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ORIGINAL PAPER

# Extremely high intracellular concentration of glucose-6-phosphate and NAD(H) in *Deinococcus radiodurans*

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**Abstract** *Deinococcus radiodurans* is highly resistant to ionizing radiation and UV radiation, and oxidative stress caused by such radiations. NADP(H) seems to be important for this resistance (Slade and Radman, Microbiol Mol Biol Rev 75:133–191; Slade, Radman, Microbiol Mol Biol Rev 75:133–191, 2011), but the mechanism underlying the generation of NADP(H) or NAD(H) in *D. radiodurans* has not fully been addressed. Intracellular concentrations of $NAD^+$, NADH, $NADP^+$, and NADPH in *D. radiodurans* are also not determined yet. We found that cell extracts of *D. radiodurans* catalyzed reduction of $NAD(P)^+$ in vitro, indicating that *D. radiodurans* cells contain both enzymes and a high concentration of substrates for this activity. The enzyme and the substrate were attributed to glucose-6-phosphate dehydrogenase and glucose-6-phosphate of which intracellular concentration was extremely high. Unexpectedly, the intracellular concentration of NAD(H) was also much greater than that of NADP(H), suggesting some significant roles of NADH. These unusual features of this bacterium would shed light on a new aspect of physiology of this bacterium.

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**Keywords** *Deinococcus radiodurans* · Glucose-6-phosphate dehydrogenase · Glucose-6-phosphate · NAD(H) · NADP(H)

**Abbreviations**

| | |
|---|---|
| ROS | Reactive oxygen species |
| GSSG | Oxidized glutathione |
| G6PDH | Glucose-6-phosphate dehydrogenase |
| G6P | Glucose-6-phosphate |
| WT | Wild type |
| GK | Glucokinase |
| NADK | NAD kinase |

## Introduction

*Deinococcus radiodurans*, an organotrophic bacterium with a proteolytic lifestyle (Murray 1986), is remarkably resistant to oxidative stress induced by reactive oxygen species (ROS), which can be produced metabolically or form upon exposure to chemical and physical agents such as hydrogen peroxide, mitomycin C, UV radiation, ionizing radiation, and desiccation (Ghosal et al. 2005; Slade and Radman 2011). Due to its extremely high resistance to ionizing radiation, *D. radiodurans* has been used for investigations of radiobiology. Recent studies suggest that an extremely high ROS-scavenging capacity is responsible for this bacterium's radiation resistance; as a result, its proteome is better protected against ROS-induced oxidative damage than those of radiation-sensitive species (Daly 2009; Daly et al. 2007; Slade and Radman 2011). Intracellular small-molecule antioxidants, including manganese, phosphate, nucleosides, and especially manganese–metabolite complexes, were recently identified as powerful ROS scavengers in *D. radiodurans* (Daly et al. 2010).

NADPH, the reduced form of $NADP^+$, is involved in anabolic reactions and protects cells against oxidative stress by donating reducing equivalents to proteins such as thioredoxin and small molecules such as oxidized glutathione (GSSG) (Lu and Holmgren 2014; Obiero et al. 2010; Slade and Radman 2011). By contrast, NAD(H) is primarily involved in catabolic reactions (Kawai and Murata 2008). Given the crucial role of NADPH in resistance to oxidative stress, it is possible that NADPH protects *D. radiodurans* against oxidative stress and other stresses such as UV and ionizing radiation (Slade and Radman 2011). Consistent with this, the thioredoxin system of *D. radiodurans* is NADPH-dependent (Obiero et al. 2010).

Glucose-6-phosphate dehydrogenase (G6PDH) is the enzyme that catalyzes the first step of the pentose phosphate pathway (PPP), which generates NADPH (Stincone et al. 2015). In general, two NADPH molecules are generated from one G6P molecule via the two oxidation reactions of the PPP. Additionally, PPP generates precursors for nucleotide and amino-acid biosynthesis, such as ribose-5-phosphate and erythrose-4-phosphate (Stincone et al. 2015). Thus, the PPP is generally important for redox homeostasis and recovery from damage caused by oxidative stresses. In *D. radiodurans*, G6PDH appears to be critical for UV resistance: a *D. radiodurans* mutant lacking the G6PDH gene grows more slowly than the wild-type (WT) strain and is more sensitive to UV, $H_2O_2$, and mitomycin C (Liu et al. 2008; Zhang et al. 2003).

Despite of the significance, the mechanism underlying generation of NADP(H) or NAD(H) in *D. radiodurans* has not fully been addressed. Intracellular concentrations of $NAD^+$, NADH, $NADP^+$, and NADPH in *D. radiodurans* are also not determined yet. In this study, we detected the high $NAD(P)^+$-reducing activity in the cell extracts of *D. radiodurans* in vitro. The activity was attributed to glucose-6-phosphate dehydrogenase and extremely high concentration of glucose-6-phosphate. We also revealed extremely high concentration of NAD(H) in this bacterium.

