Antagonistic Effect of Nickel on the Fermentative Growth of *Escherichia coli* K-12 and Comparison of Nickel and Cobalt Toxicity on the Aerobic and Anaerobic Growth

Long-Fei Wu, Clarisse Navarro, Karinne de Pina, Martine Quénard, and Marie-Andrée Mandrand

Laboratoire de Génétique Moléculaire des Microorganismes, Centre National de la Recherche Scientifique, Villeurbanne, France

The facultative anaerobic enterobacterium *Escherichia coli* requires the activity of nickel-containing hydrogenase for its anaerobic growth. Deficiency of the specific nickel transport system led to a hydrogenase-minus phenotype and slowed down the fermentative growth in the *nik* mutant. Addition of 300 μM nickel to the growth medium could restore the hydrogenase activity. This restoration resulted in the recovery of anaerobic growth. A further increase of nickel concentration inhibited growth. Thus nickel shows an antagonistic effect on the anaerobic growth of *E. coli*. To study the mechanism of nickel toxicity, two classes of nickel-resistant mutants were isolated. The *nkr* mutant was obtained by selecting colonies grown on nickel-containing minimal plate. It acquired simultaneously the resistance to cobalt. A nonspecific magnesium transport mutant corA was isolated on cobalt-containing plate. The corA mutant was also resistant to nickel. When analyzing the influence of nickel and cobalt on the bacterial growth, we obtained two interesting observations. First, anaerobic growth was less sensitive than aerobic growth to cobalt toxicity. In contrast, nickel toxicity did not vary from the growth conditions. Second, cobalt seems to abolish the growth, while nickel appears to slow down the growth rate under the condition used. — *Environ Health Perspect* 102(Suppl 3):297–300 (1994).

Key words: nickel, cobalt, toxicity, nutrition, bacteria, aerobic growth, anaerobic growth, transport, resistance, environment

Introduction

Nickel has been known for a long time to act as a toxic heavy metal to eukaryotic cells (1). Epidemiologic studies have identified nickel as potentially carcinogenic and allergenic to humans (2,3). In the carcinogenic processes, particulate nickel compounds are more offensive than water-soluble nickel salts (4). Nickel-generated formation of oxygen radicals plays a very important role in nickel carcinogenesis. Regarding its toxicity to prokaryotic cells, nickel can severely inhibit the aerobic growth of various microorganisms (5). This toxic effect depends on many biotic and abiotic environmental factors.

However, the relationship between the toxicity and oxygen was not analyzed. Nickel is also an essential trace element for many microorganisms. It forms the active center of four metalloenzymes and plays an important role in at least four biological processes: hydrolysis of urea, uptake and production of hydrogen, methanogenesis, and acetogenesis (6). These bioprocesses have an important influence on the environment and human health. The implication of nickel-containing bacterial urease in human pathogenesis has been reviewed (7). In microorganisms, there are at least three classes of proteins involved into nickel metabolism: high affinity nickel-specific transporters participating in both the sensing and transport of nickel; accessory proteins fulfilling the role of nickel incorporation into proteins; and nickel-containing metalloenzymes (8). Knowledge obtained from studies using microbes as a model system should provide fundamental insight into important aspects of nickel toxicity, carcinogenicity, and allergenicity. The facultative anaerobic enterobacterium *Escherichia coli* K-12 has three nickel-containing hydrogenase isoenzymes. The hydrogenases I and II catalyze the oxidation of hydrogen coupled with the production of energy for its anaerobic growth (9). The hydrogenase III participates in the degradation of formate into hydrogen and carbon dioxide. This pathway maintains the intracellular redox potential and keeps pH constant during fermentative growth (10). We have isolated a class of *nik* mutants defective in the specific nickel transport system. The intracellular nickel content in *nik* mutants is about 10% of the level in a wild-type parental strain. This deficiency results in hydrogenase-minus phenotype (11). Addition of 500 μM NiCl₂ to the growth medium can restore the nickel content as well as hydrogenase activity. This restoration is specific to nickel and depends on the protein biosynthesis. The nonspecific magnesium transport system(s) seems to be required for the restoration (12). We have cloned the *nik* operon. It contains five genes coding for five proteins with molecular weights ranging from 28 to 57 kDa (13). Sequence analysis shows that these proteins are homologous to the five components of periplasmic binding protein-dependent oligopeptide transporter family (14). In this article we demonstrate, by using the *nik* mutant, an antagonistic effect of nickel on fermentative growth of *E. coli*. We also describe the isolation of some...
nickel resistant mutants and compare nickel and cobalt toxicity to aerobic and anaerobic growth.

