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Determination of coenzyme A levels in *Pyrococcus furiosus* and other Archaea: implications for a general role for coenzyme A in thermophiles

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Abstract

Physiologically significant levels of intracellular coenzyme A were identified in *Pyrococcus furiosus*, *Thermococcus litoralis*, and *Sulfolobus solfataricus*, suggesting a role for CoA as an important low molecular mass thiol in the thermophilic Archaea. In *P. furiosus*, cells grown in the presence of sulfur showed significantly higher levels of oxidized CoA compared with those grown in the absence of S^0. *T. litoralis* showed strikingly similar CoA levels, although with low disulfide levels in both the presence and absence of S^0. *S. solfataricus* showed similarly high levels of CoA thiol, with correspondingly low levels of the CoA disulfide. These results are consistent with the identification of a coenzyme A disulfide reductase (CoADR) in *P. furiosus* and *horikoshii* as well as the presence of CoADR homologues in the genomes of *S. solfataricus* and *T. kodakaraensis*.

Keywords: Oxidative stress; Coenzyme A; Disulfide reductase; *Pyrococcus*; Thermophile

1. Introduction

The ability of thermophiles to thrive at temperatures as high as the recent record of 121 °C has supplied a wealth of questions to biochemists and microbiologists [1,2]. Considerable attention and progress has been made in the understanding of the factors that stabilize the structure of macromolecules [3]; moreover, the stability of small molecules may also play a significant role in microbial survival at higher temperatures. The stability of NAD(P)H at high temperatures and the levels of intracellular pyridine nucleotides in *Pyrococcus furious* have been examined [4], in one of the few examples of such studies. Small molecules may also play a role in protecting intracellular components from damage. For instance, unique intracellular solutes are thought to stabilize macromolecule structure at high temperature, and these solutes have been characterized in a range of hyperthermophilic Archaea [5]. Low molecular mass thiols are another category of small molecules that may assist in the protection of thermophiles from their environment via maintenance of the intracellular redox environment.

Thiol compounds can serve a variety of metabolic functions; in general, they comprise metabolic intermediates, such as coenzyme A, they can be used in the detoxification of zenobiotics, and they also can play

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a role in cellular redox chemistry. In mesophilic organisms glutathione has been one of the most commonly identified antioxidant thiols [6], participating in reactions such as the glutathione peroxidase catalyzed reduction of peroxides. Glutathione is also added to zenobiotics via the glutathione/glutathione-*S*-transferase system in order to increase their solubility and clearance rate [7], and acts in other detoxification reactions such as the glyoxalase-catalyzed detoxification of methylglyoxal [8]. In addition to glutathione, many other low molecular mass thiols have also been discovered, including bis-γ-glutamlycysteine of halophiles [9], mycothiol and ergothioneine in many of the actinomycetes [10,11], and the carbohydrate-containing U17 thiol of *Streptomyces clavuligerus* [12].

Pyrococcus furiosus and *horikoshii* have recently been shown to contain a coenzyme A disulfide reductase ($CoAS–SCoA + NADPH + H^+ \rightarrow 2CoASH + NADP^+$) (Fig. 1) [13]. In addition, *P. furiosus* has been found to contain intracellular levels of coenzyme A consistent with this compound playing a role in the control of the internal redox environment of *Pyrococcus* [13]. No glutathione thiol or disulfide was found, an observation consistent with the lack of homologues of glutathione synthetic enzymes or a glutathione reductase in the genome of this organism. The presence of relatively high levels of intracellular CoA, as well as the presence of a highly specific reductase of the CoA disulfide, has led to the hypothesis that the CoA thiol may play a role in *Pyrococcus* similar to the role that glutathione plays in a wide variety of organisms. Such a role for CoA has previously been characterized in the mesophilic bacterium *Staphylococcus aureus* [14]. The work described herein characterizes the response of CoA thiol and disulfide levels in *Pyrococcus* to growth in the presence of the electron acceptor sulfur, and extends these results to the related organism *Thermococcus litoralis* as well as the aerobic respiratory archaeon *Sulfolobus solfataricus*.

