Cobaltous chloride-induced mutagenesis in the supF tRNA gene of Escherichia coli

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The spectrum of mutations induced by cobalt(II) chloride (CoCl₂) was examined using plasmid pUB3 DNA, which was propagated after transfection into Escherichia coli SY1032/pKY241 host cells. The vector plasmid carried an E. coli supF suppressor tRNA gene as a target for mutations. After CoCl₂ treatment, 64 independent nalidixic acid-resistant, ampicillin-resistant and Lac⁺ (SupF⁺) clones were obtained and the altered sequences of the mutated supF⁻ genes were determined. Deletions and frameshifts were the predominant mutational event (61%) induced by CoCl₂ and base substitutions were induced to a lesser degree (29%). Analysis of sequence alterations at all the sites of mutation revealed that: (i) 18 of 19 base substitutions and eight of 10 frameshifts occurred at G:C sites, suggesting that the formation of N7G–Co(II) adducts may be responsible for premutagenic lesions of these mutations; (ii) short sequence repeats were mostly found at the sites of deletions and frameshifts. Slippage–misalignment is also suggested to be a mechanism for the induction of mutations at these sites.

Introduction

The cytotoxicity and carcinogenicity of metals and their compounds have attracted attention and been studied for a long time. Of special interest among metal compounds is cobalt. In previous studies, cobalt(II) chloride (CoCl₂) was suggested to be a mechanism for the induction of mutations in the eight of 10 frameshifts occurred at G:C sites, suggesting that the formation of N7G–Co(II) adducts may be a mechanism for the induction of mutations in the supF gene.

Materials and methods

Plasmids, bacterial strains and bacteriophage

Vector pUB3, described by Rodriguez et al. (1992), contains the tyrosine amber suppressor tRNA (supF) gene, lacZα gene and ampicillin resistance (Amp') gene used for mutagenesis studies. Escherichia coli K12 strains DL16 [leuB6 lac235 (am) recA1 rpsL151 deo1 Δ(fadE70 rpsL151 rpsL151) λ° lacY1 hsdR hsdM araD139 Δ(aroABC-λeu27E70 galU galK rpsL151)] were used to prepare plasmid pUB3 DNA and allow processing and replication of CoCl₂-treated plasmid pUB3 (Akausa et al., 1992). The indicator host bacterial strain used to distinguish wild-type and mutant pUB3 was SY1032/pKY241 [gyrA96 Δ(pro-lac) supF⁺ lacIΔΔΔΔ lacIΔΔΔΔ Δ(lacZ)ΔΔΔΔ Δ(araAB-λeu27E70) galU galK rpsL151] were used to prepare plasmid pUB3 DNA and allow processing and replication of CoCl₂-treated plasmid pUB3 (Akausa et al., 1992). The indicator host bacterial strain used to distinguish wild-type and mutant pUB3 was SY1032/pKY241 [gyrA96 Δ(pro-lac) supF⁺ lacIΔΔΔΔ lacIΔΔΔΔ Δ(lacZ)ΔΔΔΔ Δ(araAB-λeu27E70) galU galK rpsL151].

Reagents and media

CoCl₂ and other chemicals were purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan). Enzymes and reagents used for DNA manipulation and DNA sequencing were purchased from Takara Shuzo Co. Ltd (Kyoto, Japan) and PE Applied Biosystems Inc. (Foster City, CA). Compositions of L broth, L agar plates and M buffer were as described previously (Otsui et al., 1974). Minimal (M) lactose agar medium consisted of M broth containing 10 g/l lactose, 15 g/l agar and 2 mg/l thiamine.

Mutagenesis by CoCl₂

The mutagenesis was carried out by the method of Uematsu et al. (1997) with a slight modification. Plasmid pUB3 DNA (700 ng/reaction mixture), isolated from strain DL16/pUB3 according to the ‘midi’ protocol of the Quin kit (Quin Inc., Chatsworth, CA) (Lutze and Vinegar, 1990; Kimura et al., 1993), was exposed to freshly prepared solutions of 20 μM CoCl₂ for 2 h at 37°C in 0.1 M Tris–HCl buffer (pH 7.2). CoCl₂-treated pUB3 DNA was immediately transfected into competent KS40 cells which were induced by the CaCl₂/RbCl method (Maniatis et al., 1982). They were incubated overnight to attain plasmid replication and then the progeny plasmids were extracted from the cells by the alkaline lysis method (Birnboim and Doly, 1979). Progeny plasmids from individual transfections were assayed separately for the mutant supF gene to distinguish mutants that were derived from siblings. Competent SY1032/pKY241 cells were transfected with progeny plasmids and the transformants with supF mutant plasmids were plated on M lactose agar plates containing 50 μg/ml nalidixic acid (Nal), 30 μg/ml ampicillin (Amp) and 30 μg/ml chloramphenicol (Cm). To collect supF mutants, only one nalidixic acid-resistant (Nal') and Lac⁺ colony was chosen from each transformation experiment. This approach ensured that all mutants analyzed were of independent origin.

