An examination of constitutive direct light DNA repair and inducibility of DNA repair in two thermophilic bacteria

by Mary Ann Starkey Kirkpatrick

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biology

Montana State University

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Two thermophilic bacteria, Bacillus stearothermophilus and Thermus T2 were observed for response to known DNA-damaging agents, UV radiation and the chemical mutagen, Mitomycin C. The existence of a constitutive direct light DNA repair system was discovered in Bacillus stearothermophiIus. Unlike E. coli whose dark DNA repair is UV-inducible, Thermus was not found to have a UV-inducible repair mechanism. However the presence of a DNA repair system inducible by either heat or chemicals was observed in Thermus, relating temperature-associated DNA repair with survival at high temperatures.

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Mary Ann Starkey Kirkpatrick

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ABSTRACT

Two thermophilic bacteria, Bacillus stearothermophilus and Thermus T2 were observed for response to known DNA-damaging agents, UV radiation and the chemical mutagen, Mitomycin C. The existence of a constitutive direct light DNA repair system was discovered in Bacillus stearothermophilus. Unlike E. coli whose dark DNA repair is UV-inducible, Thermus was not found to have a UV-inducible repair mechanism. However the presence of a DNA repair system inducible by either heat or chemicals was observed in Thermus relating temperature-associated DNA repair with survival at high temperatures.

INTRODUCTION

Biochemistry of Thermophily

Mesophilic microorgansims have a maximum growth rate at temperatures around 30-37 C; in the case of E. coli, growth ceases at 45-48 C while temperatures of 50-52 C or greater cause cell death.

Thermophilic microorganisms can thrive at temperatures up to 85 C. They are found in geothermally active areas such as hotsprings, solar-heated desert soil, as contaminants of canned food and dairy products, in hot water heaters, and in industrial effluent. Such organisms have piqued the interest of the scientific community for reasons of their proliferation at elevated temperatures and the thermostability of their macromolecules. Basic knowledge of thermophily is applicable to studies of evolution and ecology, molecular biology and biochemistry, and could lead to utilization for industrial enzymic production of marketable products (Amelunxen and Murdoch, 1977).

Temperature is only one of the variables influencing the growth of living organisms. Other environmental factors such as pH, nutrient quality and quantity, salinity, and light interact to influence the optimal and maximum growth temperatures. Under varying conditions the interrelationship of chemical structure, conformation, and function of biological molecules may vary in adapting to stress (Hochachka and Somero, 1973). Thus, attempts to explain the special ability to live

at high temperature have evolved from the observation of general physiology to a biochemical approach with concomitant examination at the molecular level.

The hypothesis that increased rates of synthesis and turnover, either by adaptation or mutation, are responsible for rapid replacement of heat-damaged proteins was advanced by Allen (1953). However, when Brock (1967) published growth rate data of various mesophilic and thermophilic bacteria at their optimum temperatures Allen's hypothesis was discounted. Brock (1967) found that thermophiles do not grow as fast at their optima as predicted by purely theoretical calculations of effect of temperature on physiology. When Ulrich (1971) combined physiological and biochemical approaches to examine a Thermus-like organism for morphological characteristics, respiratory mechanism, and regulation of enzyme synthesis, he found no major differences between mesophile and thermophile, except thermostability.

Biochemical studies of thermophiles have determined the properties of specific cell components or molecules and compared these to their counterparts in mesophiles. Fatty acids and membranes, proteins and the protein-synthesizing machinery as well as nucleic acids of several thermophiles have been isolated and examined.

Fatty acids and membranes: The membranes of thermophiles are exposed to the environment so this component of the cell was one of the first to be examined for heat stability. The fluidity of bacterial biomembranes is constantly maintained in growing cells. Variation in

complex lipid content and structural changes in fatty acid components are suggested as mechanisms to achieve the fluidity known to be important for membrane functions (Cronan, 1978). The fatty acid content of various microorganisms is known to be affected by the temperature at which they are grown (Oshima, 1978). High proportions of unsaturated acids are found at lower temperatures, while saturated fatty acids increase with increasing temperature. The presence of highly branched, longer chain, saturated fatty acids in membranes of thermophiles has been confirmed (Oshima et al., 1976). Also, a novel glycolipid constituting up to 70% of the total lipid of two strains of Thermus has been identified (Oshima and Ariga, 1976). It is conjectured that the unique lipid content of the thermophilic membrane is responsible for successful membrane function at high temperature.

