METHODS

DNA-Bound Lipids of Thermoresistant and Thermolabile *Shigella sonnei* Strains

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Thin-layer chromatography of DNA-bound lipids of thermolabile and thermoresistant *Shigella sonnei* strains shows a lower content of neutral lipids and higher contents of polyglycerophosphatides and cholesterol in thermoresistant strains. This is regarded as a factor providing for the active state of DNA.

Key Words: *Shigella; lipids; DNA-bound lipids; thermoresistance*

Strains of *Shigella sonnei* with high thermoresistance (TR) have been identified during the last decade. Since induction of the synthesis of heat shock proteins is necessary for the realization of TR, the study of DNA-bound lipids is of interest because, together with nonhistone proteins, they play an important role in the regulation of gene expression.

We examined the DNA-bound lipids of thermolabile and TR strains of *Shigella sonnei.*

MATERIALS AND METHODS

The microorganisms were isolated from patients during sporadic outbreaks of dysentery. Thermoresistance of *Sh. sonnei* strains was determined according to methodological recommendations. Supramolecular DNA complexes (SM DNA) were isolated by phenol deproteinization (3 times, pH 8.2 [7]) of biomass prepared from *Sh. sonnei* at the stationary phase of growth. The DNA content was determined by Dische's method [9]. Supramolecular DNA was incubated with 35% ethanol for 24 h at 37°C in order to extract the weakly bound lipids. Ethanol-precipitated DNA was then incubated with DNAase I (1:10) in the presence of 0.01 M $MgCl_2$ for 2 h at 37°C [3]. Lipids were extracted as described [8] and separated by thin-layer chromatography on silicagel H plates in hexane:diethyl ether:acetic acid (73:25:2) (for neutral lipids) and chloroform : methanol : water (65:25:4) (for phospholipids) systems [10]. The values were calculated per 10 mg DNA; for individual neutral lipid and phospholipid fractions they were expressed as a percentage of total lipids, neutral lipids, and phospholipids.

RESULTS

The lipid composition of SM DNA from thermolabile and TR strains of *Sh. sonnei* is shown in Table 1.

Thermoresistant strains differ from thermolabile strains in the composition of SM DNA. The amount of total DNA-bound lipids in them is 12.7% lower due to a decreased content of neutral lipids. In TR strains, neutral lipids constitute a significantly lower percentage of the total than in thermolabile strains. The major fractions of neutral lipids (diacylglycerides, esters of higher fatty

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TABLE 1. DNA–bound Lipids of *Sh. sonnei*

	Thermolabile strains			TR strains		
	μg/10 mg DNA	% of total lipids	% of phospho-lipids[1]	μg/10 mg DNA	% of total lipids	% of phospho-lipids[1]
Total lipids	102.0±10.9		89.03±8.92			
Phospholipids	9.73±1.76	9.4±0.8		14.0±2.45	15.6±1.9*	
Phosphatidylethanolamines	4.4±1.0	4.23±0.6	44.7±4.9	4.1±0.35	4.77±0.7*	38.3±6.1
Polyglycerophophatides	5.33±1.03	5.17±0.5	55.3±4.9	9.87±2.55	10.8±2.1	68.2±7.0
Neutral lipids	91.9±9.15	90.5±0.8		75.1±7.12	84.4±2.0*	
Diacylglycerides	18.5±5.95	17.9±5.1	19.8±5.7	13.1±4.94	16.0±6.5	21.4±9.3
Fatty acids	40.6±8.83	38.9±4.8	43.1±5.6	34.9±4.22	37.5±5.1	44.9±6.7
Fatty acids esters	28.0±5.4	29.4±9.1	32.3±9.7	18.9±5.7	21.1±6.2	24.8±6.9
Cholesterol	3.47±1.5	3.19±1.1	3.54±1.2	4.6±1.5	5.22±1.9	6.28±2.4
Cholesterol : phospholipid ratio		0.326±0.099			0.305±0.087	

Note. Each group contain three strains. [1]Percentage of neutral lipids for the neutral lipid fractions. *Significance of difference of parameters between the strain ($p<0.05$).

acids, and nonesterified fatty acids) are also lower, but the cholesterol content is increased 32.5% (63.8 and 77.4% in terms of total and neutral lipids, respectively). The phospholipid content is increased 44.3% (65.5% in terms of total lipids) due to an 85.1% increase in polyglycerophosphatides (107.7 and 23.6% in terms of total phospholipids, respectively). The cholesterol:phospholipid ratio remains unchanged.

Active chromatin contains much more abundant phospholipids than chromatin in the repressed state. This is confirmed by the increased amount of phospholipids during the S-phase, primarily due to a 2-fold increase in the cardiolipin content [5]. In experiments with cycloheximide, an increase in the cardiolipin fraction was recorded at the stage of active protein synthesis, while during DNA synthesis the sphingomyelin content was elevated and the cardiolipin content lowered [1]. Procaryotes have no sphingomyelin, and therefore cardiolipin is probably the major phospholipid causing DNA relaxation and stimulating replication and transcription. From the observation that DNA-bound cholesterol also activates replication, it can be concluded that the profile of DNA-bound lipids of TR strains will determine the functionally active conformation of DNA. As far as the TR phenomenon is concerned, this is important both for the active synthesis of heat shock proteins and for the repair of heat-damaged DNA. Cholesterol and cardiolipin are involved in the attachment of DNA fibrils to the nuclear matrix and membrane [4,6]. Damage to the DNA-membrane bonds leading to DNA degradation is one of the reasons for nucleotide decompactization during gamma irradiation [2]. Since the microviscosity of cell membranes is lowered during hyperthermia, it is likely that the DNA-membrane binding is impaired during heat shock. If so, the increase in the concentrations of polyglycerophosphatides and cholesterol should also have a protective effect because the number of sites for DNA binding to the matrix and the membrane increases with the rise in their content, making for nucleotide stability.

Thus, DNA-bound lipids of TR strains of *Sh. sonnei* have high contents of phospholipids, polyglycerophosphatides, and cholesterol, which is necessary for preserving the native conformation of DNA and also for the operation of adaptive mechanisms (synthesis of heat shock proteins and methylation of membrane lipids) in the cell during hyperthermia.

REFERENCES

1. A. V. Alesenko, V. A. Krasil'nikov, P. Ya. Boikov, *et al.*, *Biokhimiya*, **54**, № 2, 328-337 (1989).
2. V. G. Bezlepkin and A. I. Gaziev, *Radiobiologiya*, **23**, № 1, 3-8 (1983).
3. Z. I. Krasichkova and N. B. Strazhevskaya, *Ibid.*, **21**, № 2, 172-179 (1981).
4. Z. I. Krasichkova and N. B. Strazhevskaya, *Ibid.*, **22**, №6, 753-758 (1982).
5. V. A. Struchkov and N. B. Strazhevskaya, *Biokhimiya*, **53**, № 9, 1449-1454 (1988).
6. V. A. Struchkov and N. B. Strazhevskaya, *Ibid.*, **55**, № 7, 1266-1275 (1990).
7. V. A. Struchkov, *Biofizika*, **7**, № 5, 538-550 (1962).
8. E. L. Bligh and W. J. Dyer, *Canad. J. Biochem. Physiol. Pharmacol.*, **37**, № 8, 911-917 (1959).
9. Z. Dische and E. Borenfreund, *J. Biol. Chem.*, **192**, 583-587 (1951).
10. H. Wagner, L. Horhammer, and P. Wolff, *Biochem. Z.*, **334**, № 2, 175-184 (1961).