## Materials and methods

### Strains, media, and growth conditions

Bacterial strains used in this study are shown in Table 1. *D. radiodurans* was grown aerobically in TY medium [1% (w/v) tryptone (Nacalai, Kyoto, Japan) and 0.5% (w/v) yeast extract (Nacalai), pH 7.1] or TGY medium [0.1% (w/v) D-glucose in TY medium, pH 7.1]. *E. coli* was grown aerobically in TGY medium. Growth in TY or TGY medium was conducted at 32 °C and 130 strokes per minutes (spm) in a PERSONAL-11 shaker incubator (TAITEC, Koshigaya, Japan). When inoculating bacterial strains into liquid media, $A_{600}$ was adjusted to 0.1. For plasmid construction, *E. coli* was aerobically grown in LB medium [1% (w/v) tryptone, 0.5% (w/v) yeast extract, and 1% (w/v) NaCl] or MMI medium [2.5% (w/v) yeast extract, 1.25% (w/v) tryptone, 0.85% (w/v) NaCl, 20 mM Tris–HCl (pH 7.2), and 0.4% (v/v) glycerol] at 32 °C. Media were solidified with 1.5% (w/v) agar. If necessary, ampicillin (Ap, at 100 μg/mL) (Wako Pure Chemical Industries, Osaka, Japan) or kanamycin [Km, at 25 μg/mL (solid media) or at 10 μg/mL (liquid media)] (Wako) was added to the media.

### Preparation of cell extract

*D. radiodurans* wild-type (WT; MK5205), G6PDH disruptant (*ΔDR1596*::$Km^r$; MK5368), and *E. coli* MG1655 strains were grown in 1.5 L TGY and TY media (32 °C, 100 spm) until $A_{600}$ reached 0.4–0.8. In the case of *D. radiodurans*, $A_{600}$ of 1.0 corresponded to $5.6 \times 10^7$ cells (colony forming units) per mL, which were determined after plating the culture onto TGY solid medium. Cells were collected by centrifugation at 6000×*g* for 10 min at 4 °C, washed once in 20 mM Tris–HCl (pH 7.5), and suspended in 20 mM Tris–HCl (pH 7.5) to 0.5 g wet weight per mL. The cell suspension was stored at −30 °C and thawed at room temperature. *E. coli* MG1655 cells were disrupted by sonication for 20 min using an INSONATOR 201 M (Kubota, Tokyo, Japan). Disruption of MK5205 and MK5368 cells

**Table 1** Bacterial strains used in this study

| Strain | Description | References |
|---|---|---|
| *Escherichia coli* | | |
| MG1655 | *E. coli* K-12 MG1655 (wild type) | Kawai et al. (2001) |
| DH5α | $F^-$ *φ80dlacZΔM15 Δ(argF - laczya)U169 deoR recA1 endA1 hsdR17*($r_k^-$, $m_k^+$) *phoA supE44* $\lambda^-$ *thi-1 gyrA96 relA1* | Toyobo |
| MK5342 | *DR_1596*_257_1502/pUC119/DH5α | This study |
| MK5346 | *DR_1596*_257_1502::$Km^r$/pUC119/DH5α | This study |
| *D. radiodurans* | | |
| MK5205 | *D. radiodurans* R1 (NBRC wild type) | NBRC |
| MK5368 | *ΔDR1596*::$Km^r$ | This study |

was performed as follows. First, the cell suspension was dispensed into screw-cap tubes (1.5 mL/tube; $1.68 \times 10^{10}$ cells/tube), and glass beads (0.1–0.2 mm diameter) were added to nearly top of the tube. Cells were disrupted by shaking the tubes for 30 s on a FastPrep24 (MP Biomedicals, Santa Ana, CA, USA) at 6.5 m/s, followed by incubation on ice for 30 s; this process was repeated a total of five times. Disrupted cells were centrifuged at 20,000×*g* for 10 min at 4 °C (MK5205 and MK5368) or 20 min (*E. coli* MG1655), and the resultant supernatant was used as cell extract. Protein concentration in the cell extract was determined by Bradford assay (Bradford 1976) using bovine serum albumin (Sigma, St. Louis, MO, USA) as a standard.

### Assay of NAD(P)$^+$-reducing activity in the cell extract

For measurement of NAD(P)$^+$-reducing activity, $A_{340}$ of the reaction mixture [800 µL; 5 mM NAD$^+$ or NADP$^+$, 100 mM Tris–HCl (pH7.5), and cell extract (1 mg/mL protein)] was monitored continuously at 32 °C. Reaction was initiated by adding NAD(P)$^+$.

For measurement of total NAD(P)$^+$-reducing activity, the same reaction mixture [100 µL per tube] was incubated at 32 °C, and the $A_{340}$ of the supernatant was measured until saturation after tenfold dilution with 10 mM Tris (pH 11) followed by centrifugation at 20,000×*g* for 3 min at 4 °C. The saturated (maximum) $A_{340}$ in the 1-mL reaction mixture was regarded as the total NAD(P)$^+$-reducing activity.

### Analysis of NAD(P)$^+$-reducing activity in the reaction mixture

Reaction mixture [300 µL; 5 mM NAD(P)$^+$, cell extract (1 mg/mL protein), and 100 mM Tris–HCl (pH 7.5)] was incubated at 30 °C for 10 min, and 100 µL of the reaction mixture was added to 150 µL of 10 mM Tris (pH 11) (no treatment), 0.17 M NaOH (alkali treatment), or HCl (acid treatment). Alkali- or acid-treated mixtures were boiled for 90 s. Absorbance spectrum of the alkali-treated, acid-treated, and untreated mixtures were measured between the wavelengths of 200 and 400 nm (0.5 nm resolution) on a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). Baselines were mixtures of 100 µL of 100 mM Tris–HCl (pH 7.5) and 150 µL of 10 mM Tris (pH 11), or acid- or alkali-treated 10 mM Tris (pH 11), as appropriate.