Proteins: Since the primary, secondary, tertiary and quaternary structure of proteins often vary as much between proteins of the same function obtained from various mesophilic organisms as between thermophilic and mesophilic proteins of the same function, it is difficult to explain the invariable stability to denaturing conditions (chemical denaturants as well as heat) of thermophilic proteins. Various investigators have proposed that enhanced stability is due to hydrophobic (Ohta, 1966), hydrogen (Barnes and Stellwagen, 1973), or ionic (Perutz and Raidt, 1975) bonding producing conformations with larger or more densely packed protein interiors (Bull and Breese, 1973), more or different secondary sturcture (Stellwagen and Barnes, 1976), more extensively laced macromolecular surfaces (Perutz and Raidt, 1975), or

more complementary intersubunit contacts (Biesecker et al., 1977). (This topic has been reviewed by Zuber, 1976; Friedman, 1978; Amelunxen and Murdock, 1977; Singleton and Amelunxen, 1973.)

Heat-resistance is often conferred by only a few amino acid changes as shown by Merkler et al. (1981) who compared the physical characteristics of proteins of closely related mesophilic and thermophilic bacilli. Argos et al. (1979), found strategically substituted amino acids increased internal hydrophobicity and increased external polarity. Hydrophobic bonds are more stable at high temperature than at low temperature. Apparently molecular interactions within polypeptide chains are sufficient to cause thermostability.

Ribosomes: Ribosomes and the other components associated with protein synthesis are also thermostable in thermophiles. Ribosomal subunits (protein and RNA) have been found to be heat stable (Yaguchi et al., 1978). Protein elongation factors which deliver and catalyze the binding of charged t-RNAs to the ribosome are required for elongation of the polypeptide chain in protein synthesis in prokaryotes. These factors have been purified from Thermus thermophilus and compared to E. coli (Arai et al., 1978). The thermophilic elongation factors are extremely stable against heat, acid, alkali, and other protein denaturants. Thermus elongation factors showed a lack of sulfhydryl groups in contrast to those of E. coli where sulfhydryls play an essential role in catalytic function. In contrast to the monomeric forms found in the mesophile the existence of multimeric forms were

demonstrated in the thermophile.

It is apparent that no single mechanism or cell component is responsible for thermophily. As the thermostability of membranes was suggested to be due to presence of novel glycolipids as well as degree of saturation of its fatty acids and the variation in protein structure allowing function at high temperature was demonstrated, the nucleic acid portion of protein synthesis was also found to be heat stable.

Nucleic acids: The nucleic acids of thermophiles have been studied in a variety of ways including base composition; presence and action of associated polyamines; isolation and study of enzymes involved in synthesis, restriction and modification of thermophilic nucleic acids; isolation and characterization of mRNA, t-RNA, r-RNA and DNA, isolation of antibiotic resistance-carrying and cryptic plasmids, as well as the cloning of thermophilic genes with subsequent expression and characterization in mesophiles.

Heat stability of thermophilic nucleic acids increases with increased G-C content producing higher corresponding increase in melting temperature (Oshima et al., 1976). Thermophilic DNA stability may arise partially from association with divalent cations as it is known that divalent cations stabilize DNA and RNA. Thiolation of nucleic acids increases with temperature and is directly correlated with thermostability of t-RNA in the cell (Quigley and Rich, 1976).

Polyamines are generally considered to be involved in important biochemical processes such as stabilizing DNA and RNA, protein

biosyntheis, DNA and RNA biosyntheses, cell division, and acclimation to environmental stress. Novel polyamines are produced by the extreme thermophile, Thermus thermophilus (Oshima, 1975 and 1982).

Alan Malcolm has proposed in a theoretical paper (1981) that the increased G-C content of m-RNA with consequent increased stability of secondary structure should also be considered as a selection pressure in the thermophilic environment and that the most common amino acid changes between mesophiles and thermophiles recorded by Argos et al. (1979) are consistent with this hypothesis. Single base changes found in the m-RNA codons of thermophiles either increase the stability of secondary structure or have little effect (none would decrease secondary structure).

Stenish and Madison (1979) compared the stability of m-RNA in mesophiles and thermophiles, found the half-life of m-RNA to decrease as growth temperature increased but discovered the "stability index" (half-life of mRNA/doubling time of cells) to be constant for each organism regardless of temperature. This supports the concept that kinetic considerations play a significant role in thermophily; the half-life of the m-RNA is a fixed fraction of the doubling time.

Nucleic acids of thermophiles and mesophiles have been shown to vary in nucleotide content, presence of novel polyamines, structure of enzymes involved in synthesis, and restriction and modification enzymes. However, there are many similarities in basic organization of genetic material and expression of information. Thermophilic DNA and RNA polymerases (Kaledin et al., 1980; Chien et al., 1976; Date,

1975), DNA methylase (Sato et al., 1980), and DNA restriction enzymes (Sato et al., 1977) have been isolated and characterized. The properties demonstrated by these enzymes are similar to those of other thermostable proteins. This is also true of enzymes produced from genes cloned from thermophiles (Nagahari et al., 1980). A circular dichroism study of the complex between promoter DNA and Thermus RNA polymerase (Tsuji, 1980) showed only more melting in the promoter region than found in E. coli, confirming the similarities of the two organisms.

