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Mutational specificity of the ferrous ion in a *supF* gene of *Escherichia coli*[1]

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ABSTRACT A plasmid, pZ189, was treated with Fe^{2+}/EDTA, and mutagenesis was determined by DNA sequencing. In the *fgp*$^+$ *Escherichia coli* host, 78% were base substitutions, with G:C->C:G transversion (58.7%) predominant, followed by G:C->T:A transversion (28.3%). In the *fpg-1* mutant, 88% were base substitutions among which 46% were G:C->C:G and 42% G:C->T:A. Fe^{2+} resulted in increased formation of 8-hydroxydeoxyguanosine (8-ohdG) in pZ189 DNA. The origin of Fe^{2+}-induced G:C->T:A transversion may be 8-ohdG; on the other hand, the origin of G:C->C:G is neither 8-ohdG nor 2,6-diamino-4-hydroxy-5-formamidopyrimidine.

Oxygen radicals are produced by cellular metabolism, ionizing radiation and environmental carcinogens (1). These radicals have been demonstrated to produce a multiplicity of DNA modifications including 8-hydroxydeoxyguanosine (8-ohdG) and 2,6-diamino-4-hydroxy-5-formamidopyrimidine (Fapy-G) (2). Recent studies show that the 8-ohdG lesion in DNA leads to G:C->T:A transversion since dATP can be misincorporated opposite an 8-ohdG lesion (3).

In *Escherichia coli*, several mutator mutants have been isolated and the genes and their products which are involved in the repair of 8-

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ABBREVIATIONS used: 8-ohdG (8-hydroxydeoxyguanosine); Fapy-G (2,6-diamino-4-hydroxy-5-formamidopyrimidine).

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ohdG and Fapy-G have been characterized (4,5). The *fpg* gene product was characterized as a DNA glycosylase that excises 8-ohdG and Fapy-G from DNA (6). Thus, knockout of the *fpg* gene led to a specific increase in G:C->T:A transversion (4).

Several recent studies have focused on the determining the molecular nature of mutations induced by oxygen radicals at the DNA sequence level. Although oxygen radicals definitely induce G:C->T:A transversion in wild type *E. coli*, oxygen radicals could induce G:C->C:G transversion (7-11). The experiments here with *E. coli fpg*$^+$ and *fpg-1* strains are directed at understanding the mutational spectra induced by Fe^{2+} generated oxygen radicals. The results suggest that 8-ohdG is responsible for G:C->T:A transversion. On the other hand, neither 8-ohdG nor Fapy-G can be the lesion responsible for G:C->C:G transversion.

Materials and Methods

Bacterial strains and plasmids *Escherichia coli* strain KS40 (*lacZ*am *gyrA*) and plasmid pKY241 were described previously (12). Strain SY16 (*fpy-1*::km) is an F factor-negative derivative of JM107; *fpg-1*::km is derived from BH20 (5). Vector pZ189 carries the *supF* gene (13).
Chemicals $FeSO_4$ and EDTA were obtained from Wako Pure Chemical Industries. Catalase (bovine liver) and superoxide dismutase (bovine erythrocytes) were purchased from Sigma.
DNA treatment pZ189 DNA (5 μg in 100 μl of reaction mixture) was treated with freshly prepared solutions of different concentration of $FeSO_4$ with a 4-fold excess of EDTA at 37°C for 30 min in 0.1 M potassium phosphate buffer (pH 7.4). The DNA was precipitated, resuspended in 30 μl of distilled water and dispensed into 6 tubes.
Transfection and mutant analysis Competent KS40 or SY16 cells were transfected with Fe^{2+}-treated pZ189. After overnight incubation, cells were harvested to extract the replicated pZ189 plasmid. Progeny plasmids from individual transfections were assayed separately for the mutant *supF* gene to distinguish mutants that were occurred from siblings. Competent KS40 cells carrying the pKY241 plasmid were transformed with the pZ189 DNA recovered and transfectants with mutant *supF* were selected (10-12). Only one mutant colony was picked from each plate to ensure an independent origin for each mutation. The DNA was sequenced with a pBR322 *Eco*RI sequencing primer.
Quantitation of 8-ohdG in DNA The amount of 8-ohdG in pZ189 DNA was determined by the HPLC-ECD method (14).