DNA sequencing

Single-strand DNA was prepared by infecting putative mutants [SY1032/pKY241/pUB3(supF⁺)] with M13KO7 phage and sequenced by the dideoxy chain termination method (Sanger et al., 1977) using an ABI automated sequencer model 373A, performed in exactly the same way as described previously (Uematsu et al., 1997). The polymerization reaction was primed.
CoCl$_2$-induced SupF$^*$ mutations

Using a vector pUB3 carrying the E. coli supF gene and the E. coli SY1032/pKY241 host cells, we analyzed the spectrum of CoCl$_2$-induced mutations. Initially, 700 ng of plasmid pUB3 DNA was exposed to 20 µM CoCl$_2$ for 2 h and then transfected into competent SY1032/pKY241 cells. The transformation frequency was ~9.7 × 10$^{-4}$, which was the same as that of untreated DNA. The mutation frequency of induced Nal$^r$ Lac$^+$ (SupF$^*$) transformants on the selective medium comprised of M lactose agar plates supplemented with Nal, Amp and Cml, averaged over eight experiments, was 2.5 × 10$^{-6}$. This value was ~8-fold higher than that of spontaneous mutations (3.1 × 10$^{-7}$). This fact indicated that 90% of the isolated mutant clones were probably induced by CoCl$_2$. Furthermore, from the treated cells, 64 Nal$^r$ Lac$^+$ (Amp$^+$) mutant clones of presumably independent origin were isolated from selective medium and vector DNA molecules containing the expected mutated supF$^*$ gene were recovered from all mutant cell clones. DNA sequences of the mutant supF$^*$ genes were determined as ~150 bp from the Pre-tRNA to the End-tRNA region, by the dideoxy chain termination method, and altered sequences were found in all 64 mutant genes. While 63 mutants carried one mutation each, one mutant, CC19, carried two base substitutions 6 bases apart. Thus we identified 65 mutations. The distribution of mutants in the supF$^*$ gene is shown in Figure 1.

Spectrum of CoCl$_2$-induced mutations

Table I lists CoCl$_2$-induced DNA sequence alterations in the supF$^*$ gene. Forty six percent of mutational events were deletions involving loss of 8–85 bp, whereas 29% were base substitutions. They differed from the spontaneous mutations, which occurred rather non-specifically (Akasaka et al., 1992). The remaining mutations were frameshifts (15%), insertion sequences (8%) and duplications (2%). Each class of mutations is hereby described in detail.

Deletions and duplication. The location and DNA sequence context at the sites of duplication and deletion mutations are presented in Table II. One duplication was recovered at site 66–73; this mutation was caused by the addition of 8 bp. Mutation at five of seven sites (55–111, 60–112, 68–129, 77–119 and 124–212) were large deletions (38–85 bp) and those at the remaining two sites (95–109 and 153–166) were deletions of 13 and 8 bp, respectively. One of these sites, 68–129, was found to be a hot-spot for CoCl$_2$ mutagenesis, since 20 mutations were recovered at this site. There were direct repeats of 10 bases (AAGGGAGCAG) flanking the fragment of 52 nucleotides to be deleted and one of the repeats was lost in the mutant sequences. This mutation was similar to spontaneous mutations in the same gene, as previously reported (Akasaka et al., 1992). A common sequence resulting from the deletion of 8–85 bp was also seen at the sites of other deletions. They had two short direct repeats of 2–7 bases and one of the repeats was missing in the mutant sequences. This unique sequence feature at the sites of deletions was frequently observed in spontaneous and topB mutant-induced mutations (Uematsu et al., 1997; Akasaka et al., 1992). We suggest slippage–misalignment (Ikehata et al., 1989; Akagi et al., 1990; Ogawa et al., 1993) to be the mechanism for CoCl$_2$ mutagenesis at the supF$^*$ system.