2. Materials and methods

2.1. Cultivation of microbes

Fifty milliliter cultures of *P. furiosus* (DSM 3638, Deutsche Sammlung von Mikroorganismen) and *T. litoralis* (DSM 5473) were grown with shaking in sealed 125 mL bottles with media described previously, except that medium was supplemented with 6 mM cellobiose, 1.5% tryptone, and 0.5% yeast extract [15]. For cells grown on S^0, approximately 0.5 grams of sulfur was added to the medium. Following addition of fresh inoculum (1%) *P. furiosus* and *T. litoralis* were grown at 95 and 85 °C, respectively, until reaching mid to late log-phase (~12 h). Fifty milliliter cultures of *S. solfataricus* (DSM 1616) were grown in 125 mL flasks in a shaking

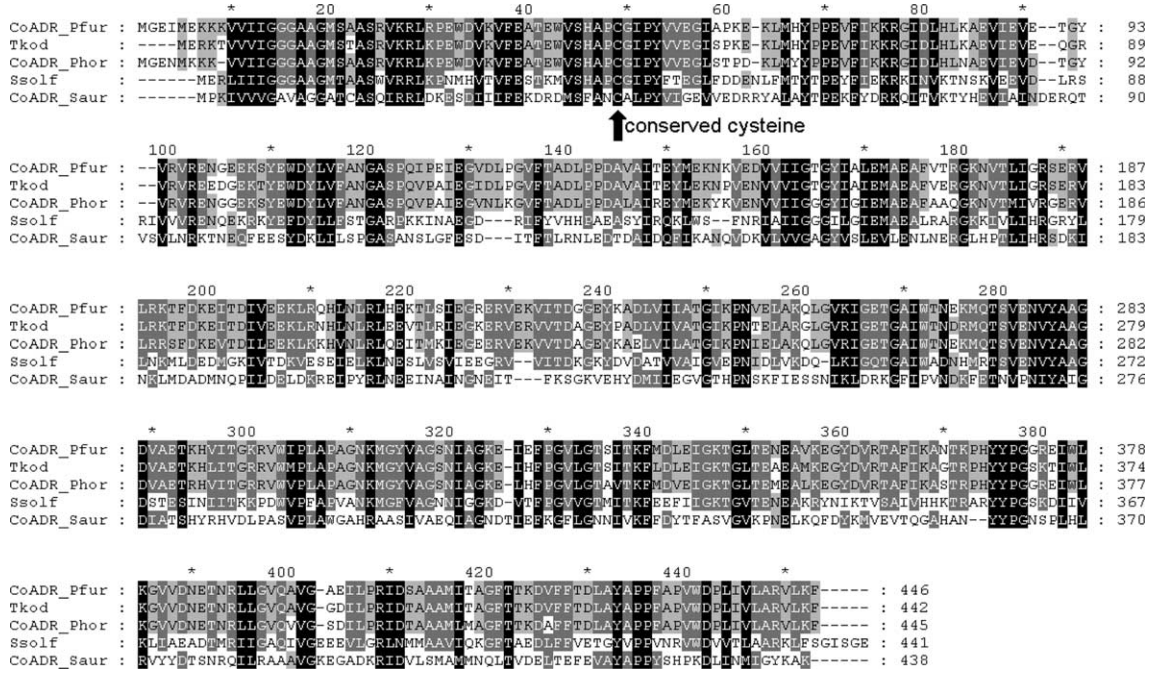

Fig. 1. Multiple sequence alignment of confirmed and putative CoADRs. The alignment was performed with CLUSTAL W. The GenBank accession numbers for the enzymes are: CoADR_Pfur, *Pyrococcus furiosus* CoADR (NP_578915); Tkod, *Thermococcus kodakaraensis* putative CoADR (YP183712); CoADR_P_hor, *Pyrococcus horikoshii* CoADR (NP_142538); Ssolf, *Sulfolobus solfataricus* putative CoADR (NP_343655); and CoADR_Saur, *Staphylococcus aureus* CoADR (AF041467).