Materials and Methods

Bacterial strains used are listed in Table 1.

Table 1. Bacterial strains.

| Strain | Genotype | Source or reference |
|--------|----------|---------------------|
| P4X    | Hfr, metB | Wu et al. (12)      |
| HPX72  | as P4X but nikA::Metil;AmpR::lacZ | Wu et al. (12) |
| CN20   | as P4X but carA | This study          |
| FNY1   | as P4X but nir | This study          |
| CGS66403 | metE163::Trn10, lvC7, 3, entA403, his-218, metE, A(lac), xyl-5, rps.l09 | Wu and Mandrand-Berthelot (11) |
| PA309  | F:: thr-1, xyl-7, leu-6, thi-1, his-1, argH1, trp-1, rps19, lacY1, matA1, mfr-2, gal-6, ara-13 | Wu and Mandrand-Berthelot (11) |

Results and Discussion

Antagonistic Effect of Nickel on the Fermentative Growth of E. coli

Under anaerobic conditions, the wild-type strain P4X showed a biphasic growth curve with a mean generation time (MGT) of 30 and 140 min for the first and the second growth phases, respectively (Figure 1, open squares; Table 2). Addition of nickel to 300 nM nickel reduced the hydrogenase activity to 60% of the wild-type strain (Table 2).

The nik mutant HPX72 contained less than 1% hydrogenase activity compared with the wild-type strain (Table 2; Figure 2). It showed a monophasic growth curve with a MGT=150 min. The maximum cell density was 60% of the level of the wild-type strain P4X (Figure 2). Addition of 300 μM nickel to the growth medium restored the hydrogenase activity and the fermentative biphasic growth (Table 2). With nickel present at 2 mM, the lag phase was extended four fold and the biphasic growth of HPX72 was abolished (Figure 2, filled circles). The MGT was two times shorter than that obtained in the absence of nickel and two times longer than that of the first growth phase with 300 mM nickel (Table 2). Thus nickel shows an antagonistic effect on fermentative growth.

Nickel-containing hydrogenases are essential for the anaerobic growth of E. coli since hydrogenase mutants could not grow in a minimal medium with hydrogen as the only source of energy (our unpublished results). In agreement with the inhibition of fermentative growth, 2 mM nickel reduced the hydrogenase activity to 60% and less than 50% in the wild-type and the nik mutant, respectively (Table 2). However, we cannot determine whether the reduction of activity results in or from the inhibition of growth.

Isolation of Nickel-resistant Mutants

With nickel in the culture, growth of the nik mutant was slightly faster than that of the wild-type strain (Table 2; Figures 1, 2). The mutant HPX72 is defective in the high-affinity nickel-specific transport system. This mutation might account for the slightly higher tolerance to nickel of the mutant than the wild-type strain. It also suggests that penetration of nickel into the cell is a crucial

Genetic Techniques

P1 cml-mediated transduction and Hfr conjugation experiments were performed as described by Miller (15).

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Table 2. Effect of nickel on growth and hydrogenase activity of Escherichia coli K-12.

| Strain | Nickel, mM | Mean generation time, min* | Hydrogenase activity |
|--------|------------|-----------------------------|---------------------|
|        |            | Phase 1 | Phase 2 |                     |
| P4X    | 0.0        | 30      | 140     | 2.13                |
|        | 0.3        | 40      | 180     | 2.07                |
|        | 0.6        | 41      | 195     | 2.51                |
|        | 2.0        | 125     | 170     | 1.31                |
| HPX72  | 0.0        | 150     |         | 0.02                |
|        | 0.3        | 35      | 150     | 2.64                |
|        | 2.0        | 85      |         | 0.96                |

Growth was carried out in a two-liter fermenter as described in Materials and Methods. *Semi-log graph was drawn by using the OD data in log scale. Two points in a straight line were taken and the mean generation time (MGT) was calculated by using the formula MGT = (time 2 - time 1)/log OD2 - log OD1/3.32. Hydrogenase activity increased in the early exponential growth phase. It reached the maximum level in the middle of exponential growth phase, then remained stable. The results presented here are the average of the data once the activity reached its maximum level. The specific activity is expressed as pmoles benzyl viologen reduced per minute per milligram bacterial dry weight. Enzyme assay was performed as described previously (17).
mutant has simultaneously acquired the resistance to nickel (Table 3).

