

Cold Shock Proteins

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Abstract: From prokaryotes to eukaryotes or from invertebrates to vertebrates, all organisms have developed various adaptive mechanisms to survive within a wide range of growth temperatures. An important part of the cold adaptation mechanism occurs at the level of the cytoplasmic membrane. Cold shock affects the membrane composition and organisation to maintain the optimum membrane function. Cold shock also affects

cell division. The temperature downshift results in a growth lag. During the lag phase the organism changes the composition of the cytoplasmic membrane and synthesis sets of specific proteins called cold shock proteins or cold induced proteins.

Key Words: Antifreeze glycoproteins, cold shock proteins, membrane fluidity

Introduction

All living organisms must adapt to changes in the environment. Adaptation to environmental stress is essential for the survival of organisms since dramatic changes such as cold shock, heat shock, acid shock, pressure and osmotic stress are lethal for most organisms (1).

The study of the adaptation to cold began at the end of the 1960's (2). It is known that a highly regulated sequence of physiological events begins for all living organisms for adaptation to winter (3). For example, certain species of fish can live at the freezing point of seawater, which is about -1.9°C (2,4,5). The freezing points of blood from Antarctic fishes are between -2.0 and 2.1°C (6). These unusual low freezing points are the result partly of the presence of high sodium chloride concentrations and partly of some glycoproteins also known as AFGP (antifreeze glycoproteins) found in blood (2,4-9). Some beetles and pine needles are also able to withstand temperatures as low as -3.0°C . In addition, to synthesize polyols that act as true antifreeze, such organisms gain additional protection from AFGP macromolecules (10).

The structure of antifreeze glycoproteins

The glycoproteins isolated from serums of the Antarctic fishes *Trematomus borchgreviki*, *Trematomus bernacchi* and *Dissostichus mawsoni* have a repeating tripeptide $(\text{Ala-Ala-Thr})_n$ and N-acetylgalactosamine, and galactose units attached to threonine residues (5,6). It was also stated that the glycoproteins of *Trematomus borchgrevinki* differ from those antifreeze glycoproteins

by the presence of proline, and contain approximate proportions of $(\text{Ala}):7$ $(\text{Thr}):2$ $(\text{Pro}):1$ (7). Arginine containing antifreeze glycoprotein was also isolated and characterized from *Eleginus gracilis* (11). The molecular weights of these glycoproteins are 2600-33000 Da (4,9).

It is thought that AFGPs decrease the freezing temperature of water by binding to ice, presumably through the hydrogen bonds involved in the hydroxyl groups of carbohydrates, and inhibit the growth of ice (8,12). In a crystallographic study, it was demonstrated that an antifreeze polypeptide from the winter flounder is a single α helix which interacts with ice crystal planes and retards both 'a' and 'c' axis growth (4).

In recent years, the most extensive studies have been done about cold adaptation in both prokaryotes and eukaryotes. In some bacteria, a group of proteins which can be induced at low temperatures were chemically identified and called CIPs (cold-induced proteins) or CSPs (cold shock proteins) (13-18).

Cold shock and membrane composition

Temperature plays a very important role in the composition, organization and function of biological membranes. Membranes adjust their unsaturated fatty acid composition according to the changes in the environmental temperature. If the temperature decreases, the ratio of unsaturated fatty acids increases as a function of temperature (19,20). Most of the data for understanding the molecular mechanism of the organization and thermal adaptation of membrane lipids have been obtained by using the thermotolerant strain

Tetrahymena pyriformis NT-1, which is a ciliated protozoan (21-23). This eukaryotic cell can grow well at 39.5°C and adapt quickly to sudden changes in temperature by altering its lipid composition and membrane fluidity for the optimal functions (21,24). *Tetrahymena pyriformis* NT-1 had varying lipid composition when grown at 39.5°C or 15°C. At 39.5°C, the cells contained 25% γ -linoleic acid, but at 15°C, they contained 31% (24). In another study, the cells were grown at 28°C, and then chilled to 10°C. At the beginning of the experiment, 23.9% saturated fatty acids were obtained and there were 163 double bonds. After 16 h, the amount of saturated fatty acids had fallen to 13.4%, but the double bonds had increased to 180. It is thought that, this process is accomplished by the activity of an enzyme called fatty acid desaturase (24-26). When *Tetrahymena pyriformis* NT-1 was grown in a medium at 39.5°C, and then transferred into a medium at 15°C, an increase in palmitoyl-CoA desaturase activity was also observed. However, *Tetrahymena pyriformis* was unable to grow or live at 5°C. Palmitoyl-CoA desaturase activity localized in microsomal membranes in *Tetrahymena* cells was first characterized in 1977 and it was documented that the cells can quickly adapt to lower temperatures by increasing the palmitoleic acid (27). The desaturase activity may be regulated by the degree of membrane fluidity (26).

