial epithelial cells both contained the activity. Although the fibroblasts were slightly more active in this respect on a per protein basis, the 2 cell types have similar activities on a per cell basis, i.e., 26,000 (epithelial cells) and 29,000 (fibroblasts) alkyltransferase molecules/cell. Previous estimates of alkyltransferase activity in cultured human cells similarly indicate high levels in skin fibroblasts (13), while different human tumor cell lines range from nondetectable levels to 2 × 10^5 alkyltransferase molecules/cell (4, 23). The activity of the bronchial cells is about one-half of the activity present in freshly isolated rat hepatocytes (18) but is very much greater than in cultured V79 hamster lung fibroblasts which contained very little activity. The activity of V79 cells was so low that it was barely detectable in extracts obtained from large numbers of cells and amounts to only 200 molecules/cell. Although Waldstein et al. (21) found 94,000 molecules/cell in their cultures of V79 cells, results from other studies agree with the low activity of O6-alkylguanine-DNA alkyltransferase reported here (2, 4, 14, 23).

In conclusion, several human tissue and cell types demonstrate a capacity to repair O6-mGua lesions in DNA. For all tissues compared, this capacity is generally several-fold higher than in rats, in which N-nitroso compounds cause the formation of O6-alkylguanine lesions in cellular DNA and tumors in many organs (12). Further studies are required to elucidate the relationship, if any, of the comparatively high levels of O6-alkylguanine-DNA alkyltransferase activity in normal human tissues and cells and oncogenic susceptibility to N-nitroso carcinogens in humans.

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