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SUMMARY

A polynucleotide containing G-C8-AF residues was obtained by treatment of poly(dG-dC) with the carcinogen N-hydroxy-2-aminofluorene. The resulting product [³H]-AF-poly(dG-dC) was further incubated in 0.1 N NaOH for 24 hours at 37°C, which resulted in the conversion of 60% of the G-C8-AF residues to their imidazole ring-opened derivative (iro-G-C8-AF). This modified polynucleotide was used as substrate for the Fapy-DNA glycosylase of E. coli. H.P.L.C. analysis of the products of the reaction shows that the pure Fapy-DNA glycosylase excised the ring-opened derivative (iro-G-C8-AF). In contrast, the primary lesion (G-C8-AF) was not removed. These results show that the Fapy-DNA glycosylase of E. coli excises imidazole ring-opened purines which are modified at the C8 position. These observations suggest that the Fapy-DNA glycosylase may have a broad substrate specificity which includes all imidazole ring-opened purines modified at the N7 or C8 position in DNA.

INTRODUCTION

The N7 and C8 positions of guanine in DNA are major targets for mutagens and carcinogens (Singer and Grunberger, 1983). Modified guanine residues are considerably less stable than the parent bases. This instability may result in the formation of ring-opened derivatives after cleavage of the imidazole ring across N7-C8 or C8-N9 bonds. Such decomposition products have been identified in the DNA of animals treated by chemical carcinogens, including: N-methylnitrosourea (Kadlubar, et al., 1984), aflatoxin B1 (Essigman, et al.,
1984), and 2-naphthylamine (Kadbular, et al., 1981).

The ring-opened form of N7-methylguanine (iro-me7-G or Fapy) is removed from the DNA by a specific DNA glycosylase in E. coli (Chetsanga and Lindahl, 1979; Boiteux, et al., 1984) and in mammalian cells (Margisson and Pegg, 1981; Laval, et al., 1988). The gene coding for the Fapy-DNA glycosylase of E. coli has been cloned and sequenced (Boiteux, et al., 1987). The Fapy DNA glycosylase of E. coli exhibits a substrate specificity which is not limited to iro-me7-G, since it excises the ring-opened form of guanine residues modified at the N7 position by aflatoxin B1 (Chetsanga and Frenette, 1983), or phosphoramide mustard (Chetsanga, et al., 1982). These observations suggest that the ring-opened form of alkylated purines might play a significant role in biological processes leading to mutagenesis and/or cell death by chemical carcinogens.

In this paper, we show that the Fapy-DNA glycosylase of E. coli removes the imidazole ring-opened form of guanine residues modified at the C8 position by the carcinogen N-hydroxy-2-aminofluorene.

**MATERIAL AND METHODS**

**Preparation of poly(dG-dC) containing [³H]-G-C8-AF and [³H]-iro-G-C8-AF residues**

Poly(dG-dC) (Boehringer Mannheim) was reacted with [³H]-N-OH-AF (140 mCi/mmol) as previously described (Fuchs and Seeberg, 1984). The specific activity of the [³H]-AF-poly(dG-dC) was 1650 cpm/µg. The imidazole ring-opened derivative was obtained after incubation of [³H]-AF- poly(dG-dC) with 0.1 N NaOH for 24 hours at 37°C. Polynucleotides containing [³H]-me7-G or [³H]-iro-me7-G were prepared as described (Boiteux, et al., 1984).

**Analysis of the reaction products**

The authentic markers (G-C8-AF and iro-G-C8-AF) were prepared as described by Kriek and Westra (1980). The products were separated by H.P.L.C. using a C18 µBondapack column (Waters). The mobile phase was 20 mM NH₄H₂PO₄, pH 4.5, containing 60% methanol (V/V). The column was isocratically eluted at 1.0 ml/minute. The products were detected by UV absorption at 280 nm and by scintillation counting of fractions.

**Purification of the Fapy-DNA glycosylase of E. coli**

LB-broth medium (2.5 l) containing 50 µg/ml ampicillin was inoculated with 50 ml of an overnight culture of E. coli strain JM 105 carrying the pFPG220 (fpg) plasmid (O'Connor, et al., 1988). The bacteria were grown at 37°C for 2 hours, then supplemented with 0.5 mM IPTG and further incubated for 17 hours under vigorous agitation. The cells (10 g wet weight) were harvested and the Fapy-DNA glycosylase was purified according to Boiteux, et al., 1987.