In *Bacillus megaterium* ATCC 14581, there are three control mechanisms which regulate the level of $\Delta 5$ -desaturase:

a) One control process mediated by temperature is that of desaturase induction. A culture growing at 35°C does not synthesize unsaturated fatty acids. When the culture is transferred to 20°C, the synthesis of desaturase begins and continues at an accelerating rate for at least one hour.

b) A second control process is the irreversible inactivation of the enzyme. The rate of inactivation of desaturase is extremely sensitive to slight changes in temperature. The enzyme is inactivated over 20°C. However, at temperatures near 20°C, a decrease of less than 2°C in the temperature of the incubation medium results in a more than 2-fold increase in the half-life of the enzyme.

c) A third process is the decay of the desaturase synthesizing system. When a culture is transferred back

to 34°C, the desaturase synthesizing system immediately begins to decay at a rate which follows zero order kinetics (20).

Bacillus subtilis grown at high temperatures synthesizes saturated fatty acids, because $\Delta 5$ -desaturase is poorly functional. It was also shown that, when the culture is transferred to 20°C, the synthesis of unsaturated fatty acids is induced (28). There is considerable evidence that a family of desaturases exists, each having its own substrate specificity (19).

When *Bacillus megaterium* cultures are transferred to a low temperature (20°C) from a high temperature (35°C), the level of unsaturated fatty acids increases in the membrane since bacteria do not exhibit any desaturase activity at 35°C. This process occurs in a very short time. Synthesis of desaturase begins within 5 min and reaches its maximum rate at about 15 min, and continues at this high rate for up to 90 min after the shift to 20°C. This "hyperinduction" process (so called because the rate of desaturase synthesis after the transfer of culture from 35°C to 20°C far exceeded the rate found in comparable cultures growing from inoculum at 20°C) was dependent on protein synthesis and RNA synthesis initiated after the transfer. Experimental evidence suggests that the turn-off of hyperinduction at 20°C, was the result of a temperature-sensitive modulator protein which was absent at 35°C but was produced at 20°C (29).

Cold shock and cell division

In addition to the effects on membrane composition and function, cold shock also affects cell division. In experiments, studies have been carried out at two extreme temperatures in general.

When *Tetrahymena pyriformis* NT-1 cultures grown at 15°C for several days were transferred to 39.5°C, their growth rate increased to that of 39.5°C and a lag period was not seen. However, there was a lag period of about 7 h if the cultures were transferred to 15°C from 35°C (24). In another study, first cultures were grown at 28°C, and then temperature was decreased 10°C and it was demonstrated that cell division was inhibited for approximately 16 h (21).

Prokaryotic microorganisms have different optimal growth temperatures and can be classified according to their range of growth temperatures. Thermophiles grow between 4 and 100°C; mesophiles 10-50°C; psychrophiles 15-20°C; and psychrotrophs, 15-40°C (1).

Escherichia coli has a wide growth temperature range and can maintain its growth between 10°C and 49°C (12). In *Escherichia coli*, there are no physiological changes between 20°C and 37°C. However, at extreme temperatures below 20°C and above 40°C, some physiological changes are seen (28). The cold shock response of this bacteria was first reported in 1987 (12). When a culture of *Escherichia coli* ML30 growing at 37°C was shifted to 10°C, growth decreased for about 4.5 h and there was no net synthesis of DNA, RNA or protein. When the cultures were grown at 10°C, protein synthesis started at 4 h and synchronous division occurred at about 11 h after shifting to 10°C (31).