For HPLC analysis, the original reaction mixture was diluted fivefold in 10 mM Tris (pH 11) for detection of NADH. For detection of NADPH, the original reaction mixture was diluted 2.5-fold in 10 mM Tris (pH 11). After dilution, reaction mixtures were passed through a Cosmonice filter W (pore size 0.45 µm, Nacalai). The filtered mixture (10 µL) was analyzed with HPLC equipped with a COSMOSIL/COSMOGEL 5C18-AR-300 (4.6 × 150 mm; Nacalai) column. Solvent composition was changed from a 50 mM Tris–HCl (pH 7.5):methanol ratio of 100:0 to 90:10 over 40 min, and the final solvent composition was maintained for an additional 40 min (Mori et al. 2005). Signals were detected at $A_{340}$.

### Disruption of the G6PDH gene in *D. radiodurans*

Disruption of *DR_1596*, the G6PDH gene of *D. radiodurans* (Zhang et al. 2003), was performed as described previously (Nishida and Narumi 2002) with some modifications. Details are provided in Supplementary Information.

### Assay of G6PDH activity in cell extract

G6PDH activity was assayed by continuously monitoring $A_{340}$ of the reaction mixture [800 µL; 5 mM $MgCl_2$, 5 mM G6P, 5 mM NAD(P)$^+$, cell extract, and 100 mM Tris–HCl (pH 7.5)] at 32 °C. Protein concentration in the mixture was adjusted to 0.5 mg/mL. Reaction was initiated by adding G6P. One unit of G6PDH activity was defined as the activity that generated 1 µmol of NAD(P)H in 1 min at 32 °C. Apparent kinetic constants were determined from the Lineweaver–Burk plot.

### UV resistance assay

*D. radiodurans* WT (MK5205) and G6PDH-disruptant (MK5368) strains were grown in 4 mL TGY or TY media until $A_{600}$ reached 0.4–0.8; $A_{600}$ of 1.0 corresponds to $5.6 \times 10^7$ cells (colony forming units) per mL as described above. Cultures were diluted in sterilized water (Zhang et al. 2003) and the cells corresponding to 2,000–4,000 cells were spread on TGY or TY agar plate (diameter = 8.5 cm). The plates were irradiated using a UV transilluminator (BIO CRAFT, Tokyo, Japan) equipped with Vilber-Lourmat T-8C, UV-C 4E (diameter = 1.5 cm; 5 lamps). The distance between the lamps and the plate was 2.5–3.0 cm. After exposure to UV, plates were incubated at 30 °C for 2–3 days. Colonies were counted, and survival rate was calculated relative to non-radiated samples.

### Measurement of G6P in cell extract

Cell extracts of *D. radiodurans* WT (MK5205), G6PDH disruptant (MK5368), and *E. coli* MG1655 strains were diluted with 20 mM Tris–HCl (pH 7.5) to adjust protein concentration to 2 mg/mL, and then cleared by centrifugation at 20,000×*g* for 5 min at 4 °C after the diluted cell extracts were boiled for 5 min. In the case of *D. radiodurans*, the cell extract (approximately 1 mL containing 5.5 mg protein) was prepared. The total amount of G6P in the cell extract ($G6P_t$) was measured based on the increased $A_{340}$ of the mixture

[total 500 µL; 1:1 of cleared supernatant: 2× reaction mixture consisting of 10 mM $MgCl_2$, 1 mM $NADP^+$, 200 mM Tris–HCl (pH 7.5), and 0.2 U G6PDH (Sigma)] incubated at 37 °C for 30–60 min until $A_{340}$ became stable. After boiling for 5 min in this condition, no degradation of G6P was observed. Assuming that *D. radiodurans* cells are spherical bodies 1 µm in diameter (Slade and Radman 2011), the volume of a single cell ($4/3\pi r^3$) is $5.24\times10^{-10}$ µL. Thus, the total volume of $1.68\times10^{10}$ cells per tube, from which cell extracts were prepared, was 8.80 µL. Assuming that intracellular G6P was extracted thoroughly from $1.68\times10^{10}$ cells, the intracellular G6P concentration could be calculated by dividing $G6P_t$ by 8.80 µL. It should be noted that the calculated G6P concentration represents a minimum value, as some cells might have remained intact.

### Estimation of intracellular $NAD(P)^+$ and NAD(P)H concentration

*D. radiodurans* was cultured in TGY media until $A_{600}$ reached 0.8–1.0. Cells ($2.5\times10^8$ cells/tube) were collected by centrifugation at 15,000×*g* for 2 min at 4 °C, washed once with 1 mL of 20 mM Tris–HCl (pH 7.5), and immediately stored at −80 °C. Extraction of $NAD(P)^+$ and NAD(P)H was performed as previously reported (Leonardo et al. 1996) using 125 µL/tube of 0.2 M HCl [for extraction of $NAD(P)^+$] or 0.2 M NaOH [for extraction of NAD(P)H]. Assays of extracts containing $NAD(P)^+$ and NAD(P)H were performed by cycling assay (details provided Supplementary Information) (Bernofsky and Swan 1973; Leonardo et al. 1996). As described above, the volume of a single cell was calculated as $5.24\times10^{-10}$ µL. $NAD(P)^+$ and NAD(P)H were extracted from $2.5\times10^8$ cells, so the total cell volume was 0.131 µL. Assuming that intracellular $NAD(P)^+$ and NAD(P)H were extracted thoroughly into 125 µL of extract, intracellular $NAD(P)^+$ and NAD(P)H concentration ($C_{in}$) could be calculated from that of the cell extract ($C_{ex}$) using the equation: $C_{in} = (C_{ex} \times 125)/0.131$.

