Reverse gyrase and genome stability in hyperthermophilic organisms

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Abstract

Reverse gyrase is a DNA topoisomerase that is peculiar in many aspects: it has the unique ability to introduce positive supercoils into DNA molecules; it comprises a type IA topoisomerase fused to a helicase-like domain; although it is a type IA topoisomerase, its reaction is ATP-dependent; and it is the only hyperthermophile-specific protein. All these features have made reverse gyrase the subject of biochemical, structural and functional studies, although they have not shed complete light on the evolution, mechanism and function of this distinctive enzyme. In the present article, we review the latest progress on structure–function relationships of reverse gyrase, and discuss old and recent data linking reverse gyrase to DNA stability, protection and repair in hyperthermophilic organisms.

Introduction

DNA topoisomerases are essential enzymes that regulate the topological state of DNA in all cells throughout the life cycle. They are needed to solve the topological problems associated with all DNA activities and co-operate with multiple proteins in essential processes such as DNA replication, repair, recombination, chromosome segregation and regulation of gene expression [1,2]. Whereas most topoisomerases relax unwanted superhelical turns, two peculiar enzymes are able to actively introduce torsional stress of opposite sign: the bacterial gyrase, which induces negative supercoiling, and reverse gyrase of thermophilic micro-organisms, which induces positive supercoiling. However, these enzymes are not related either structurally or functionally. Whereas gyrase is a type II topoisomerase, reverse gyrase is a unique enzyme comprising a topoisomerase IA module fused to a helicase-like domain (for recent reviews, see [3,4]). Although this arrangement is peculiar to reverse gyrase, the association of helicase and topoisomerase activities is known to play a role in different aspects of DNA metabolism in several (if not all) organisms. In particular, members of the type IA topoisomerase III family, conserved in organisms of the three domains (Eukarya, Bacteria and Archaea) associate physically with helicases of the RecQ family, forming a complex which is strikingly conserved across evolution, from bacteria to humans, and is involved in replication, recombination and repair [5,6]. Whether reverse gyrase might function analogously to helicase–topoisomerase complexes is currently not clear. In the present paper, we review biochemical and functional studies on reverse gyrase that have provided evidence both in favour and against this hypothesis.

We anticipate, however, that we still do not have the final word on the activity and function of this molecular machine.

A thermophile-specific enzyme

Since the discovery of reverse gyrase in a hyperthermophilic archaeon, it was suspected that this unique enzyme might be a distinctive trait of adaptation to high temperature [7]. This prediction turned out to be strikingly correct many years afterwards, when the increasing number of genomes sequenced allowed thorough phylogenomic analyses, from which reverse gyrase was identified as the only hyperthermophile-specific protein [8,9]. In the most recent update of such analysis, Brochier-Armanet and Forterre [9], searching several hundred genomes whose sequences were available, found one (in euryarchaea and bacteria) or two (in some crenarchaea) reverse gyrase gene(s) in all microorganisms living above 80°C (hyperthermophiles) and in some, although not all, of those living between 65 and 80°C (thermophiles). With no exceptions, the reverse gyrase gene was not found in the genomes of mesophilic organisms. Analysis of the genomic context suggested that reverse gyrase originated in an archaeon and was transferred later on to (hyper)thermophilic bacteria through lateral gene transfer events [9,10]. This unique distribution pattern led to the hypothesis that reverse gyrase was essential for the evolution of a hyperthermophilic lifestyle, and possibly helpful for organisms living between 60 and 80°C. In contrast, its presence might be dispensable or even harmful for mesophilic organisms [9]. However, this hypothesis is still controversial, since reverse gyrase was shown to be not essential in the archaeon Thermococcus kodakaraensis [11] (see below).

An enigmatic enzyme

Reverse gyrase is a large (1050–1200 amino acid residues) bimodular enzyme (Figure 1). Its N-terminal domain contains sequence motifs typical of the SF2 (superfamily 2)
helicases, including an ATP binding site and a so-called DEAD (Asp-Glu-Ala-Asp) box. The C-terminal domain shares approx. 30% sequence identity with IA-type topoisomerases and contains the essential catalytic tyrosine residue [12].