Eichhorn and Shin (1968) and Zimmer et al. (1974) reported that CoCl$_2$ reacts with DNA producing two major adducts [the N7G–Co(II) and -O$^3$PO–Co(II) adducts] and a chelate [N7G–Co(II)–OPO$_3$] at the N7 position of guanine (N7G) or/and the phosphate residue (-O$^3$PO$^-$). It has also been shown that induction of DNA strand breaks generated by oxygen free radicals arose in vivo by reaction of Co(II) ions with superoxide...
Table I. Classes of CoCl₂-induced and spontaneous mutations in an E.coli supF tRNA gene

<table>
<thead>
<tr>
<th>Class of mutation</th>
<th>Spontaneous mutations</th>
<th>CoCl₂-induced mutations</th>
<th>χ² value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base substitution</td>
<td>33 (63%)</td>
<td>19 (29%)</td>
<td>7.52</td>
</tr>
<tr>
<td>Transition</td>
<td>5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>G→A</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>A→G</td>
<td>28</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>G→T</td>
<td>9</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>A→T</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Frameshift γ</td>
<td>2 (2%)</td>
<td>1 (15%)</td>
<td>31.0</td>
</tr>
<tr>
<td>Deletion</td>
<td>5 (10%)</td>
<td>30 (46%)</td>
<td>76.4</td>
</tr>
<tr>
<td>Duplication</td>
<td>0</td>
<td>1 (2%)</td>
<td>–</td>
</tr>
<tr>
<td>Insertion sequence</td>
<td>13 (25%)</td>
<td>5 (8%)</td>
<td>0.64</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>Mutation frequency</td>
<td>3.1×10⁻⁷</td>
<td>2.5×10⁻⁶</td>
<td></td>
</tr>
</tbody>
</table>

aData from Akasaka et al. (1992).
bBased on experiments performed 398 times, 52 spontaneous mutants were isolated (Akasaka et al., 1992). In the case of CoCl₂-induced mutagenesis, we performed experiments >75 and <100 times and obtained 64 mutants. Thus, for calculating χ² values, we assumed that we performed experiments 100 times. χ²(0.01) is 6.63.

Table II. Deletions and duplication

<table>
<thead>
<tr>
<th>Site</th>
<th>Incidence</th>
<th>Sequence context</th>
<th>Nucleotide change</th>
<th>Mutant gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>55-111</td>
<td>1</td>
<td>GUGGCCAT...GTTCCCGAGC→GUGCAGCG</td>
<td>-53 bp</td>
<td>CC6</td>
</tr>
<tr>
<td>60-112</td>
<td>1</td>
<td>GUTCCCGAGC→GTTCCCGAGC→GTTCCCGAGC</td>
<td>-46 bp</td>
<td>CC4</td>
</tr>
<tr>
<td>66-73</td>
<td>1</td>
<td>CCGGTAAGGCGAGC→</td>
<td>+8 bp</td>
<td>CC63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CCGGTAAGGCGAGC→</td>
<td></td>
<td></td>
</tr>
<tr>
<td>68-129</td>
<td>20</td>
<td>GATAGGGGAGGTGAC...GAAAGGAGGACT</td>
<td>-52 bp</td>
<td>CC1, CC3, CC5, CC11, CC14, CC17, CC18, CC22, CC23, CC24, CC28, CC30, CC34, CC35, CC39, CC41, CC42, CC48, CC51, CC66</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GATAGGGGAGGTGAC...</td>
<td></td>
<td></td>
</tr>
<tr>
<td>77-119</td>
<td>2</td>
<td>GUGCAGACAT...GUGCAGAGAG→GCAGGCCAAG</td>
<td>-38 bp</td>
<td>CC15, CC38</td>
</tr>
<tr>
<td>95-109</td>
<td>1</td>
<td>TTACCGCA...GTTCCCGAG→TTACCGCAAG</td>
<td>-13 bp</td>
<td>CC53</td>
</tr>
<tr>
<td>124-212</td>
<td>2</td>
<td>AGCGCGGAG→AGCGCGGAG→AGCGCGGAG</td>
<td>-85 bp</td>
<td>CC50, CC52</td>
</tr>
<tr>
<td>153-166</td>
<td>3</td>
<td>GATCCCAAGAGGCGAGTCC→GATCTGCTCAGGCAGTCC</td>
<td>-8 bp</td>
<td>CC27, CC32, CC60</td>
</tr>
</tbody>
</table>

aData numbers indicated are the nucleotide number of E.coli supF DNA; numbering is in accordance with Hauser et al. (1986).