### Bioinformatics

Bioinformatic searches were conducted using The SEED Viewer (http://pubseed.theseed.org/seedviewer.cgi) (Overbeek et al. 2005) or GenomeNet (http://www.genome.jp).

## Results

### $NAD(P)^+$-reducing activity in *D. radiodurans* cell extract

During our experiments using cell extract of *D. radiodurans*, we observed an obvious increase in $A_{340}$ in a simple reaction mixture consisting of the extract, 5 mM $NAD^+$ or $NADP^+$, and 100 mM Tris–HCl (pH 7.5) alone (Fig. 1a; TGY, black bar), suggesting that $NAD(P)^+$ was reduced to NAD(P)H via an endogenous $NAD(P)^+$-reducing enzymatic activity using endogenous substrates in the extract.

To confirm that $NAD(P)^+$ was reduced to NAD(P)H, we incubated the reaction mixture for 10 min and analyzed it by absorption spectroscopy and HPLC (Fig. 2a, b). Absorption spectra of the incubated reaction mixture, as well as a NaOH-treated reaction mixture, exhibited a peak near 340 nm, whereas the spectrum of HCl-treated reaction mixture did not (Fig. 2a). This observation supports the idea that $NAD(P)^+$ is reduced to NAD(P)H, which is alkaline-stable and acid-unstable. In the HPLC analysis, NADPH was detected in the incubated reaction mixture (Fig. 2b). When the reaction mixture contained $NAD^+$, the peak was observed at a slightly later elution time (~2 min); we attributed this to the degradation of the NADH formed in the incubated reaction mixture. Considering these results, we concluded that $NAD^+$ and $NADP^+$ were reduced to NADH and NADPH, respectively, when they were incubated for 10 min with cell extract of *D. radiodurans* and Tris–HCl (pH 7.5).

The $NAD(P)^+$-reducing activity was saturated in the presence of >2.5 mM $NAD(P)^+$, and exhibited higher affinity for $NAD^+$ than for $NADP^+$ (Fig. 1b; Supplementary Fig. S1). Moreover, $NAD^+$-reducing activity was higher than $NADP^+$-reducing activity (Fig. 1a, b; Supplementary Fig. S1). The total $NAD(P)^+$-reducing activity was calculated as 3.5–4.0 $mg^{-1}$ (Fig. 1c; black bar). By contrast, when cell extract of TGY-grown *E. coli* was used, we detected no $NAD^+$-reducing activity and only a little $NADP^+$-reducing activity (Fig. 1a; white bar). The total $NAD(P)^+$-reducing activity of *E. coli* cell extract was also much lower than that of TGY-grown *D. radiodurans* (Fig. 1c; white bar).

In TGY-grown *D. radiodurans*, the intracellular concentrations of $NAD^+$, NADH, and NADPH were calculated to be $6.5\pm0.8$ mM ($NAD^+$), $37\pm1$ mM (NADH), and $0.72\pm0.39$ mM (NADPH), respectively, whereas $NADP^+$ was not detectable (Fig. 3; black bar, Table 2).

### Contribution of G6P and G6PDH to $NAD(P)^+$-reducing activity

Taking into account previous observations that G6PDH plays a critical role in survival of *D. radiodurans* under oxidative stress (Liu et al. 2008; Zhang et al. 2003), we sought to understand how this enzyme is involved in NAD(P)H generation. To this end, we constructed a *D. radiodurans* G6PDH disruptant (MK5368). Cell extract from TGY-grown *D. radiodurans* WT strain exhibited $NADP^+$- and $NAD^+$-dependent G6PDH activity, with higher affinity for $NADP^+$ (apparent $K_m$: $NADP^+$, 12 µM; $NAD^+$, 53 µM; apparent $V_{max}$: $NADP^+$, 0.032 U/

