Multiresistance and Lipid Peroxidation System of Thermoresistant *Shigella sonnei* Strains

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The content of lipid peroxidation products increases in lipids isolated from *Shigella sonnei* after heating. Thermoresistant strains are characterized by higher activity of superoxide dismutase and catalase and by stable activity of glutathione reductase and glutathione transferase compared with thermolabile strains. Thermoresistant strains are also resistant to some antibiotics and hydrogen peroxide.

Key Words: *Shigella sonnei; thermoresistance; lipid peroxidation*

Thermoresistance reflects the common biological process of adaptation of microbial population to environmental factors. However, biochemical mechanisms responsible for bacterial resistance to high temperatures remain obscure. It is known that lipids of thermoresistant *S. sonnei* are modified (methylated) [4]. Induction of the synthesis of heat shock proteins plays an important role in some thermoresistant bacteria [7]. However, neither lipid methylation nor synthesis of heat shock proteins are specific mechanisms: they are activated by various unfavorable factors.

In the present study we determined the activity of antioxidant enzymes and the content of lipid peroxidation products (LPO) before and after heating of *Shigella sonnei* strains. The resistance of these microorganisms to some antibiotics and H_2O_2 was also studied.

MATERIALS AND METHODS

Experiments were performed on 315 *S. sonnei* strains isolated from children and adults during sporadic outbreaks of dysentery. The thermoresistance of these strains was determined according to the methodological recommendations [2]. The minimal inhibiting concentration of antibiotics was measured in slightly alkaline agar (1 ml) by the conventional method in the concentration range 0.5-100 μg/ml. The H_2O_2 sensitivity of thermolabile and thermoresistant *S. sonnei* strains was evaluated by the method described elsewhere [3].

Lipids from bacterial cells were extracted as described [6]. The content of Schiff bases and diene conjugates was measured fluorimetrically [8] and spectrophotometrically [13], respectively. For the antioxidant enzyme activity assay shigellas were disintegrated with ultrasound. The protein content was measured as described [10], the catalase activity by the rate of hydrogen peroxide degradation [5], the superoxide dismutase activity (SOD) by the method of Nishikimi, the glutathione reductase activity by the accumulation of oxidized NADPH [12], and the activity of glutathione S-transferase by the accumulation of reduced glutathione conjugated with 1-CI-2,4-dinitrobenzene [9].

RESULTS

According to the resistance to high temperatures, the strains were divided into thermolabile (TLS), which died at 70°C, and thermoresistant (TRS), which remained alive at 85-90°C for 5-15 min.

Thermoresistant strains were characterized by high SOD and catalase activities: by 31.4 and 206.8%, respectively, higher than those of TLS. The glutathione reductase and glutathione transferase activities were the same in TRS and TLS.

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TABLE 1. Constitutive Activity of Antioxidant Enzymes of *S. sonnei* and the Content of LPO Products before and after Heating (*M*±*m*, *n*=10)

Parameter	TLS	TRS
SOD, U/mg protein×10^3	7.69±0.85	10.21±0.75*
Catalase, mcat/g protein×10^3	7.34±1.9	22.52±4.64*
Glutathione S-transferase, U/g protein×10^3	1.24±0.3	1.19±0.26
Glutathione reductase, U/g protein×10^3	28.35±3.85	32.7±2.01
Diene conjugates, nm/mg total lipids×10^{-5}		
before heating	3.074±1.04	4.344±1.44
after heating	5.832±0.89*	5.81±1.17
Schiff bases, units fluor./mg total lipids		
before heating	232.08±26.0	260.14±23.0
after heating	347.0±39.8*	316.8±81.6

Note. Here and in Table 2: asterisk indicates values statistically significant at $p<0.05$.