Research on DNA repair and mutagenesis in mesophilic bacteria, initial observations of the filamentous highly thermophilic bacterium, Thermus, and recent reports that a protective response to heat stress can be induced in mesophilic prokaryotes and eukaryotes by agents that can induce changes in DNA repair activity associated with filamentation suggested to Dr. Guylyn Warren a possible natural association between growth at high temperature, DNA damage and repair.

DNA Damage and Repair

DNA Damage: Four main types of DNA alterations or damage have been studied: 1) Dimerization of two adjacent pyrimidines on the same DNA strand when the pyrimidines become connected by a cyclobutane ring, 2) chemical alteration of bases by deamination or aklylation, 3) introduction of covalent crosslinks between bases on two strands, and 4) breaks in one or both strands. Any of these damages can result in lethality or an altered coding property or mutation.

Mutations can be spontaneous, possibly arising from enzymatic

dysfunction during DNA replication or recombination. Mutation, in a broad sense, although a heritable change, may not affect the phenotype or be recognized. However, the term will be used in this manuscript to refer to a heritable change in nucleotide sequence of an organism which is recognized by its effect on the phenotype of the organism.

A mutagen is an agent which causes changes, as described above, in genomic nucleic acid and increases the mutation rate above the spontaneous level as observed phenotypically. Known mutagens present in the environment include radiation (UV and X-rays), chemical mutagens (alkylating and deaminating agents, base analogs, intercalating agents, and cross-linking agents) and transposons.

1. Radiation.

a) Ultraviolet (UV) light causes formation of dimers between pyrimidine bases on the same strand (intrastrand) of DNA. The pyrimidines become connected by a four carbon cyclobutane ring. (See diagram and detail in UV section below.)

b) X-rays cause breaks in the phosphodiester backbone in one or both strands of DNA.

2. Chemical mutagens effect modifications in DNA bases in situ by deamination, alkylation, or the addition of a variety of bulky adducts. Chemical mutagens also include: structural analogs which vary in bonding with the partner base; intercalating agents which insert during replication, distort the base pairing and leave after replication resulting in a gap or an added base in the newly synthesized strand; and cross-linking agents which form interstrand cross-

links posing an absolute block to replication and transcription.

3. Transposons, or units of DNA that have the capability of moving from one DNA moleule to another, result in rearrangements and deletions in the molecule that was left and insertion and disturbance of DNA coiling in the molecule entered.

DNA Damage - UV and MC: UV and Mitomycin C were chosen for examination of the thermophiles' response to mutagens. The pyrimidine dimer caused by UV is the best researched lesion. The cross-linking mechanism of Mitomycin C provided a second mechanism of DNA damage for observation.

UV Damage: DNA efficiently absorbs light in the range of 240-300 nm resulting in excited energy states of the bases and causing a variety of photochemical reactions (Wang, 1976). The principle product, pyrimidine dimers, causing the principle biological effects, lethality and mutagenisis, is formed when two adjacent pyrimidine bases on a strand are linked together by a four-carbon ring (Fig. 1). The two bases are pulled out of alignment, the hydrogen bonds to complementary bases are broken, and the DNA backbone is distorted, preventing the correct pairing of the two bases on each side of the dimer. The presence of a single dimer can interrupt transcription or replication. Even if replication resumes on the other side of the dimer, a gap is left in the newly synthesized strand, blocking transcription of the entire transcription unit and aborting replication in the next cycle (Hanawalt et al., 1979).

Mitomycin C (Fig. 2) is metabolically reduced by a quinone reductase in the cell to a hydroquinone derivative which alkylates and extensively cross-links DNA (Iyer and Szybalski, 1963). The biological significance of interstrand cross-linking is evident from studies on transforming DNA and bacterial viruses (Kohn et al., 1963; Becker et al., 1964)). One interstrand cross-link is sufficient to cause the inactivation of at least 3,000 base pairs within a DNA molecule, presumably as a consequence of blocking complete strand separation for replication. E. coli mutants defective in one or more uvr genes (excision repair) are more sensitive to Mitomycin C than wild type strains. While simple alkylation damage is not repaired by excision repair, cross-linking caused by the bifunctional Mitomycin C requires excision repair (Fishbein et al., 1970; Cole et al., 1976).

DNA Repair: For every organism, life and continuity from generation to generation depend on the long-term stability of its hereditary material, the DNA. Since all cells are sensitive to damage by radiation and chemical agents in the environment, a system of removal of lesions and restoration of the intact DNA appears to have been adopted. It is not possible for DNA polymerase III to replicate areas of DNA containing dimers or cross-links, although it can restart after the damaged region has been passed. Howard-Flanders (1975) has shown that daughter DNA molecules replicated from UV-damaged DNA contain gaps approximately the size of one or more Okazaki fragment indicating