Base substitutions. Base substitution mutations were found in 19 of 65 mutations (29%) in the supF gene, of which four were transitions (G→A:T) and 15 were transversions (eight G:C→T:A, six G:C→C:G and one A:T→C:G) (Tables I and III). CoCl₂-induced base substitution mutation frequency was 7.3×10⁻⁷ (= 2.5×10⁻⁶×29%), which is 3.6-fold higher than the frequency of spontaneous base substitution, i.e.
Eight of 10 frameshifts and 18 of 19 base substitutions occurred weakly but significantly (Table I). The distribution of CoCl$_2$-induced base substitution sites was similar to that of spontaneous base substitution sites (Figure 1). There was an 18:1 bias in favor of the G:C base pair as the site of spontaneous base substitutions, while transitions were predominant over transitions. The strong preference for CoCl$_2$-induced base substitutions at G:C sites suggests that the N7G–Co(II) adduct is the lesions responsible for inducing these mutations.

In contrast, Tkeshelashvili et al. (1991) demonstrated that Cu(I) and/or Cu(II) ions induced C$\rightarrow$T transitions caused by oxygen free radicals and G$\rightarrow$T transversions mediated by binding of copper ions to the N7G in $\delta$X174 am3 DNA. A different result was observed with FeSO$_4$-induced base substitutions in the same supF gene of E.coli (Akasaka and Yamamoto, 1995). The mutation spectra induced by Fe(II) ion-generated oxygen free radicals suggested that 8-hydroxydeoxyguanosine (8-OHdG) is responsible for G:C$\rightarrow$T transitions, whereas neither 8-OHdG nor 2,6-diamino-4-hydroxy-5-formamidopyrimidine can be the lesions responsible for inducing these mutations. Furthermore, this phenomenon also seems to support our previous study (Ogawa et al., 1992), where CoCl$_2$ induced specific frameshift mutations in the Ames Salmonella tester strains TA1537 and TA2637, but not in strains TA98 and TA100. Specifically the hisC5036 frameshift mutant had an additional G base in the -GGG- site of the hisC gene, whereas reverse mutations were detectable depending on the deletion of one G (Ames et al., 1973, 1975).

Insertion sequences. Five mutants, CC31, CC56, CC57, CC59 and CC64, were found to contain the sequences of IS insertion elements; the frequency was $2.5\times10^{-8}$ among spontaneous mutations (the frequency is $3.1\times10^{-7}$ among CoCl$_2$-induced mutations, Table I). Four of five were IS50R insertions and one was an IS186 insertion. IS50R and IS186 were inserted into sites 124–125 and 114–115, respectively. IS50R was probably derived from the Tn5 in strain DL16 (Akasaka et al., 1992; Berg and Howe, 1989).

We conclude that exposure of plasmid pUB3 DNA to CoCl$_2$ primarily induced deletions and frameshifts followed by base substitutions, although very weakly, in the supF gene of E.coli. Eight of 10 frameshifts and 19 base substitutions occurred

\[3.1\times10^{-7}\times63\% = 2.0\times10^{-7}\]. Thus, CoCl$_2$ can induce base substitution weakly but significantly (Table I). The distribution of CoCl$_2$-induced base substitution sites was similar to that of spontaneous base substitution sites (Figure 1). There was an 18:1 bias in favor of the G:C base pair as the site of spontaneous base substitutions, while transitions were predominant over transitions. The strong preference for CoCl$_2$-induced base substitutions at G:C sites suggests that the N7G–Co(II) adduct is the lesions responsible for inducing these mutations. Furthermore, this phenomenon also seems to support our previous study (Ogawa et al., 1992), where CoCl$_2$ induced specific frameshift mutations in the Ames Salmonella tester strains TA1537 and TA2637, but not in strains TA98 and TA100. Specifically the hisC5036 frameshift mutant had an additional G base in the -GGG- site of the hisC gene, whereas reverse mutations were detectable depending on the deletion of one G (Ames et al., 1973, 1975).
at G:C sites. Thus, N7G–Co(II) adds contributed to 40% (26 of 65) of CoCl₂ mutagenesis. Slippage–misalignment was the suggested mechanism for generation of deletions, including CoCl₂–induced frameshifts. Furthermore, use of the SY1032/pKTY241 system may provide an easy approach to DNA sequence analysis of metal ion mutagenesis in E.coli.

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References


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