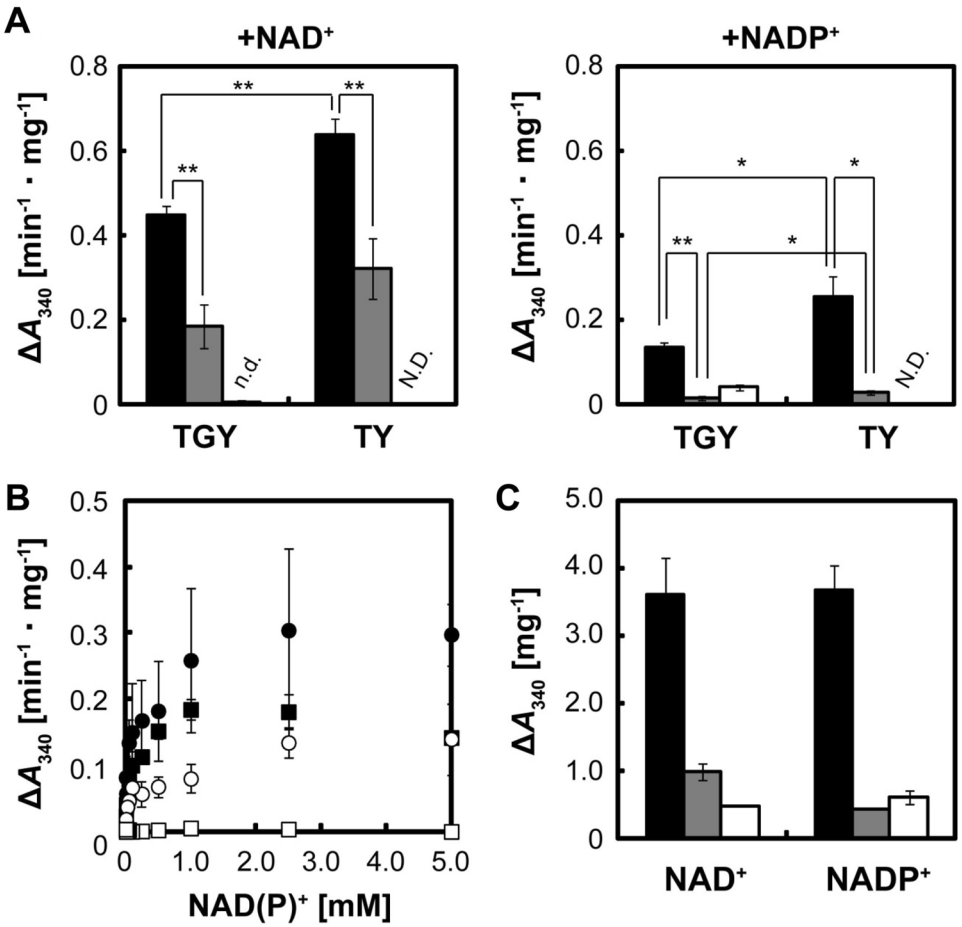


**Fig. 1** NAD(P)$^+$-reducing activity in *D. radiodurans* cell extract. **a** NAD$^+$-reducing activity (*left* panel) and NADP$^+$-reducing activity (*right* panel) in cell extracts of *D. radiodurans* WT (*black*), *D. radiodurans* G6PDH disruptant (*gray*), and *E. coli* MG1655 (*white*) grown in TGY or TY medium. $n=3$, Ave. ± SD. *$p<0.05$, **$p<0.01$, *nd* not detected. *ND* not determined. **b** Effect of the concentrations of NAD$^+$ (*closed symbols*) and NADP$^+$ (*open symbols*) on NAD(P)$^+$-reducing activities in cell extracts of *D. radiodurans* WT (*circles*) and G6PDH disruptant (*squares*) at NAD(P)$^+$ concentrations of 0–5 mM. $n=2$, Ave. ± SD. **c** Total NAD(P)$^+$-reducing activity in cell extracts of TGY-grown cells, as in **a, b**. $n=2$, Ave. ± SD

mg; NAD$^+$, 0.053 U/mg) (Supplementary Fig. S2). No NADP$^+$-/NAD$^+$-dependent G6PDH activity was detectable in cell extract from the *D. radiodurans* G6PDH disruptant (data not shown). In agreement with previous reports (Liu et al. 2008; Zhang et al. 2003), the *D. radiodurans* G6PDH mutant grew more slowly than the WT strain and was more sensitive to UV (Fig. 4). Next, we examined the NAD(P)$^+$-reducing activity of this disruptant (Fig. 1; TGY, gray bar). Notably, very little of NADP$^+$-reducing activity was detected in the mutant, whereas the NAD$^+$-reducing activity was approximately 50% of that of the WT strain (Fig. 1a, b; Supplementary Fig. S1). These data indicated that NAD(P)$^+$-reducing activity, that was observed in vitro, was attributed to G6PDH.

Thus, we expected that G6P would be present at a high concentration in *D. radiodurans* cells. Accordingly, an intracellular G6P concentration was 106 mM (173 µmol/g protein) (Fig. 5; black bar, Table 3). In cleared cell extract from TGY-grown *D. radiodurans* G6PDH disruptant, approximately the same level of G6P was detected (Fig. 5). TGY-grown *E. coli* was treated using the same method, but no G6P was detected in the cleared cell extract (Fig. 5; black bar).

Intracellular concentrations of NAD$^+$, NADH, NADP$^+$, and NADPH in the TGY-grown *D. radiodurans* G6PDH disruptant were calculated as 6.1 ± 2.6 mM (NAD$^+$), 11 ± 2 mM (NADH), 0.084 ± 0.118 mM (NADP$^+$), and 0.26 ± 0.07 mM (NADPH) (Fig. 3, gray bar; Table 2).

### Effect of glucose on NAD(P)$^+$-reducing activity and concentration of G6P

We examined the effect of glucose in the medium on NAD(P)$^+$-reducing activity, G6P content, and UV sensitivity (Figs. 1a, 4, 5; white bar). Glucose had no effect on growth (data not shown) or UV sensitivity (Fig. 4b), but unexpectedly, NAD(P)$^+$-reducing activity was higher in extract of TY-grown cells (without glucose) than in extract of TGY-grown cells (with glucose) (Fig. 1a).

