BioMetals (2006) 19:31–37
DOI 10.1007/s10534-005-4320-7

Characterization of a Mn-dependent fructose-1,6-bisphosphate aldolase in *Deinococcus radiodurans*

Yong-Mei Zhang[1], Jong-Kang Liu[2], Mohammad Reza Shouri[3] & Tit-Yee Wong[1,*]
[1]*Department of Microbiology and Molecular Cell Sciences, University of Memphis, Memphis, TN, 38152, USA;* [2]*Department of Biological Sciences, National Sun Yat-sen University, Taiwan, Republic of China;* [3]*Department of Anatomy & Neurobiology, University of Tennessee Health Science Center, Memphis, TN, 38163, USA; *Author for correspondence (E-mail: tywong@memphis.edu; Tel: +901-678-4462; fax +234-(0)-36-232-401)*

Received 10 August 2004; accepted 21 March 2005

Key words: class II aldolase, *Deinococcus radiodurans*, glycolysis, manganese-dependent, protein expression

Abstract

The key enzyme of the glycolytic pathway of *Deinococcus radiodurans*, fructose-1,6-bisphosphate aldolase, could be induced independently by glucose and Mn. The enzyme exhibited the characteristics of the metal-dependent Class II aldolases. Unlike most Class II aldolases, the deinococcal aldolase preferred Mn, not Zn, as a cofactor. The *fbaA* gene encoding the deinococcal aldolase was cloned and the protein overproduced in various *Escherichia coli* expression hosts. However, the overexpressed deinococcal enzyme aggregated and formed inclusion bodies. Dissolving these inclusion bodies by urea and subsequent purification by nickel affinity chromatography, resulted in a protein fraction that exhibited aldolase activity only in the presence of Mn. This active aldolase fraction exhibited masses of about 70 kDa and 35 kDa by gel filtration and by SDS gel electrophoresis, respectively, suggesting that the active aldolase was a dimer.

Introduction

Deinococcus radiodurans is a Gram-positive bacterium highly resistant to radiation, dryness and toxic chemicals. The bacterium is considered one of the deep-rooted groups that branched out early from the phylogenetic tree of the bacterial domain (Brooks & Murray 1981; White *et al.* 1999). The complete genome of this organism is available at the website of The Institute for Genomic Research (TIGR, White *et al.* 1999). The divalent metal ion manganese protects *D. radiodurans* from radiation and oxidative stress through its effects on multiple cellular activities. For example, Mn plays an important role in the pyrimidine dimer UV endonuclease for DNA repair (Evans & Moseley 1985). The binding of Mn to chromosomes of intact cells may contribute to resistance of this organism to radiation (Leibowitz *et al.* 1976). Furthermore, Mn is a cofactor for deinococcal superoxide dismutase (Juan *et al.* 1991). However, addition of Mn to the culture of *D. radiodurans* resulted in reduced resistance to UV irradiation (Chou & Tan 1990). We have previously shown that this adverse effect was largely due to the induction of a Mn-induced glycolytic pathway which oxidized glucose to CO_2 rapidly without converting the carbon source from glucose into cell mass. Additionally, the induction of this glycolytic pathway reduced the flux of glucose to the pentose phosphate (PP) pathway, the products of which were needed for excision repair (Zhang *et al.* 2000). An open reading frame (DR1589) corresponding to the *D. radiodurans* fructose-1,6-bisphosphate aldolase gene *fbaA* has been annotated. Disruption of this gene in the deinococcal chromosome resulted in mutants defective in the glycolytic pathway. Importantly, *fbaA* defective mutants were more resistant to UV light than were the wild-type cells (Zhang *et al.* 2003).

Fructose-1,6-bisphosphate aldolase is a marker enzyme of the glycolytic pathway. It catalyzes the reversible condensation reaction of fructose-1,6-bisphosphate to dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. There are two types of aldolases, designated as Class I and Class II aldolases. The Class I aldolases are tetramers and are generally found in plant and animal tissues. This class of aldolase catalyzes the reaction by the Schiff base mechanism (Perham 1990). Some bacteria also possess Class I aldolase activity. For example, a novel Class I aldolase from *E. coli*, encoded by the *dhnA* gene, has been identified and characterized (Thomson *et al*. 1998; Schurman & Sprenger 2001; Thorell *et al*. 2002). Additionally, some Class I aldolases, such as those identified in hyperthermophilic archaea *Thermoproteus tenax* and *Pyrococcus furiosus*, show no significant DNA sequence similarity when compared with the classical Class I enzymes. A new sub-class name, fructose-1,6-bisphosphate aldolase Class IA, was proposed for these enzymes (Siebers *et al*. 2001).

