SHORT COMMUNICATION

Correlation of glutathione peroxidase—glutathione reductase—NADP-linked glyceraldehyde 3-phosphate dehydrogenase activity with cellular degradation in *Strep. mutans* JC 2 strains

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Introduction

The isolation of rapidly growing mutants from *Strep. mutans* JC 2 and HS 6 or *Strep. salivarius* SS 2 has been successfully carried out¹⁾. Previously, we reported changes in enzymatic activity correlated with cellular lyses of *Strep. mutans* HS 6-Ly mutant and *Strep. salivarius* SS 2-Ly mutant strains²⁾. These lytic mutants were easily destroyed by H₂O₂ or did not lyse by addition of reducing agents. Thus, it may be speculated that these mutants do not develop protective mechanisms against oxidative damage.

Glutathione peroxidase (GSH-Px) functions to detoxicate and protect cellular membranes from oxidative damage³⁾ by coupling with the reactions of both glutathione reductase (GR) and NADP-linked glyceraldehyde 3-phosphate dehydrogenase (NADP-linked GA3PDH). To demonstrate the correlation of enzymatic activity with cellular destruction, we compared the activities of *Strep. mutans* JC 2⁺, JC 2-Ly and the nonlytic mutant, JC 2-NL for both the lytic and nonlytic states. The *Strep. mutans* JC 2 strain was found to repair oxidative damage through enzymatic activity.

Materials and Methods

Strains of *Strep. mutans* JC 2⁺, JC 2-Ly and JC 2-NL were used in this study. *Strep.*

mutans JC 2-Ly was the lytic mutant obtained from JC 2⁺ strain¹⁾. *Strep. mutans* JC 2-NL was the nonlytic mutant isolated from JC 2-Ly lytic mutant as more resistant to H₂O₂ than JC 2-Ly. The media and culturing methods have been described in a previous paper⁴⁾.

Aliquots (0.5 ml) of overnight cultures of the three TSMG grown strains were inoculated in 200 ml of a TSMG+0.5 mM glucose broth and incubated at 35 and 39°C overnight. The bacteria were harvested by centrifugation for 30 min at 2,500×g and washed twice with 0.05 M potassium phosphate buffer (pH 7.8)/0.1 mM ethylenediaminetetraacetic acid·2Na (EDTA). The washed bacterial pellets were suspended in 3.0 ml of 0.05 M potassium phosphate buffer (pH 7.8)/0.1 mM EDTA, and disrupted by sonication at 0°C for 10 min using a sonicator (T-A-4201, Kaijyo Denki Co., Japan). The suspension was then centrifuged for 30 min at 18,000×g at 4°C. The clear supernatant was used as the cell-free extract after being dialyzed against the same buffer overnight at 4°C.

Glucose 6-phosphate dehydrogenase (G6PDH) (EC 1.1.1.49) activity was estimated by the method of Yamada and Carlsson⁵⁾. NADP-linked (EC 1.2.1.12) glyceraldehyde 3-phosphate dehydrogenase (GA3PDH) activity was estimated by the methods of Brown and Wittenberger⁶⁾. NAD(P)H oxidases were estimated by the

procedure of Carlsson *et al.*⁷⁾ Glutathione peroxidase (GSH-Px) (EC 1.11.1.9) and glutathione reductase (GR) (EC 1.6.4.2) activity was estimated as follows. The reaction mixture for the GSH-Px assay consisted of 0.06 M potassium phosphate buffer (pH 7.0)/0.1 mM EDTA/1.0 mM NaN₃/0.2 mM NADPH/one unit per ml GR (Oriental Yeast, Japan)/1.0 mM GSH/1.5 mM cumene hydroperoxide or 0.25 mM H₂O₂/100 μ l cell-free extract, total 1.0 ml. The reaction mixture for the GR assay (Total 1.0 ml) contained 0.069 M potassium phosphate buffer (pH 7.6)/2.0 mM EDTA/0.1 mM NADPH/3.0 mM GSSG/100 μ l cell-free extract. Enzymatic activity was estimated on the basis of an increase or decrease in optical density at 340 nm and the results were expressed in μ moles of NAD(P)H oxidized or NAD(P) reduced, per min per mg protein. The amounts of protein were determined by the method of Lowry *et al.*⁸⁾

Results

The changes in the enzymatic activity of *Strep. mutans* JC 2⁺, JC 2-Ly and JC 2-NL strains induced at different temperatures are shown in Table 1. Cellular lysis occurred at an incubation temperature of 39°C and above but not at 35°C in *Strep. mutans* JC 2-Ly mutant strain. As evident from Table 1, changes in the activity of GSH-Px, GR and NADP-linked GA3PDH are closely correlated to cellular lysis, but not so in the case of G6PDH, NAD-linked GA3PDH and