## Discussion

In this study, we observed an increase in the $A_{340}$ of *D. radiodurans* cell extract when NAD(P)$^+$ was added, which was due to NAD(P)$^+$ reduction by endogenous enzymes

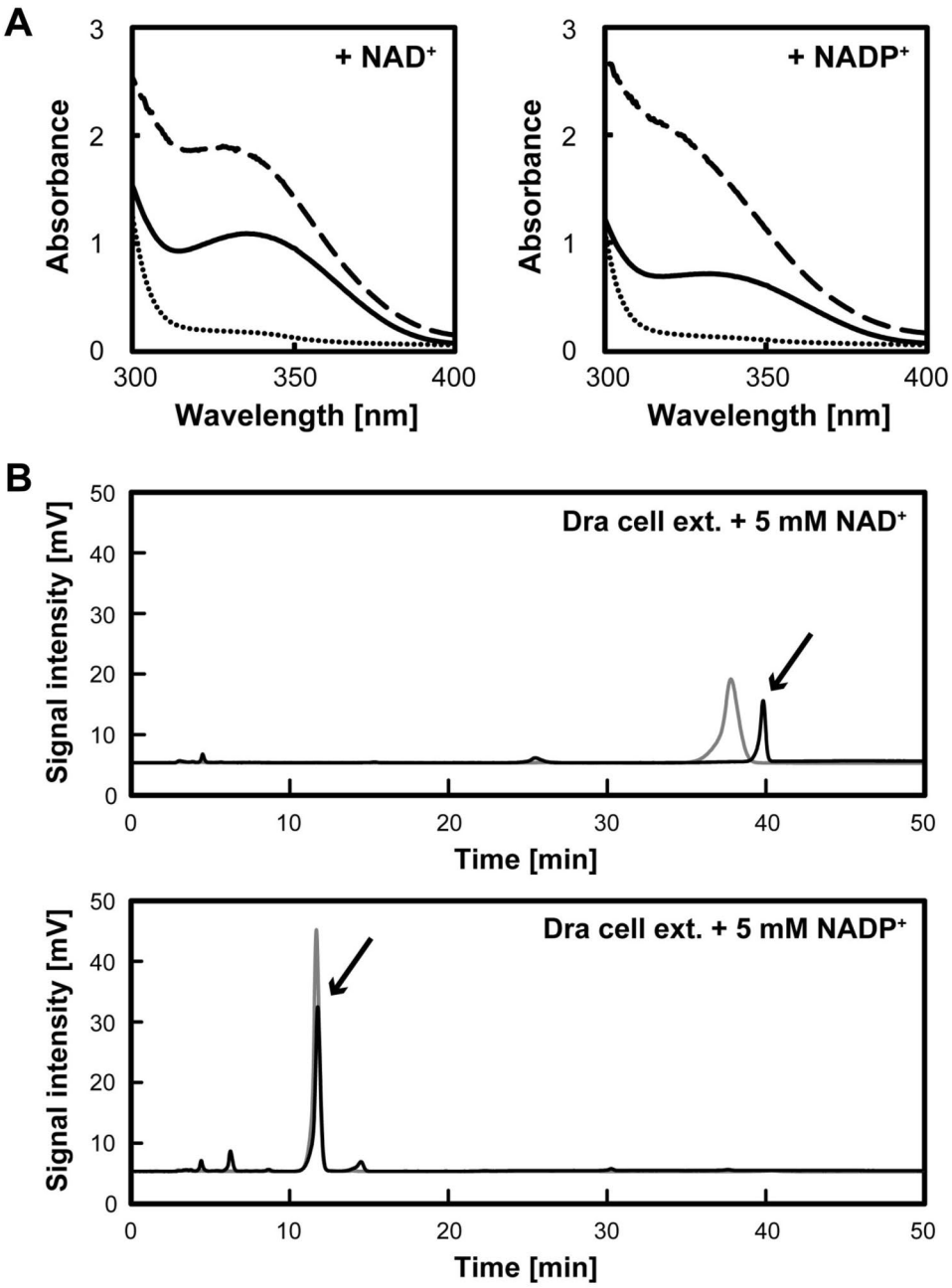


**Fig. 2** Reduction of NAD(P)$^+$ to NAD(P)H. **a** UV-absorption spectrum of a mixture containing NAD$^+$ (*left panel*) or NADP$^+$ (*right panel*). Reaction mixture was subjected to no treatment (*solid lines*), alkaline treatment (*dashed lines*), or acid treatment (*dotted lines*). **b** Chromatograms of HPLC analysis. *Upper panel* 0.2 mM NADH (*gray line*), mixture consisting of 5 mM NAD$^+$ and *D. radiodurans* cell extract (*black line*). *Lower panel* 0.2 mM NADPH (*gray line*), mixture consisting of 5 mM NADP$^+$ and *D. radiodurans* cell extract (*black line*). *Arrows* indicate peaks corresponding to the generated NAD(P)H

and substrates. The enzyme and substrate responsible for the observed reducing activity was attributed to G6PDH and G6P in *D. radiodurans* cells (intracellular concentration of G6P was 106 mM, corresponding to 173 µmol/g protein). Compared with those of other microorganisms (*E. coli, Saccharomyces cerevisiae, Mycobacterium smegmatis, Corynebacterium glutamicum*), this value of *D. radiodurans* is extremely high (Table 3).