The Class II aldolases are found in bacteria and yeast. These enzymes are dimers and utilize a divalent metal ion, mostly zinc, to perform the essential catalytic role (Kadonaga & Knowles 1983; Perham 1990; Berry & Marshall 1993). Each subunit of the Class II aldolase binds one Zn ion (Szwergold *et al*. 1995). The Zn metal can be replaced by Mn to yield a partially active enzyme (Kobes *et al*. 1969), and Mn could compete for the same binding site with Zn (Mildvan *et al*. 1971). Nuclear relaxation studies showed that Mn could effectively transmit the electrophilic effect to the carbonyl group of the substrate through a hydrogen bond (Smith *et al*. 1980). Some microorganisms, including *E. coli*, *Euglena gracilis*, *Chlamydomonas mundana* and *Chlamydomonas rheinhardii*, possess both Class I and Class II aldolases (Lebherz & Rutter 1973). In this study, we have cloned, expressed, purified and characterized a unique Mn-dependent Class II aldolase of *D. radiodurans*.

Materials and methods

Bacterial strains and growth conditions

Deinococcus radiodurans R1 was a gift from Dr. S.-T. Tan, National Tsing Hua University, Taiwan, Republic of China. TY medium was composed of 0.5% Difco tryptone and 0.3% Difco yeast extract. TGY medium was TY medium plus 0.1% D-glucose. The Mn concentration in the Mn-enriched TGY and TY (TGY + Mn and TY + Mn) media was 0.1 mM. *D. radiodurans* was grown at 30 °C in TGY medium with shaking at 200 rpm. *E. coli* (ATCC 25922) was grown on TGY medium at 37 °C. *E. coli* BL21-CodonPlus(DE3)-RIL (Stratagene) and Tuner (Novagen) strains were grown at 37 °C in LB medium (1% peptone tryptone, 0.5% yeast extract and 0.5% NaCl). Ampicillin, when needed, was added to the LB medium to the final concentration of 100 μg/ml.

Determination of Mn concentration

The endogenous concentration of Mn in TY growth medium was analyzed by Inductivity Coupled Plasma-Mass Spectrometer (Perkin Elmer) and was shown to be about 0.18 μM. The endogenous concentration of Mn in LB medium was about 0.42 μM.

Preparation of cytosol fractions

The deinococcal cells were harvested at mid-log phase by centrifugation at 4300 × g for 10 min. Cells were washed with ice-cold buffer (50 mM Tris-HCl, pH 7.5) and suspended in the same buffer at about 0.1 g wet weight of cells per milliliter. All of the subsequent procedures were performed at 4 °C. Cells were disrupted by passing through a French pressure cell (SLM Instruments, Inc.) at 15000 lb/in^2. Cellular debris and unbroken cells were removed by centrifugation at 23000 × g for 20 min. The supernatant was removed and further centrifuged at 193000 × g for 2 h. The resultant supernatant (cytosol) was stored at −80 °C until used.

Fructose-1,6-bisphosphate aldolase assay

Aldolase activity was determined at 25 °C by the spectrometric method described in Worthington Enzyme Manual (1993). The reaction mixture included: 2 mM hydrazine and 2.4 mM fructose-1,6-bisphosphate in 50 mM Tris-HCl (pH 7.5). Glyceraldehyde-3-phosphate produced from fructose-1,6-bisphosphate reacts with hydrazine to form an aldehyde-hydrazone adduct which was monitored at 240 nm with a dual beam spectrophotometer (Varian DMS 100S). Enzyme reaction

was initiated by the addition of fructose-1,6-bisphosphate. Reaction mixture without cytosol was used as a reference blank. One unit of the specific enzymatic activity was defined as a one unit change of the absorbance at 240 nm at 25 °C per minute per mg protein. The protein concentration was determined using the Bradford method with bovine serine albumin (BSA) as the standard.

