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Influence of DnaK and DnaJ Chaperones on *Escherichia coli* Membrane Lipid Composition

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A b s t r a c t

The content of fatty acids extracted from the membranes of *E. coli* MC1061 harboring the wild-type *dnaKdnaJ* alleles and its Δ*dnaJ* and Δ*dnaKdnaJ* derivatives was compared. It was demonstrated that dodecanoic acid was missing in NPLs fraction extracted from both mutants grown at 42°C. Phospholipids extracted from mutant strains were deprived of hexadecanoic acid methyl ester and octadecanol, the latter being correlated with the presence of octadecanoic acid. The amount of LPS extracted from Δ*dnaKdanJ* mutant was significantly lower when compared with wild-type strain and Δ*dnaJ* mutant.

K e y w o r d s: *Escherichia coli*, chaperones DnaJ, DnaK, membranes, fatty acids composition

Escherichia coli chaperones, proteins DnaK and DnaJ, influence the proper folding, oligomeric assembly, stabilization and degradation of other proteins and also protein export across the membranes (Hartl, 1996; Hendrick and Hartl, 1993; Mayhew and Hartl, 1996). Abundant information about the cellular functions of DnaK and DnaJ indicates their involvement in a variety of cellular functions such as growth (Tsuchido *et al*., 1986), replication of bacterial chromosome, plasmids and bacteriophages (Sakakibara, 1988; Liberek *et al*., 1988; Wolska *et al*., 1999), gene expression (Ohki *et al*., 1992; Karpiński *et al*., 2002), antibiotic resistance (Wolska *et al*., 2000a). *E. coli dnaK* and *dnaJ* mutants are severely impaired in protein secretion (Wild *et al*., 1992; Wild *et al*., 1996; Wolska *et al*., 2000b). The deprivation of DnaK and DnaJ proteins also caused cell filamentation (McCarty and Walker, 1994; Paciorek *et al*., 1997), therefore McCarty and Walker suggested that DnaK may play a direct role in the septation pathway *via* an interaction with FisZ. These last defects point to the possible role of DnaK and DnaJ chaperones in the structure, composition and/or synthesis of cell membranes.

We decided to check the content of fatty acids isolated from phosphorus-containing and non-phosphorus-containing lipids (NPLs) extracted from Δ*dnaJ* and Δ*dnaKdnaJ* mutants. In addition we compared the structure of lipopolysaccharide extracted from these mutants with their wild-type parental strain.

Mutant strains KW86 and KW69 used in this study were constructed by transferring deletions Δ*dnaJ* and Δ*dnaKdnaJ* to MC1061 background by P1 transduction. Deletion mutants were selected by screening for Kan^R cassette which replaced wild-type genes after spreading the transduction mixture on agar plates with kanamycin (50 μg ml^{-1}). The presence of the deletions was confirmed by temperature-sensitive growth and the inability to propagate bacteriophage λ. The extraction of NPLs and phospholipids was carried out following the modified procedure of Knox *et al.* (1967). Cells were grown in LB medium at 30°C to an early exponential phase and then each culture was divided in two portions, one of which was incubated further at 30°C and the second transferred to 42°C for 2 h. NPLs were extracted with chloroform and phospholipids were extracted with the mixture chloroform: methanol (65:35, v/v) and then hydrolyzed with 0.1 N HCl.

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Table I
Comparison of fatty acids isolated from NPLs of Δ*dnaJ* and Δ*dnaKdnaJ E. coli* mutants and their wild-type parent

Compound	Bacterial strain					
	MC1061 (wild-type)		KW86 (Δ*dnaJ*)		KW69 (Δ*dnaKdnaJ*)	
	30°C	42°C	30°C	42°C	30°C	42°C
Cyclohexenol	–	–	+	–	–	–
Dodecanoic acid	+	+	+	–	+	–
Tetradecane	–	–	–	+	–	–
Hexadecanoic acid	–	+	+	+	+	+
Octadecanoic acid	–	+	+	–	–	+
Octadecenoic acid	–	+	–	+	–	+
Hydroxyhexadecanoic acid methyl ester	–	–	–	–	+	–

Table II
Comparison of fatty acids isolated from phospholipids of Δ*dnaJ* and Δ*dnaKdnaJ* mutants and their wild-type parent