NAD(P)H oxidases. In the lytic mutant of JC 2-Ly incubated at 39°C, the activity of GSH-Px and GR decreased to 77% and 38% of that at 35°C and the NADP-linked GA3PDH activity of the NADPH synthesizing enzymatic system decreased to 28% of that at 35°C. In the nonlytic mutant JC 2-NL incubated at 39°C, the activity of GSH-Px, GR and NADP-linked GA3PDH decreased to 92%, 85% and 80% of that at 35°C, respectively. In the parental JC 2⁺ strain incubated at 39°C, the activity of GSH-Px decreased to 94%, that of GR increased to 100% and that of NADP-linked CA3PDH decreased 52% of the activity at

Fig. 1 Change in the turbidity of JC 2-Ly (○), JC 2-NL (●) and 2⁺ (△) strains incubated at 37°C in a TSMG medium and those in the JC 2⁺ strain incubated at 37°C in the same medium +3.2 mM GSSG (□) or +2.6 mM NADH (■).

Table 1 Enzyme activity in cellular lysis

Strains	Temp.	GSH-Px	GR	G6PDH	GA3PDH		oxidase	
					NADP	NAD	NADPH	NADH
JC 2 ⁺	35°C	0.016	0.19	ND*	0.10	2.9	0.0013	0.086
	39°C	0.015	0.20	ND	0.053	2.7	0.0019	0.043
JC 2-Ly	35°C	0.012	0.046	ND	0.069	2.6	0.0009	0.0076
	39°C	0.0089	0.017	ND	0.019	3.7	0.0003	0.0050
JC 2-NL	35°C	0.013	0.036	ND	0.066	3.0	0.0010	0.017
	39°C	0.012	0.031	ND	0.053	3.7	0.0005	0.0040

JC 2⁺, JC 2-Ly and JC 2-NL cells were incubated in TSMG+5.0 mM glucose media.

The results are expressed as units per mg protein. * ND=Not detectable.

35°C, respectively. But as shown in Table 1, the activity of GR at 35°C in the JC 2-Ly and JC 2-NL mutant strains decreased to 19–25% of that in the JC 2⁺ parental strain. The activity of NADH oxidase at 35°C in the JC 2-Ly and JC 2-NL mutant strains also decreased to 10–22% of that in the JC 2⁺ parental strain.

As shown in Fig. 1, the JC 2⁺ parental strain grew slower than the JC 2-Ly or JC 2-NL mutant strains in the TSMG media at 37°C. However, the growth of the JC 2⁺ parental strain was accelerated by the addition of 3.2 mM GSSG, but the addition of 6.4 mM GSH, 2.9 mM NAD, 2.5 mM NADP, 2.6 mM NADH or 2.2 mM NADPH failed to do so. Only the data of the GSSG and NADH addition appear in Fig. 1.

Discussion

Correlation of changes in enzymatic activity with cellular degradation was evident from the activity of GSH-Px, GR and NADP-linked GA3PDH (Table 1). Thus, a coupled eliminating system of GSH-Px—GR—NADP-linked GA3PDH against oxidative damage may exist in *Strep. mutans* JC 2 strain. This coupled enzymatic system may possibly act to eliminate lipid peroxide or H₂O₂ as its substrate and recover the rigidity of the cell membrane²⁾. The oxidative portion of the pentose monophosphate shunt was absent from the strains of *Strep. mutans* and *Strep. salivarius*. These organisms showed different methods for generating NADPH which appeared to be expressed by the presence of NADP-linked GA3PDH (EC 1.2.1.9) in the glycolytic pathway^{5,6,9)}. This was found to be the case in the JC 2⁺ parental, JC 2-Ly and JC 2-NL mutant strains which showed changes in the activity of NADP-linked GA3PDH concomitant with cellular degradation. *Strep. mutans* possesses two isozymes of GA3PDH specific to NAD and NADP respectively⁶⁾ and NAD-linked GA3PDH of *Strep. mutans* was completely inhibited by OSCN⁻ but not NADP-linked GA3PDH⁷⁾. Our data suggest that NADP-linked GA3PDH activity changed with cellular damage.

The activity of GR and NADH oxidase both decreased simultaneously with an increase in the growth rate (Table 1). This strongly suggests that GSSG, NADPH or NADH are restored intracellularly so as to ensure the accelerated growth of the JC 2-Ly and JC 2-NL mutant strains. In fact, the addition of 3.2 mM of GSSG actually accelerated the growth rate of JC 2⁺ parental strain, as shown in Fig. 1. The accumulation of sulfhydryl compounds in the medium and deletion of O₂ by NADP-dependent reduction may be the principal mechanisms by which *Strep. mutans* creates a reducing environment in the extracellular medium¹⁰⁾. However, GSH had no enhancing effect on the growth rate of the JC 2⁺ parental strain and the activity of GR and NADH oxidase decreased in the aeration-resistant mutants of JC 2-Ly and JC 2-NL. Thus, it may be concluded that GSSG and NADH possibly participate in the synthesis of certain macromolecules or ATP in aeration-resistant mutants. There was only a trace amount of the activity of NADPH oxidase, regardless of cellular degradation or rapid growth.

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