Effects of Mn and Zn on the aldolase activity of the EDTA-pretreated cytosols of D. radiodurans and E. coli

The cytosol preparations of *D. radiodurans* (grown on TGY, TGY + Mn, TY and TY + Mn media) were used to determine the specific effects of metal ions on the aldolase activity. About 7 min after the reaction was started, 0.67 mM EDTA was added to the reaction mixture. The rate of hydrazone formation was monitored continuously at 240 nm at 25 °C. After 10 min incubation in the presence of EDTA, 1 mM of $MnCl_2$ or $ZnCl_2$ was reconstituted to the reaction mixture. The cytosol of *E. coli* (ATCC 25922) grown on TGY medium was prepared similarly and used for a comparative study.

Construction of the expression plasmid of Deinococcal aldolase

Primers were designed to introduce an NdeI site in the beginning of *fbaA* gene with the forward primer 5′-ACCATATGCTCGTTACCGGTAAAGAC-3′ (NdeI site underlined) and a BamHI site at the end of the gene with the reverse primer 5′-CTTCGGATCCTAGGGCAAGTATAGG-3′ (BamHI site underlined). A 1026-bp DNA fragment was amplified and purified before being ligated into pCR2.1 vector (Invitrogen). Then the DNA insert was sequenced using the universal primers flanking the multiple cloning site to ensure there was no PCR error. NdeI and BamHI were used to digest the pCR2.1 vector with the correct DNA insert, which was then ligated into the expression plasmid pET-15b (Novagen), already restricted with the same enzymes. The *fbaA* gene was inframe with an N-terminal His-tag in the expression plasmid.

Overexpression and purification of Deinococcal aldolase

The expression plasmid was introduced into BL21-CodonPlus(DE3)-RIL cells using electroporation. Transformants were selected on LB/Ampicillin (100 μg/ml) agar plates. Single colonies were picked and grown in LB/Amp broth overnight to inoculate fresh LB/Amp broth (1% inoculation). The expression of deinococcal aldolase was induced by adding 1 mM IPTG (isopropyl β-D-thiogalactopyranoside) into the mid-log phase culture (Klett unit at about 80). The cells were further incubated at 37 °C for another 3 h before being harvested by centrifugation. About 1 ml of cells before and after the addition of IPTG was saved to run an expression gel (12% SDS mini-gel) to assess the expression level.

Cell pellet was resuspended in MCAC buffer (20 mM Tris-HCl, pH 7.9, 0.5 M NaCl, 10% glycerol). Cells were incubated in 1 mg/ml lysozyme on ice for 10 min before 0.1% TritonX-100 and 1 mM of phenylmethylsulphonyl fluoride (PMSF) were added. The mixture was frozen and thawed (−80 °C and 4 °C) 3 times. Cytosol was obtained by centrifugation at 193000 g at 4 °C for 1 h. The resultant supernatant (S_3, cell extract) was then loaded onto a charged Ni-nitrilotriacetic acid agarose column (Qiagen). After the cell extract flowed through, the column was washed with 10-bed volumes of MCAC followed by 10-bed volumes of MCAC plus 40 mM imidazole. The bound protein was eluted in 1-bed volume fractions of MCAC plus 200 mM imidazole. Fractions with the highest protein content were pooled together and dialyzed overnight against 10 mM Tris-HCl pH 7.6, 0.1 mM DTT and 0.5 mM EDTA at 4 °C. The dialyzed protein solution was concentrated using Centricon-30 tubes (Amicon). To get soluble expression products, the incubation temperature was decreased from 37 °C to 25 °C. Instead of inducing the expression for 3 h after addition of 1 mM IPTG, the cells were harvested after 30 min, 1, 2, and 3 h after induction. A different strain of BL21 cells, the Tuner strain, was also used to express deinococcal aldolase. Since this strain does not have the LacY permease for IPTG, the inducer can enter the cells only by passive diffusion. The rate of IPTG transport into the cell is dictated by the concentration of IPTG outside the cell. Thus, the expression level can be controlled by adjusting the concentration of IPTG. A range of IPTG concentrations, 50 μM, 100 μM, 0.5 mM and 1 mM, were used to induce protein expression. The expression level at different IPTG concentrations was examined with 12% SDS gels.

Denaturation and refolding of the insoluble aldolase inclusion body

The ultracentrifugation pellet (R_3) was suspended in MCAC buffer. An equal volume of ice-cold 8 M urea was added to the solution. The protein precipitate was removed by centrifugation at 5000 × g for 5 min. The supernatant was subsequently diluted 10 times with MCAC buffer. The diluted sample was loaded onto a freshly charged Ni-affinity column. The His-tagged protein was purified following the procedures described above, and the purified protein was dialysed against 20 mM phosphate buffer, pH 7.2.