Cold shock and protein synthesis

In *Escherichia coli* it has been reported that, during the growth lag, the number of proteins synthesized were dramatically reduced and only 28 proteins were detected from two-dimensional gel autoradiograms. These proteins were grouped according to the rate of synthesis: those whose differential rate of synthesis decreased, those whose differential rate of synthesis stayed the same and those whose differential rate of synthesis increased. A total of 15 proteins were called CSPs. During the fourth hour, shortly before the resumption of growth, synthesis of an additional 50 polypeptides was detected. One of the CSPs synthesized, F10.6, was detectable only during growth at low temperatures (13,17). This protein was dramatically induced within the first 2 h after being shifted from 37°C to 15°C and it was also named CS7.4 or CspA protein. It is a 7400 Da cytoplasmic protein. It is reported that when temperature decreased, mRNA of CspA increased. This indicates that, the synthesis of this protein is regulated at the level of transcription (32).

Ribosomes can act as sensors of the cold shock response in *Escherichia coli* on the basis of the observation that the cold shock response can be induced by a group of antibiotics which includes chloramphenicol, tetracycline, erythromycin, spiramycin and fusidic acid (33). A pulse-labelling experiment was carried out to investigate the cold shock response induced by chloramphenicol. In the absence of chloramphenicol, CS7.4 could not be detected at 37°C, since mRNA of *cspA* (gene of CS7.4) was unstable (32). After temperature downshifts to 15°C, CS7.4 was induced during the first 2 h, which corresponded to the lag time of the cell growth caused by cold shock. However, in the presence of chloramphenicol, a constitutive increase in the level of

cspA transcript and constitutive production of CS7.4 was observed (15). When kanamycin, an antibiotic that induces the heat shock response but not the cold shock response (33) was used, the results showed no induction of *cspA* expression. When rifamycin was added 30 min after the cold shock treatment, and the amount of *cspA* mRNA was measured at 15, 30 and 60 min after the addition, it was demonstrated that the *cspA* mRNA induced by cold shock was degraded with a half-life of approximately 15 min at 15°C (15).

In another study, it was also demonstrated that CS7.4 is regulated at the transcription level in *Escherichia coli*, and its gene *cspA* is regulated and induced only at low temperatures. Synthesis of CS7.4 was very low at 37°C, since the mRNA of *cspA* was unstable at the temperature mentioned above. It has not been shown if any protein factor(s) is required for the stabilization of this major cold shock mRNA at low temperatures (17).

It has been established that CS7.4 was produced at a level of 13% of total cellular protein synthesis upon a temperature shift from 37°C to 10°C (32). *Escherichia coli* has five additional genes besides *cspA*, each encoding a protein highly similar to CspA. Therefore, these proteins were grouped under the name of the CspA family. On the other hand, *cspG* encodes a cold shock inducible analog of CspA and CspB. This gene is located at 22 min on the *Escherichia coli* genetic map, apart from the other *cspA* family genes. Its gene product (70 amino acids) is 73% and 77% identical to CspA (70 amino acids) and CspB (71 amino acids), respectively (30). The CspA family consists of nine proteins (CspA to CspI), of which CspA, CspB and CspG have been shown to be cold shock inducible (16). CspD is induced in stationary-phase and starvation. It has also been thought that this protein plays a role in the nutrition-stress response (34). The *cspI* gene is located at 35.2 min on the *Escherichia coli* chromosome map, and CspI shows 70%, 70% and 79% identity to CspA, CspB and CspG, respectively. The *cspI* mRNA is very unstable at 37°C but is stabilized upon cold shock and CspI production is maximal at or below 15°C (16).

When the effects of kanamycin and chloramphenicol (inhibitors of protein synthesis) on cold shock inducibility of CspA, CspB and CspG were examined, it was observed that cell growth was completely blocked at 37°C in the presence of kanamycin (100µg/ml) or chloramphenicol (200µg/ml). After 10 min of incubation with the antibiotics at 37°C, cells were cold shocked at 15°C.

Surprisingly, the synthesis of all these cold shock proteins was induced at a significantly high level, virtually in the absence of any other proteins, indicating that the cold shock proteins are able to bypass the inhibitory effects of the antibiotics (17).