To what extent G6P is synthesized in *D. radiodurans* cells? Although G6P can be synthesized by the PTS-dependent glucose uptake system (Deutscher et al. 2006), no genes encoding components of this system have been detected in the genome of *D. radiodurans* (The SEED Viewer and GenomeNet). G6P is also synthesized from glucose by glucokinase (GK) (Kawai et al. 2005); two GK genes are present in the *D. radiodurans* genome (*DR_0823* and *DR_2296*). However, in *D. radiodurans* grown without glucose, the intracellular G6P level was not significantly different from that of *D. radiodurans* grown with glucose (Fig. 5), indicating that GK is not responsible for the production of G6P in this bacterium. *D. radiodurans* utilizes protein as its main carbon source (Slade and Radman

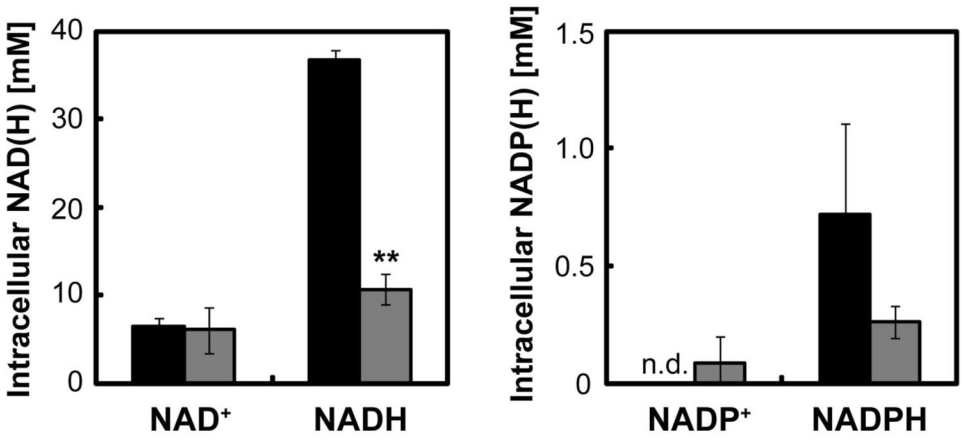


**Fig. 3** Intracellular concentrations of NAD(P)$^+$ and NAD(P)H in TGY-grown *D. radiodurans*. Intracellular concentrations of NAD(H) (*left panel*) and NADP(H) (*right panel*) of *D. radiodurans* WT (*black bars*) and G6PDH disruptant (*gray bars*). $n=3$, Ave. ± SD, $^{**}p<0.01$, *nd* not detected

**Table 2** Comparison of intracellular NAD(P)$^+$ and NAD(P)H concentrations

| Species/strain | Intracellular concentration (mM) | | | | References |
|---|---|---|---|---|---|
| | NAD$^+$ | NADH | NADP$^+$ | NADPH | |
| *D. radiodurans* | | | | | This study |
| WT | 6.5 ± 0.8 | 37 ± 1 | nd | 0.72 ± 0.39 | |
| Δ*g6pdh* | 6.1 ± 2.6 | 11 ± 2 | 0.084 ± 0.118 | 0.26 ± 0.07 | This study |
| *E. coli* | 0.90 | 0.40 | 0.17 | 0.22 | Lundquist and Olivera (1971) |
| *S. cerevisiae* | 1.2 | 1.2 | NR | NR | Sporty et al. (2009) |
| *S. enterica* | 0.90 ± 0.10 | 0.16 ± 0.04 | 0.54 ± 0.10 | 0.18 ± 0.07 | Grose et al. (2006) |

*nd* not detected, *NR* not reported, *S. cerevisiae S. cerevisiae* BY4742, *S. enterica Salmonella enterica*

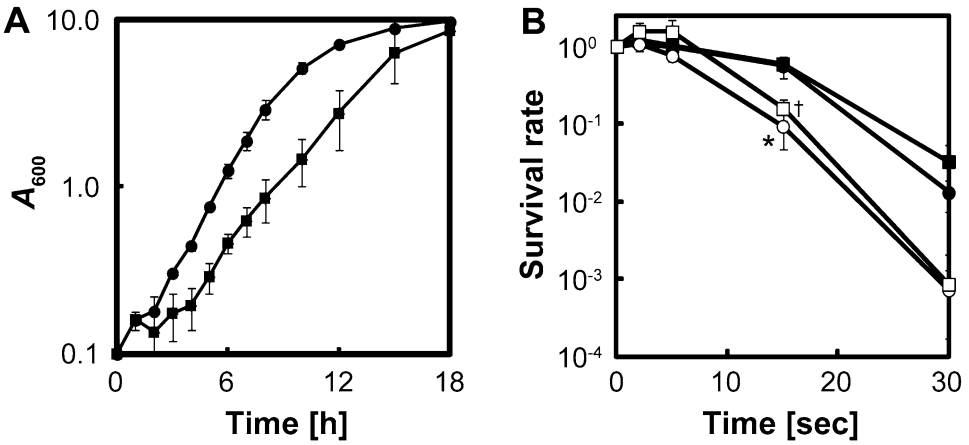


**Fig. 4** Contribution of G6PDH and G6P. **a** Growth of *D. radiodurans* WT (*closed circles, n*=3) and G6PDH disruptant (*closed squares, n*=2) in TGY media. Ave. ± SD. **b** Sensitivity to UV of *D. radiodurans* WT (*closed symbols*) and G6PDH disruptant (*open symbols*) grown in TGY (*circles*) or TY (*squares*) media. Survival rate was defined as the ratio of colony count after UV exposure to the count in non-UV-irradiated samples. Ave. ± SD, $n=3$, $^{*}p<0.05$ (vs. WT in TGY medium), $^{\dagger}p<0.01$ (vs. WT in TY medium)

2011), and glucose is a less optimal carbon source than amino acids and fructose (Slade and Radman 2011; Venkateswaran et al. 2000). The *D. radiodurans* genome contains genes encoding all enzymes of gluconeogenesis. Taking these facts into consideration, it is likely that *D. radiodurans* synthesizes G6P via gluconeogenesis rather than GK or the PTS system.