Determination of molecular weight by gel filtration analysis

The native molecular weight of the aldolase was estimated by gel filtration chromatography. The affinity-purified and urea-treated protein was adjusted to 0.5 mM $MnCl_2$ and concentrated by pressure dialysis with a DIAFLO ultrafilter YM30 (Amicon) under nitrogen atmosphere. The concentrated sample was applied to a Sephadex G-200 column (30 × 2.5 cm) previously equilibrated with 20 mM phosphate buffer (pH 7.2) and eluted with the same buffer. Fractions were monitored by a UV light detector at 280 nm. Authentic globular protein markers (Sigma Kit MW-ND-500) were used to calibrate the molecular weight.

Results

We analyzed the aldolase activities in cytosol fractions of *D. radiodurans* grown on media without glucose or Mn (TY), with glucose (TGY), with Mn (TY + Mn), and with both glucose and Mn (TGY + Mn). We also tested the dependency of aldolase on Mn or Zn by treating the cytosols with EDTA prior to the addition of a surplus amount of Mn or Zn to the reaction mixtures. Results are summarized in Table 1.

Deinococcal aldolase preferred Mn as cofactor

There was no detectable aldolase activity in cytosol obtained from cells grown on TY medium (Table 1), which suggested that the aldolase was not constitutively expressed in *D. radiodurans*. Aldolase activities were readily detected in all the other cell preparations. The most active aldolase activities were found in the samples from cells grown on Mn-enriched media (TY + Mn and TGY + Mn). The level of activity in both preparations was comparable to that in the *E. coli* cytosol. Like other metal-dependent Class II aldolase, the deinococcal enzyme was completely inhibited by EDTA (Table 1). Replenishing a surplus amount of $MnCl_2$(1 mM) to the reaction mixture restored aldolase activities to the initial levels in both preparations. In contrast, a supplement of 1 mM of $ZnCl_2$ to the EDTA-pretreated cytosols of these cells recovered less than 20% of

Table 1. Aldolase activity in the cytosols of D. radiodurans grown on different media and the effects of divalent metals on the EDTA-pretreated cytosols[a]

Cytosol	Specific activity ($\Delta OD_{240\ nm}$/min/mg protein)[b] (Percent activity)			
	Initial activity	+ 0.67 mM EDTA	+ EDTA + 1 mM $MnCl_2$	+ EDTA + 1 mM $ZnCl_2$
D. radiodurans				
TGY	0.038 ± 0.002 (100%)	UD[c]	0.061 ± 0.003 (160%)	0.012 ± 0.001 (31%)
TGY + Mn	0.081 ± 0.005 (100%)	UD	0.084 ± 0.004 (103%)	0.013 ± 0.001 (16%)
TY	0.006 ± 0.001 (100%)	UD	0.004 ± 0.000 (67%)	0.001 ± 0.001 (17%)
TY + Mn	0.078 ± 0.002 (100%)	UD	0.083 ± 0.003 (106%)	0.011 ± 0.001 (14%)
E. coli (ATCC 25922)				
TGY	0.088 ± 0.006 (100%)	0.042 ± 0.001 (48%)	0.058 ± 0.002 (66%)	0.068 ± 0.001 (77%)
E. coli overexpressed and purified deinococcal FbaA protein				
	UD	UD	0.194 ± 0.018	0.048 ± 0.008

[a]Data shown are average ± SD from three independent experiments. [b]Data in brackets are percentages of the initial activity of each sample. [c]Undetectable.

the initial activity (Table 1). These results indicated that that deinococcal aldolase preferred Mn to Zn as its cofactor.

The cytosol of the deinococcal cells grown on TGY also exhibited aldolase activity. Despite the lower initial activity in TGY-grown cells to those cells obtained from Mn-enriched media, addition of Mn to the EDTA-pretreated cytosol not only fully restored the aldolase activity, but also stimulated activity to 156% of the initial aldolase level (Table 1). This suggested that a portion of the aldolase enzyme in TGY-grown cells was in the form of an apoenzyme when Mn was limited.