On the other hand, we isolated nickel resistant mutants by spreading P4X cells on minimal N-glucose plates as described in Materials and Methods. One of them, PNV1, was characterized and is presented in Table 3. By conjugation experiment, we have localized the nkr (nickel resistance) mutation on the chromosome map of E. coli. When PNV1 was used as donor and PA309 was used as receiver, the coinheritance of nickel resistance with thr (0 min) was 100% and that with xyl (80 min) was 70%. Therefore, this mutation is likely to be located close to 0 min on the E. coli chromosome and thus is different from the corA mutation.

In bacteria, inorganic ion transport systems can be coded by either chromosomal or plasmid DNA. Chromosomally based systems are usually responsible for the uptake of essential ions, while plasmid-based systems play an important role in the toxic ion efflux (18,19). In this study, we demonstrate that deficiency in specific nickel transport slightly increases the tolerance to nickel in the nkr mutant. Further, our results show that chromosomal mutations corA, which is impaired in the non-specific magnesium transport system, and nkr also can confer to cells a resistance to both nickel and cobalt (Table 3). Further characterization of these mutations is currently in progress.

### Table 3. Toxic effect of nickel and cobalt on the growth of wild-type and mutant strains of E. coli.

| Strain * | P4X | CN20 | PNV1 |
|----------|-----|------|------|
| Genotype | Wild-type | corA | nkr |
| OD after Growth | Wild-type | corA | nkr |
| Without metal | O₂⁻ | O₂⁺ | 96 min | O₂⁻ | O₂⁺ | 96 min | O₂⁻ | O₂⁺ | around 0 min |
| 0.44 | 1.4 | 0.41 | 1.68 | 0.40 | 1.62 |
| +Nickel, 25 μM | 0.30 | 1.42 | 0.35 | 1.76 | 0.36 | 1.67 |
| +Nickel, 50 μM | 0.08 | 0.07 | 0.11 | 0.15 | 0.12 | 0.11 |
| +Nickel, 100 μM | 0.04 | 0.07 | 0.11 | 0.15 | 0.12 | 0.11 |
| +Nickel, 200 μM | 0.03 | 0.03 | 0.10 | 0.06 | 0.05 | 0.06 |
| (0.25) | (1.75) | (0.26) | (1.22) | (0.26) | (1.16) |
| +Cobalt, 12.5 μM | 0.37 | 0.10 | 0.40 | 1.19 | 0.37 | 1.21 |
| +Cobalt, 25 μM | 0.29 | 0.08 | 0.40 | 0.15 | 0.36 | 0.17 |
| +Cobalt, 50 μM | 0.09 | 0.08 | 0.10 | 0.34 | 0.34 | 0.09 |
| +Cobalt, 100 μM | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 |
| (0.05) | (0.32) | (0.39) | (0.39) | (0.39) | (0.39) |
| +Cobalt, 200 μM | 0.03 | 0.05 | 0.08 | 0.04 | 0.05 | 0.05 |
| (0.03) | (0.03) | (0.06) | (0.06) | (0.06) | (0.06) |

*Genotype of the strains: P4X, Hfr, metB, CN20, same as P4X, but corA, PNV1, same as P4X but resistant to nickel. *The corA mutation was localized by P1 cml-mediated transduction as described by Miller (15). CN20 was transduced to tetracycline resistance with phage P1 cml grown on the strain CSGC6403 (metE162; Tn10, ixbC). The Tn10 insertion was found to be 45% linked to cobalt resistance and 20% to ixbC. *The mutation was localized by conjugation using PNV1 as donor and PA309 (thr-1, xyl-7) as recipient. Coconjugation frequencies of nickel resistance were 100% with thr (0 min) and 70% with xyl (80 min). *Cells were grown in minimal N-glucose medium (see Materials and Methods). Nickel or cobalt chloride was added at the concentrations indicated. Optical density (OD) was measured after 15 hr or after 3 days (presented in parentheses) incubation. Aerobic growth (+O₂) and anaerobic growth (-O₂) were performed as described in Materials and Methods.

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