In order to assess the significance of free radical oxidation for the development of thermoresistance, the content of LPO products in *S. sonnei* lipids was measured before and after heating (60°C, 30 min). Lipids isolated from TRS had a higher content of diene conjugates and Schiff bases irrespective of high SOD and catalase activities. This points to a "higher" level of LPO regulation by pro- and antioxidant systems in TRS, which may be one of the mechanisms providing for thermotolerance. The contents of diene conjugates and Schiff bases in the TLS lipids increased by 89.7 and 49.5%, respectively, the differences being significant compared with the baseline values, while in the lipids of TRS the differences were insignificant (Table 1). Bacterial cells are known to have a high content of Fe^{2+} [1]. Heating leads to the accumulation of H_2O_2 [11], which may trigger the Fe^{2+}-induced formation of hydroxyl radical ($OH^{\cdot}$). Presumably, in TRS this is controlled by the higher activities of SOD and catalase, while in TLS uncontrolled free radical oxidation (due to low activity of these enzymes) causes cell death.

In order to find out whether these mechanisms are universal, we tested the *S. sonnei* strains for resistance to some antibiotics and H_2O_2. The minimal inhibiting concentration of antibiotics that alter the membrane permeability (polymyxin, ampicillin, and carbenicillin) for TRS was 6-fold higher than that for TLS (15.07±2.03 and 2.35±0.21 μg/ml, respectively).

TABLE 2. Survival of *Shigella sonnei* in H_2O_2 during a 10-min Period (*M*±*m*, *n*=5)

Concentration of H_2O_2, M	Survived cells, %	
	TLS	TRS
0.03	26.3±7.69	77.7±12.10*
0.3	2.06±0.549	8.5±1.92*
1.0	0.0±0.0	6.6±1.47*

The inhibiting concentrations of antibiotics suppressing protein synthesis (streptomycin and tetracycline) were virtually the same for TLS and TRS: 38.44±4.63 and 43.6±2.17 μg/ml, respectively.

At a concentration of 1 M, hydrogen peroxide displayed high bactericidal activity towards TLS, while TRS were resistant to this concentration, 6.6% of cells surviving (Table 2). More thermoresistant cells survived during incubation with lower concentrations of H_2O_2.

Thus, the constitutively high activities of the antioxidant enzymes and the "high" level of LPO regulation are probably involved in the development of cross-resistance of *S. sonnei* strains.

REFERENCES

1. I. S. Kurbanov, I. V. Zlatkin, D. I. Nikin, *et al.*, *Izv. Akad. Nauk SSSR, Ser. Biologiya*, No. 3, 443-447 (1990).
2. L. V. Pozhalostina, N. I. Abramova-Obolenskaya, and A. M. Amerkhanova, *Methodological Recommendations on the Determination of Shigella's Resistance to High Temperatures* [in Russian], Moscow (1985).
3. I. I. Samoilenko, E. I. Vasil'eva, I. B. Pavlova, and M. A. Tumanyan, *Zh. Mikrobiol.*, No. 12, 30-33 (1983).
4. A. P. Shepelev, A. A. Ryndich, V. D. Rabinovich, *et al.*, *Ibid.*, No. 6, 16-17 (1993).
5. R. F. Beers and I. W. Sizer, *J. Biol. Chem.*, **195**, No. 1, 133-140 (1952).
6. E. L. Bligh and W. J. Dyer, *Can. J. Biochem Physiol.*, **37**, No. 8, 911-917 (1959).
7. M. F. Christman, R. W. Morgan, F. S. Jacobson, and B. N. Ames, *Cell*, **41**, 753-762 (1985).
8. B. Fletcher, C. Dillard, and A. Tappel, *Anal. Biochem.*, **52**, No. 1, 1-10 (1973).
9. W. H. Habig, M. J. Pabst, and W. B. Jacoby, *J. Biol. Chem.*, **249**, 7130-7139 (1974).
10. O. H. Lowry, N. J. Rosebrough, A. H. Farr, *et al.*, *Ibid.*, **193**, 265-275 (1951).
11. B. M. Mackey and C. M. Derrick, *J. Appl. Bacteriol.*, **53**, No. 2, 233-242 (1982).
12. R. E. Pinto and W. Bartley, *Biochem. J.*, **112**, No. 1, 109-115 (1969).
13. R. O. Recknagel and A. K. Choshal, *Nature*, **210**, 1162-1163 (1966).