The cold shock proteins of *Escherichia coli* can be categorized into two groups. Class I proteins are expressed at an extremely low level at 37°C and are dramatically induced to very high levels after a shift to a lower temperature. In contrast, Class II cold shock proteins are present at a certain level at 37°C and are induced a few-fold from their steady-state levels after a downshift in temperature. The mRNAs of Class I proteins have a long 5' untranslated region which plays an important role in stability and transcription attenuation (1).

The minimum temperature for growth of *Escherichia coli* is in the vicinity of 7.8°C (35). A number of scientists have studied the effects of low temperature on *in vivo* and *in vitro* protein synthesis by *Escherichia coli* (a mesophile), and by *Pseudomonas fluorescens* (a psychrotroph). After shifting to 5°C, proteins were synthesized at a slowly decreasing rate for 1 h by both organisms. However, *Pseudomonas fluorescens* synthesized proteins at a rate corresponding to its 5°C growth rate in contrast to *Escherichia coli*, which did not synthesize at a measurable rate. It is possible that there was an initiation problem in *Escherichia coli* related to energy levels (ATP or GTP) in the cell (36).

Bacillus subtilis have wide growth range temperatures and it has been identified that this class of microorganisms synthesizes a subset of protein when heat shocked (37). *Bacillus subtilis* have a cold shock inducible gene, *cspB*, which is induced upon a shift from 37°C to 10°C. CspB is an acidic protein and has 67 amino acid residues with a molecular weight of 7365 Da. CspB shows 61% sequence identity to the CspA of *Escherichia coli*. This result indicates that there are high levels of conservation at the DNA and amino acid sequence (38). CspB consists of an antiparallel five-stranded β -barrel with strands connected by turns and loops (39). The nucleic acid binding properties of CspB and also CspA have been characterized in *Escherichia coli* (40). Both proteins show 40% identity with the nucleic acid-binding domain of the Y-box factors, which is referred to as CSD (cold shock domain) (38,41). Therefore, CspB and CspA are the members of the CSD family, which is widespread

among pro- and eukaryotes. CspB binds to the pentamer sequences CCAAT with higher affinity in single stranded DNA and can act as a transcriptional activator of cold shock genes by recognizing putative ATTGG-box elements shown to be present in promotor regions of genes and they are induced under cold shock conditions (39-41).

In *Bacillus subtilis*, after a shift from 37 to 15°C, a total of 38 proteins were reproducibly expressed at a high level. These CIPs can be grouped into three categories. Twenty-one proteins were induced only after a cold shock, not in response to heat shock or salt-stress, and thus represent true cold shock and cold stress-induced proteins. Six proteins were induced in response to cold as well as heat shock (48°C) and were therefore named temperature-induced proteins. An enhanced expression of seven proteins was detected after cold shock and salt-stress (1M NaCl) but not after heat shock. These proteins were named stress and cold shock proteins (42).

The growth temperature of *Bacillus cereus* (a psychrotrophic bacterium) is between 7°C and 30°C. It has been described that *Bacillus cereus* has five small proteins which have RNA and DNA binding motifs. It was shown that the amino acid sequence of *Bacillus cereus* CspA is similar to the cold shock proteins of *Escherichia coli* CspA (63%), *Bacillus subtilis* CspB (71%) and *Streptomyces clavuligerus* SC7.0 (58%) (43).

Aquifex aeolicus is one of the earliest diverging thermophilic bacteria known. This organism can grow at 95°C. Complete genome sequences of this organism have been determined and a gene for a cold shock protein has been found (44).

The cold shock response and the heat shock response may have an inverse relationship. After cold shock, the cold shock protein synthesis increases, but heat shock protein synthesis decreases (45).

Cold shock proteins from mesophiles and thermophiles differ widely in their stabilities, but show close structural similarity. *Thermotoga maritima* is a hyperthermophilic bacterium, and TmCsp shows 76% homology (61% identity) to Csp from mesophilic *Bacillus subtilis* (CspB), and its thermal stability (Tm: 87°C) exceeds that of CspB by 35°C (46).

CspA-like proteins have also been identified in psychrotrophic organisms: *Bacillus cereus* WSBC 10201 (43), *Pseudomonas fragi* (47), *Arthrobacter globiformis*

SI55 (48) and in a psychrotolerant pathogen *Yersinia enterocolitica* (49).