The aldolase activity in the *E. coli* cytosol exhibited both metal-independent Class I and metal-dependent Class II characteristics. This observation was consistent with those reported in the literature (Lebherz & Rutter 1973). About 52% of the initial aldolase activity in the *E. coli*cytosol was inhibited by EDTA (Table 1), indicating that each Class I and Class II accounted for half of the total aldolase activity in *E. coli*. The partially-inhibited aldolase activity of the *E. coli* cytosol was recovered slightly more with Zn than with Mn (Table 1).

Expression of Deinococcal aldolase in various E. coli host strains

Attempts were made to purify the endogenous deinococcal aldolase from the cellular lysate by conventional protein purification procedures. However, the yield of the aldolase protein was poor. Because the gene sequence of this enzyme is known, we cloned the *fbaA* gene into pET-15b plasmid for overexpression of a His-tagged aldolase in *E. coli* hosts and subsequently purified the protein (Figures 1, 2). The high G + C content of the deinococcal *fbaA* gene (64.13%) and differences in codon usage between *D. radiodurans* and *E. coli* host strains could prevent overexpression of the deinococcal protein. To avoid these problems, we used the *E. coli* BL21-CodonPlus(DE3)-RIL strain to express the deinococcal aldolase. Induction with 1 mM IPTG resulted in very high level expression of deinococcal aldolase in all four clones tested. Over 50% of the total cellular protein was committed to the protein molecule (Figure 1). Unfortunately, after differential centrifugation, the overexpressed aldolase protein was retained in the pellet fraction R_3 (Figure 1), likely in the form of inclusion bodies. Decreasing the growth temperature to 25 °C, reduction of incubation time, or a combination of both did not prevent protein aggregation. Attempts to control the expression of aldolase in Turner cells at very low IPTG concentration and a lower growth temperature all failed to yield a soluble deinococcal aldolase.

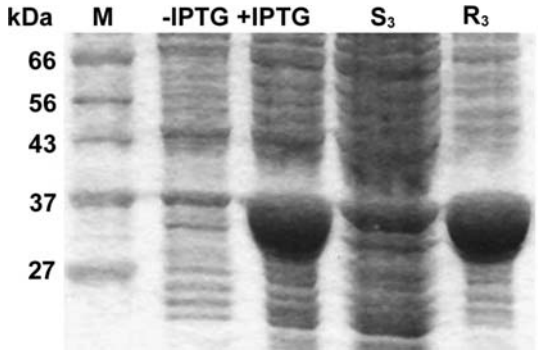

Figure 1. Overexpression of deinococcal aldolase induced by IPTG and formation of inclusion body. Addition of 100 μM IPTG induced significant overexpression of the 35 kDa deinococcal aldolase in BL21-CodonPlus(DE3)-RIL cells carrying the expression plasmid of *fbaA* gene. Protein profile of samples after differential centrifugation showed that the aldolase protein was not soluble and was retained in the pellet fraction (R_3) after the ultracentrifuge step. S_3 was the resultant supernatant fraction from the ultracentrifugation. Proteins in the first lane (M) on the gel were markers with known molecular weights.

Urea treatment and native mass analysis of aldolase

To solublize the aldolase protein from the inclusion body, we treated the protein aggregate in the R_3 fraction with 4 M urea. The denatured protein was purified by Ni-affinity chromatography and dialyzed against 20 mM phosphate buffer, pH 7.2, overnight. Analysis of the dialyzed sample by SDS-gel electrophoresis showed a single protein with a mass of 35 kDa (Figure 2). The eluted and dialyzed protein did not exhibit any aldolase activity, but it was activated by the addition of 0.5 mM $MnCl_2$. The Mn-reconstituted protein has a specific activity of about 0.194 units, and the activity was completely inhibited by 1 mM EDTA (Table 1). Zinc only partially restored the aldolase activity of the renatured protein to 0.048 units. Elution of the Mn-reconstituted enzyme by gel filtration chromatography resulted in an active fraction corresponding to a mass of 70 kDa. This suggested that the active form of the deinococcal aldolase was a dimer.