Compound	Bacterial strain					
	MC1061 (wild-type)		KW86 (Δ*dnaJ*)		KW69 (Δ*dnaKdnaJ*)	
	30°C	42°C	30°C	42°C	30°C	42°C
Hexadecanoic acid	–	+	–	+	–	–
Hexadecanoic acid methyl ester	+	–	–	–	–	–
Octadecanol	–	+	–	–	–	–
Octadecanoic acid	–	+	+	+	+	+
Octadecanoic acid derivative	+	+	+	+	+	+
Nonacosane	–	–	–	+	–	+
Triacontane	–	–	+	–	+	–

The samples were analyzed by high pressure liquid chromatography. For analysis of fatty acids HPLC-MS Waters Integrity System was used. Samples (20 μl each) were chromatographed on C-18 symmetry column (2.1×150 mm, pore size 0.5 μm, Waters) with gradient of acetonitryle (A) – water (B), 70A:30B. Identification of the components was achieved by computer peak analysis using Waters Millenium 3.2 software with Willey Registry of Mass Spectra Data, 7th Edition. Lipopolysaccharide (LPS) was extracted using two standard methods described by Hitchock and Brown (1983) and Westphal and Jann (1965). LPS bands were resolved by SDS-PAGE (Darveau *et al*., 1983) and visualized by staining with silver reagent.

Tables I and II, respectively, compare the content of fatty acid extracted from NPLs and from phospholipids of Δ*dnaJ* and Δ*dnaKdnaJ* mutants and MC1061 strain. Analysis of fatty acids extracted from NLPs (Table I) demonstrated that dodecanoic acid was missing in the membranes isolated from mutant cells grown at 42°C. Octadecenoic acid was present in all strains grown at elevated temperature but its saturated form, octadecanoic acid, was absent in the membranes of KW86 (Δ*dnaJ*) strain, even when grown at 42°C. The presence of hexadecanoic acid was reported only in membrane extracted from wild-type strain grown at 30°C. The analysis of the fatty acids extracted from phospholipids (Table II) showed that hexadecanoic acid was present only in the wild-type strain and Δ*dnaJ* mutant grown at 42°C. Moreover it was demonstrated that the presence of octadecanoic acid and hexadecanoic acid methyl ester were mutually exclusive. Finally, octadecanol was found only in the membranes of MC1061 grown at 42°C and saturated hydrocarbons, nonacosane (C29) and triacontane (C30) were present exclusively in the membranes of mutant strains.

The data presented above did not allow to draw general conclusions. It can only be mentioned that the lack of octadecanol correlated with the presence of octadecanoic acid in the membranes isolated from both mutant strains could point to the elevated activity of alcohols oxidation by the mutant strains. In turn, the presence of long-chain, saturated hydrocarbons in the membranes of Δ*dnaJ* and Δ*dnaKdnaJ* could be explained on the basis of efficient decarboxylation of waxes in cells deprived of DnaK, DnaJ chaperones.

The structure of LPS isolated from Δ*dnaJ* and Δ*dnaKdnaJ* mutants using two forementioned methods was also determined (data not shown). All strains studied are of the K-12 serotype which is characterized by "deep rough" phenotype and therefore the strains are deprived of long O-specific polysaccharide chains. Analysis of polyacrylamide gels stained with silver allowed to conclude only that the lack of DnaJ and DnaK chaperones does not severely influence the structure of LPS but the amount of LPS isolated from Δ*dnaKdnaJ* mutant was repeatedly lower, irrespective of growth temperature.

The molecular basis of our data pointing to the involvement of DnaK and DnaJ molecular chaperones on the content of *E. coli* membrane fatty acids can not be explained without further experiments. The literature dealing with this problem is very scarce. The paper by Tsvetkova *et al*. (2002) is the only one worth mention. The authors studied the effect of small heat-shock proteins (sHsp, *i.e*. α-crystallin and *Synechocystis* HSP17) on model membranes formed of synthetic and cyanobacterial lipids and found that both proteins strongly stabilized the liquid-crystalline state. It was determined that the nature of sHsp-membrane interactions depends on lipid composition and the extent of lipid unsaturation and sHsps can regulate membrane fluidity. These data also suggest that the association between sHsps and membranes may constitute a general mechanism that preserves membrane integrity during thermal fluctuations.

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