Alfa casein seems to have some characteristics of a cold shock protein, and its chaperon-like activity increases with a decrease of temperature (50).

Beta crystallin is expressed endogenously in N1E-115 cells (from a mouse neuroblastoma cell line) upon heat shock at 43°C or 55°C, or cold shock at 30°C (51).

The cold shock domain protein zfY1 in zebrafish has been identified and characterized. This protein contains a sequence of 68 amino acids and shares substantial similarity (55%) to *Escherichia coli* cold shock proteins, CspA and CspB (52). In Table 1, the major properties of some AFGPs and CSPs are given.

Table 1. Antifreeze Glycoproteins and Cold Shock Proteins.

Organism	Cold Shock Protein	Biochemical Characteristics	References
<i>Escherichia coli</i>	CspA	β -barrel structure and five antiparallel β -strands	1,13,15,30
	Polynucleotide phosphorylase	MW: ~ 7 kDa	
	NusA	15 cold induced proteins	
	Initiation factor2 α		
	Initiation factor2 β		
	RecA		
	Dihydroliipoamide acetyltransferase		
	Pyruvate dehydrogenase		
	F14.7		
	F84.0		
	G41.2		
	G55		
	G74		
<i>Bacillus subtilis</i>	CspB	MW: 7.365 kDa. CspB consists of an antiparallel five-stranded β -barrel, pI 4.31	14,29,39
	CspC	MW: 8 kDa	
	CspD	MW: 13 kDa 16 cold-induced proteins	
<i>Bacillus cereus</i> WSBC 10201	CspA of <i>Bacillus cereus</i>	MW: 7.5 kDa, pI 4.9	43
	Other cold stress proteins	MW: 30 kDa, pI 5.1 MW: 35 kDa, pI 4.7 5 cold-induced proteins	
<i>Pseudomonas fragi</i>	C7.0	MW: 7 kDa	47
	C8.0	MW: 8 kDa The role of these proteins is not known.	
<i>Arthrobacter globiformis</i> S155	A9	CS7.4-like protein MW: 9 kDa, pI 4.5	48
<i>Trematomus borchgrevinki</i>	There are three distinct groups of freezing point-depressing glycoproteins	They are composed primarily of Thr (16%), Ala (23%), N-acetylgalactosamine (29%), and galactose (28%). MW: 10.5, 17 and 21.5 kDa	7
	A proline-containing glycopeptide has been determined in the blood of this Antarctic fish.	The approximate proportions of amino acids: Ala: 7, Thr: 2, Pro: 1	8

Table 1. (Continued)

<i>Dissostichus mawsoni</i>	There are three distinct groups of freezing point depressing glycoproteins.	Thr (16%), Ala (23%), N-acetylgalactosamine (29%), and galactose (28%).	7
Winter flounder	A single α -helix polypeptide		4
Marine fish	Four distinct macromolecular antifreezes have been isolated.	The glycoprotein antifreezes MW: 2.5-33 kDa, (Ala-Ala-Thr) _n , disaccharide attached to the threonyl residues. Type I is Ala-rich amphiphilic, α -helix MW: 3-5 kDa Type II MW: 14 kDa with a high content of reverse turns and five disulfide bridges Type III MW: 6-7 kDa with no distinguishing features of secondary structure or amino acid composition.	9
<i>Eleginus gracilis</i>	Arg containing antifreeze glycoprotein (EgAF 2) EgAF 8R	MW: 30 kDa MW: 3 kDa	11
<i>Pagothenia borchgrevinkii</i>	Antifreeze glycoprotein 4 Antifreeze glycoprotein 8	MW: 17.5 kDa MW: 2.6 kDa	11
<i>Tetrahyena pyriformis</i>	Membrane-associated fatty acid desaturase	Fatty acid desaturase activity regulates membrane fluidity at adaptation to cold.	25-27
<i>Bacillus megaterium</i> ATCC 14581	Hyperinduction of desaturase		29

Conclusion

Temperature is an important environmental stress and requires adaptive responses, such as to synthesize AFGPs or CSPs. The cellular contents of CSPs and membrane fluidity change due to the growth temperature of many organisms. However, cellular responses to a decrease in temperature are not well known. Therefore, this area is open to new investigations.

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