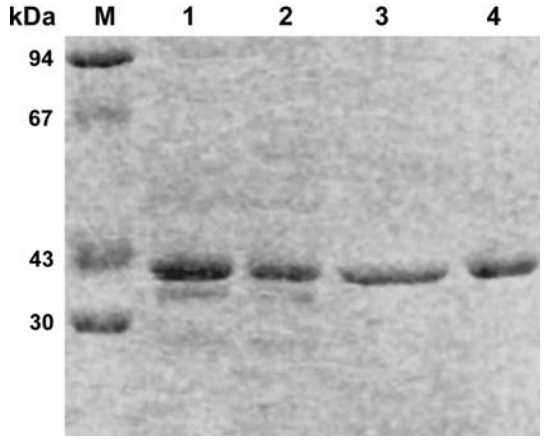

Figure 2. Purification of the renatured deinococcal aldolase protein. The insoluble aldolase protein in the R_3 fraction (Figure 1) was denatured by urea as described in 'Material and methods'. The soluble protein (Lane 1) was separated from the precipitate (Lane 2) by centrifugation. The His-tagged deinococcal aldolase in the soluble fraction was purified by Ni-affinity chromatography (Lane 3) before being dialysed and further concentrated by ultrafiltration (Lane 4). Protein markers were in Lane M.

Discussion

The divalent metal ions Mn and Zn can be used interchangeably in many enzyme systems. For example, Zn can be replaced by Mn to yield a fully active aldolase in yeast (Kobes *et al*. 1969). In bovine lens, Mn could replace Zn in restoring the EDTA-inhibited sorbitol dehydrogenase (Marini *et al*. 1997). Analysis of the *fbaA* gene of *D. radiodurans* showed that the deinococcal aldolase amino acid sequence was very similar to other Class II aldolases and contained the signature sequence (-HxDH-) common for Zn-binding bacterial aldolases (data not shown). But, unlike the typical bacterial Class II enzymes, the deinococcal aldolase preferred Mn to Zn as cofactor (Table 1). The active form of the purified recombinant deinococcal aldolase existed as a dimer with a mass of about 70 kDa, similar to other Class II aldolase enzymes.

Deinococcus radiodurans has a peculiar affinity toward Mn. The endogenous Mn in the TY growth medium (0.18 μM) seems to satisfy all the requirements for normal growth. Yet, exogenously added Mn could induce additional rounds of cell division known as Mn-induced cell division (Chou & Tan 1990) and thus stimulate a glycolytic pathway (Zhang *et al*. 2000). It should be noted that the occurrences of Mn-induced cell division and the Mn-induced glycolytic pathway were two different, unrelated events because most of the biomass generated via Mn-induced cell division did not come from the glucose consumed by the glycolytic pathway (Zhang *et al*. 2000). More importantly, in this report we showed that exogenously added Mn alone could induce the activity of aldolase, even in the absence of the glucose substrate in the growth medium (Table 1). In addition, our results showed that supplementing the medium with glucose stimulated the aldolase activity in *D. radiodurans*in a Mn-independent manner.

Attempts to express a soluble and functional deinococcal aldolase in *E. coli* hosts were unsuccessful. Perhaps, an overexpression of a soluble and functional deinococcal aldolase was toxic to the *E. coli* host, as this protein could quench the pool of Mn needed for normal cell growth. Converting this undesirable aldolase protein into inclusion bodies could prevent Mn depletion. We could not test if addition of Mn to the *E. coli* growth medium could improve the solubility of the recombinant protein, because the growth of *E. coli* was inhibited when Mn was added to the medium at 100 μM, this was the concentration needed to get a fully active aldolase in *D. radiodurans*. Alternatively, the proper folding of the Mn-dependent *D. radiodurans* aldolase may require a unique chaperon protein that was absent from the *E. coli* hosts.

Whole cell studies (Zhang *et al*. 2000) showed that *D. radiodurans* growing on TGY medium without Mn lacked a functional glycolytic pathway. The inability to hydrolyze glucose by glycolysis, despite the presence of aldolase enzyme in the TGY-grown cells (Table 1), might be due to the cell's inability to incorporate the endogenous Mn into the aldolase apoenzyme. Although we detected aldolase activity in the cytosol of TGY-grown cells (Table 1), this activity might be the result of Mn contamination during the cytosol preparations. The metal French press cell and the phosphate buffers used could be probable sources of Mn contamination. Alternatively, Mn might be prevented from being incorporated into the apoenzyme by some unknown regulatory mechanism *in vivo*. Lysis of the cells likely released any enzyme regulation and a portion of the apo-aldolase became active.

References

Berry A, Marshall KE. 1993 Identification of zinc-binding ligands in the Class II fructose-1,6-bisphosphate aldolase of *Escherichia coli*. *FEBS Lett* **318**, 11–16.