Generally, NADP$^+$ is biosynthesized from NAD$^+$ by NAD kinase (NADK) (Kawai and Murata 2008). However, no homologue of NADK gene was found in the genome of

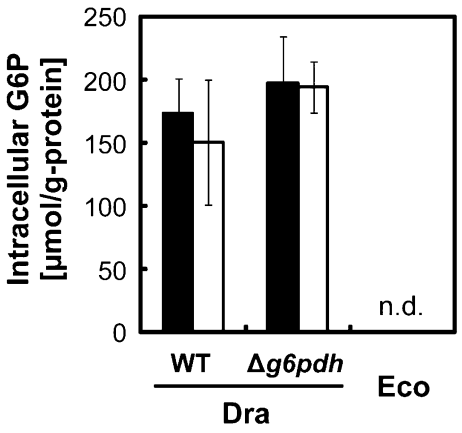


**Fig. 5** Intracellular G6P. Intracellular G6P levels of *D. radiodurans* (Dra) WT, G6PDH disruptant (Δ*g6pdh*), and *E. coli* MG1655 (Eco) grown in TGY (*black bars*) or TY (*white bars*) medium ($n=3$–5, Ave. ± SD). Values are shown as μmol of G6P per 1 g of cellular protein. *nd* not detected

**Table 3** Comparison of intracellular G6P

| Species | Intracellular G6P | | References |
|---|---|---|---|
| | (mM) | (μmol/g protein) | |
| *D. radiodurans* | ~106 | 173 ± 28 | This study |
| *E. coli* | ND | 0.23 ± 0.06 | Hasan et al. (2010) |
| *S. cerevisiae* | ND | 0.93 ± 0.01 | Theobald et al. (1997) |
| *M. smegmatis* | ND | 30.5 ± 0.9 | Hasan et al. (2010) |
| *C. glutamicum* | 8 ± 3 | 29 | Dominguez et al. (1998), Hasan et al. (2010) |

ND, no data; *S. cerevisiae, Saccharomyces cerevisiae; M. smegmatis, Mycobacterium smegmatis; C. glutamicum, Corynebacterium glutamicum*

*D. radiodurans* using The SEED Viewer and GenomeNet. Also, intracellular concentrations of $NADP^+$ and NADPH, as well as $NAD^+$ and NADH in *D. radiodurans* had also not been determined yet. In this study, for the first time, we detected NADPH in *D. radiodurans* WT and $NADP^+$ in the G6PDH disruptant (Fig. 3; Table 2). This result indicates that *D. radiodurans* is able to synthesize NAD(P)H. The reason why $NADP^+$ was undetectable in cell extract of *D. radiodurans* WT (Fig. 3; Table 2) was speculated that intracellular $NADP^+$ would be reduced into NADPH to the level below detection.

In this study, we found that *D. radiodurans* cells contain a high concentration (37 mM) of NADH (Fig. 3; Table 2), possibly consistent with the observation that the $NAD^+$-reducing activity was higher than the $NADP^+$-reducing activity (Fig. 1a). Generally, NADH and $NAD^+$ engage in catabolic reactions, and the intracellular concentration of $NAD^+$ tends to exceed that of NADH; the intracellular $NAD^+$/NADH ratio is on the order of 700 to 1 (Ying 2008). However, the intracellular concentration of NADH in *D. radiodurans* that we measured in this study is higher than those in other species (Table 2); consequently, the intracellular $NAD^+$/NADH ratio was much lower (Table 4), suggesting some significant roles of NADH in *D. radiodurans*. These unusual features of this bacterium would shed light on a new aspect of the physiology of *D. radiodurans*.

The *D. radiodurans* G6PDH disruptant grew more slowly than the WT strain and was more sensitive to UV, as reported previously (Liu et al. 2008; Zhang et al. 2003). Zhang et al. reported the survival index (% survival of mutant/% survival of WT) of the *D. radiodurans* G6PDH disruptant after exposure to UV were 0.16–0.49 (Zhang et al. 2003), that is similar to the survival index (0.17) of the G6PDH disruptants after exposure to UV for 15 min (Fig. 4). Zhang et al. attributed the UV-sensitivity of the G6PDH disruptant to the low levels of UMP and IMP that are demanded for excision repair to repair its DNA (Zhang et al. 2003). Our data suggest that the high concentration of G6P in *D. radiodurans* would guarantee a good supply of substrates and reducing power such as NADH demanded for several cellular processes including the protection from UV-damage.

**Table 4** Comparison of the $NAD^+$/NADH, $NADP^+$/NADPH, and NAD(H)/NADP(H) ratios

| Species/strain | $NAD^+$/NADH | $NADP^+$/NADPH | NAD(H)/NADP(H) | References |
|---|---|---|---|---|
| *D. radiodurans* | | | | |
| WT | 0.18 | nd | 60 | This study |
| Δ*g6pdh* | 0.57 | 0.32 | 49 | This study |
| *E. coli* | 2.3 | 0.77 | 3.3 | Lundquist and Olivera (1971) |
| *S. cerevisiae* | 6.5 | 3.4 | 9.4 | Moreira dos Santos (2004) |
| Mouse | 4.6 | 0.16 | 8.3 | Gao et al. (2007) |

*nd* not determined

*S. cerevisiae, S. cerevisiae* CEN.PK113-7D. Mouse, 3T3 embryonic fibroblasts

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