Results

Formation of 8-ohdG

Treatment of DNA with Fe^{2+} yielded increasing amounts of 8-ohdG, and the formation of 8-ohdG was partly inhibited by catalase and superoxide dismutase (Table I). The results therefore suggest that the multiplicity of DNA lesions produced by Fe^{2+} is due to formation of oxygen-free radicals by catalyzing the reduction of oxygen in vitro.

Fe^{2+}-induced mutagenesis

Mutagenesis with 125 μM Fe^{2+}-treated pZ189 was 6- and 9-fold greater than in the controls using an *fpg*$^+$ host and an *fpg-1* host, respectively. Since *fpg-1* is defective for Fapy-G and 8-ohdG DNA glycosylase and Fe^{2+} induces 8-ohdG (Table I), the higher level of induction of mutation in *fpg-1* suggests that these adducts can be responsible for mutagenesis.

Type and spectrum of ferrous ion-induced mutations

Tables II and III summarize the class distribution and the types of the mutations. The majority of changes were single base substitutions and the rest were insertion sequence, -1 frameshift and deletion changes. Of the base substitutions obtained, 98% of the mutations were

Table I. Induction of 8-ohdG formation by Fe^{2+} and the effects of catalase and superoxide dismutase

additions[a)]	8-ohdG per 10^5 dG
none	5.9
Fe^{2+} (75 μM)	22.7
Fe^{2+} (125 μM)	49.1
+catalase (20 units/ml)	35.3
+catalase (200 units/ml)	15.9
+SOD (20 units/ml)	39.4

[a)] 50 μg of plasmid pZ189 was incubated in 0.1 M potasium phosphate buffer.
(pH 7.4) with different concentrations of Fe^{2+} at 37°C for 30 min.

Table II. Distribution of the Fe^{2+}-induced supF mutants by class

Class of change	Number detected (% of total mutants examined)	
	wild-type host	*fpg-1* host
Base substitution	46 (78%)	50 (88%)
Insertion sequence	6 (10%)	3 (5%)
Deletion[a)]	3 (5%)	2 (4%)
Frameshift	4 (7%)	2 (4%)
Total	59	57

[a)] 52 basepair deletions which were flanked by 10 nucleotide direct repeats were exclusively observed (positions 68 to 77 and 120 to 129, see Fig. 1).

in the G:C base pairs. G:C->T:A transversion was more frequent in the *fpg-1* host than in the *fpg*$^+$ host. *Fpg-1* strain is unable to repair 8-ohdG which is known to lead to G:C->T:A transversion (15). Therefore, formation of 8-ohdG in pZ189 (Table I) can contribute to the increase in G:C->T:A transversion in the *fpg-1* host.

Fig. 1 shows the spectrum of base substitutions and -1 frameshifts in the *supF* gene of pZ189 DNA. Mutations were seen at many sites throughout the gene, and there are hotspots at position 133.

Table III. Types of base substitution among Fe^{2+}-induced supF mutations

Type of mutation	Number detected (% of total substitutions)	
	wild-type host	*fpg-1* host
Transition		
G:C -> A:T	5 (10.9%)	5 (10%)
A:T -> G:C	0 (-)	0 (-)
Transversion		
G:C -> T:A	13 (28.3%)	21 (42%)
G:C -> C:G	27 (58.7%)	23 (46%)
A:T -> T:A	0 (-)	0 (-)
A:T -> C:G	1 (2.2%)	1 (2%)
Total	46 (100%)	50 (100%)