Brooks BW, Murray RGE. 1981 Nomenclature for "*Micrococcus radiodurans*" and other radiation-resistant cocci: Deinococcaceae fam. nov. and *Deinococcus* gen. nov., including five species. *Int J Syst Bacteriol* **31**, 353–360.

Chou FI, Tan ST. 1990 Manganese(II) induces cell division and increases in superoxide dismutase and catalase activities in an aging deinococcal culture. *J Bacteriol* **172**, 2029–2035.

Evans DM, Moseley BE. 1985 Identification and initial characterisation of a pyrimidine dimer UV endonuclease (UV endonuclease beta) from *Deinococcus radiodurans*; a DNA-repair enzyme that requires manganese ions. *Mutat Res* **145**, 119–128.

Juan JY, Keeney SN, Gregory EM. 1991 Reconstitution of *Deinococcus radiodurans* aposuperoxide dismutase. *Arch Biochem Biophys* **286**, 257–263.

Kadonaga JT, Knowles JR. 1983 Role of mono- and divalent metal cations in the catalysis by yeast aldolase. *Biochemistry* **22**, 130–136.

Kobes RD, Simpson RT, Vallee BL, Rutter WJ. 1969 A functional role of metal ions in a Class II aldolase. *Biochemistry* **8**, 585–588.

Lebherz HG, Rutter WJ. 1973 A Class I (Schiff base) fructose diphosphate aldolase of prokaryotic origin. *J Biol Chem* **248**, 1650–1659.

Leibowitz PJ, Schwartzberg LS, Bruce AK. 1976 The in vivo association of manganese with the chromosome of *Micrococcus radiodurans*. *Photochem Photobiol* **23**, 45–50.

Marini I, Bucchioni L, Borella P, Del Corso A, Mura U. 1997 Sorbitol dehydrogenase from bovine lens: purification and properties. *Arch Biochem Biophys* **340**, 383–391.

Mildvan AS, Kobes RD, Rutter WJ. 1971 Magnetic resonance studies of the role of the divalent cation in the mechanism of yeast aldolase. *Biochemistry* **10**, 1191–1204.

Perham RN. 1990 The fructose-1,6-biphosphate aldolases: same reaction, different enzymes. *Biochem Soc Trans* **18**, 185–187.

Schurmann M, Sprenger GA. 2001 Fructose-6-phosphate aldolase is a novel class I aldolase from *Escherichia coli* and is related to a novel group of bacterial transaldolases. *J Biol Chem* **276**, 11055–11061.

Siebers B, Brinkmann H, Dorr C, *et al*. 2001 Archaeal fructose-1,6-bisphosphate aldolases constitute a new family of archaeal type class I aldolase. *J Biol Chem* **276**, 28710–28718.

Smith GM, Mildman AS, Harper ET. 1980 Nuclear relaxation studies of the interaction of substrates with a metalloaldolase from yeast. *Biochemistry* **19**, 1248–1255.

Szwergold BS, Ugurbil K, Brown TR. 1995 Properties of fructose-1,6-bisphosphate aldolase from *Escherichia coli*: an NMR analysis. *Arch Biochem Biophys* **317**, 244–252.

Thomson GJ, Howlett GJ, Ashcroft AE, Berry A. 1998 The *dhnA* gene of *Escherichia coli* encodes a class I fructose bisphosphate aldolase. *Biochem J* **331**, 437–445.

Thorell S, Schurmann M, Sprenger GA, Schneider G. 2002 Crystal structure of decameric fructose-6-phosphate aldolase from *Escherichia coli* reveals inter-subunit helix swapping as a structural basis for assembly differences in the transaldolase family. *J Mol Biol* **319**, 161–171.

White O, Eisen JA, Heidelberg JF, *et al*. 1999 Genome sequence of the radioresistant bacterium *Deinococcus radiodurans* R1. *Science* **286**, 1571–1577.

Zhang Y-M, Wong TY, Chen LY, Lin CS, Liu JK. 2000 Induction of a futile Embden-Meyerhof-Parnas pathway in *Deinococcus radiodurans* by Mn: possible role of the pentose phosphate pathway in cell survival. *Appl Environ Microbiol* **66**, 105–112.

Zhang Y-M, Liu JK, Wong TY. 2003 The DNA excision repair system of the highly radioresistant bacterium *Deinococcus radiodurans* is facilitated by the pentose phosphate pathway. *Mol Microbiol* **48**, 1317–1323.