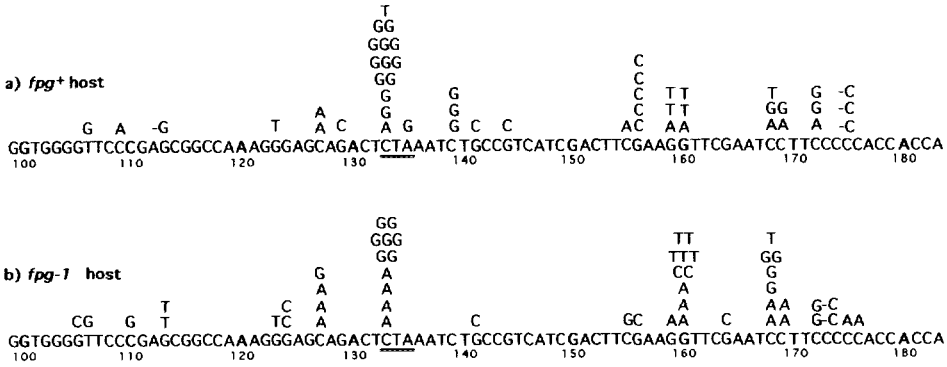

Figure 1. Location of base substitution and -1 frameshift mutations of the *supF* gene after transformation of a) an *fpg*$^+$ host or b) an *fpg-1* host with Fe2$^+$-damaged DNA. -C and -G indicate frameshift. Underline indicates the anticodon.

The dominance of mutations at G:C residues, the high frequency of G:C->C:G transversion (Table III), and the hotspots observed (Fig. 1) for mutagenesis by Fe^{2+} were observed in the hydrogen peroxide- and lipid peroxidation-induced mutations (10,11).

Discussion

We have investigated the molecular nature of Fe^{2+}-induced mutations in the *supF* gene by DNA sequencing. The data show that base substitutions constitute the majority of the *supF* mutations. These point mutations occurred predominantly at sites that can be specifically damaged by Fe^{2+}, i.e., G:C base pairs. Although we can not state which of these two bases is actually responsible for the mutation, our data together with other data (8) clearly suggest that the extracellular modification of plasmid pZ189 by Fe^{2+} treatment results in the formation of miscoding guanine damage upon replication in *E. coli* cells.

The G:C->C:G transverion is the most frequent base substitution observed. The dominance of G:C->C:G transversion was demonstrated in the mutagenesis experiments by γ-ray irradiation, methylene blue plus light, hydrogen peroxide and lipid peroxidation (7,9-11). All the treatments including Fe^{2+} produced oxygen radical lesions in the DNA.

DNA lesions produced after incubation with hydrogen peroxide (2) include 8-ohdG, thymine glycol, dihydroxycytidine and Fapy-G. Thus, it is reasonable to assume that, in addition to inducing the formation of 8-ohdG (Table I), Fe^{2+} treatment induces thymine glycol, dihydroxycytidine and Fapy-G. The lesion(s) responsible for G:C->C:G, however, is not one of the lesions mentioned above; i) McBride et al (8,9) observed that oxygen radicals could induce G->C, but not C->G, transversion in single-stranded M13mp2, suggesting that dG lesions involve G:C->C:G transversion. Therefore, thymine glycol and dihydroxycytidine cannot be candidates for G:C->C:G transversion. ii) 8-ohdG is known not to form a basepair with dG (3), and thus cannot lead to G:C->C:G. iii) G:C->T:A transversion was more frequent in the *fpg-1* host than in the wild-type host, but there was no difference in G:C->C:G transversion (Table III) which suggests that Fapy-G and 8-ohdG are not the lesions responsible for G:C->C:G. The origin of G:C->C:G is unidentified.

G:C->T:A transversion was the second most frequent class of base substitution observed. This implies that the 8-ohdG induced in the DNA by Fe^{2+} (Table I) can basepair with dA during DNA replication, resulting in G:C->T:A transversion (3,15). Since the *fpg-1* mutant cannot repair 8-ohdG in DNA, G:C->T:A transversion increased in the host (Table III).

The finding that incubation of Fe^{2+} with DNA produces DNA lesion (Table I) and a variety of mutations (Tables II and III) brings into focus the possibility that intracellular iron is mutagenic. Iron is found in strong association with cellular DNA (16) and could serve to generate hydroxyl radicals, particularly in the presence of hydrogen peroxide (17). Therefore, iron can be a source of spontaneous mutations.

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