The resolution of the three-dimensional structure of the reverse gyrase of the archaeon Archaeoglobus fulgidus confirmed the two-domain structure and revealed other interesting features [13]. The C-terminal domain forms a toroidal shape typical of IA-type topoisomerases, comprising four subdomains arranged around a central ‘hole’ for the passage of DNA. If compared with the Escherichia coli topoisomerase I, reverse gyrase does not show a C-terminal extension containing three zinc-finger motifs important for DNA binding in the E. coli enzyme. In reverse gyrase, a putative zinc finger is located in each of the N- and C-terminal domains (Figure 1A). Three-dimensional EM (electron microscopy) studies of reverse gyrase from Sulfolobus tokodai showed that the enzyme contacts dsDNA (double-stranded DNA) both in the helicase-like and in the topoisomerase domains [14]. The reverse gyrase N-terminal half folding is similar to the ATP-binding domain found in several classes of ATPase, including SF2 helicases and E. coli RecA. The two domains interact mainly through a subdomain called latch (residues 360–418) that may be involved in the functional communication between the N-terminal ATPase and the C-terminal topoisomerase, coupling nucleotide hydrolysis to DNA supercoiling [13,15] (Figure 1B).

Despite the structural similarities and the clear evolutionary relatedness, reverse gyrase displays a unique activity. Starting from topologically closed, negatively supercoiled or relaxed DNA molecules, reverse gyrase induces a progressive Lk (linking number) increase in an ATP hydrolysis-dependent reaction (Figure 2). Like all Type IA topoisomerases, the enzyme cuts only one DNA strand, and needs short ssDNA (single-stranded DNA) regions; however, unlike Type IA enzymes, reverse gyrase activity is ATP-dependent. Other NTPs, as well as the ATP analogue ATP[S] (adenosine 5′-[γ-thio]triphosphate), also support the supercoiling reaction, although with reduced efficiency [16,17].

The function of the two domains in each reaction step has been investigated using deletion mutants. Work with the Sulfolobus acidocaldarius and Sulfolobus solfataricus reverse gyrase showed that the isolated C- and N-terminal domain can be produced in stable form and retain their own DNA-binding and enzymatic activities [18,19]. As a typical Type IA topoisomerase, the C-terminal domain performs an ATP-independent relaxation reaction, but is unable to produce positive topoisomers (Figure 2). The N-terminal domain is endowed with DNA-dependent ATPase activity, although it is much less efficient than the full-length enzyme [19]. Strikingly enough, although all the signatures of SF2 helicases are present, neither the full-length enzyme nor the N-terminal half show helicase activity or the ability to translocate along DNA [18,19].

Interestingly, when combined, the two domains form a specific physical and functional interaction, restoring the unique ATP-dependent positive supercoiling activity. Both domains co-operate in all individual steps of the reaction (DNA binding, ATPase, strand passage) and exert a reciprocal strong stabilization effect [19]. When assayed on linear DNA molecules, reverse gyrase binds dsDNA, ssDNA and mixed substrates; binding does not require high temperature. The isolated C-terminal and N-terminal domains bind DNA independently, as the full-length reverse g
gyrase, and they show higher affinity for ssDNA than for dsDNA. On the other hand, the full-length enzyme, but not the two separate halves, shows higher affinity for substrates containing dsDNA–ssDNA junctions [19]. The ability of reverse gyrase to recognize such structures, reminiscent of arrested replication forks or recombination intermediates, might suggest a role of the enzyme in (one of) these processes.

Although the N-terminal domain comprises all the essential ATPase motifs, it has a very weak ATPase activity on its own; interestingly, this activity is stimulated by the C-terminal domain. As shown in the model in Figure 1(B), the latch might be involved in the co-ordinated regulation of the ATPase activity by the two domains. Finally, whereas the two separate domains are only stable for few minutes at 80°C, co-incubation rescues the typical reverse gyrase stability [19].

These results show how the interaction between two universal modules, endowed with their own independent activity, could give rise to a completely novel enzyme activity, displaying the peculiar binding specificity, positive supercoiling activity and thermostability of reverse gyrase. It is tempting to speculate that the high environmental temperature applied a strong pressure to recruit pre-existing modules for the evolution of an activity beneficial to coping with high temperature. The evolution of reverse gyrase, in turn, might have determined the evolution of hyperthermophilic micro-organisms. However, it is also possible that reverse gyrase originated in a thermophilic organism from already thermostable polypeptides.

Whatever its evolutionary history, the molecular mechanism of reverse gyrase reaction is still obscure. A number of models have been proposed to explain the positive supercoiling reaction, but none has received experimental support so far. Initially, based on a presumptive ATP hydrolysis-driven helicase activity, it was proposed that, translocating along DNA, the enzyme generates two topological domains, one with reduced Lk and one with increased Lk; selective relaxation of negative supercoiling by the C-terminal topoisomerase would result in net positive supercoiling [12]. Such helicase/translocase activity has not been demonstrated [18,19]; however, binding of reverse gyrase to DNA does induce local unwinding without translocation [18]. Two models were based on this observation: the first suggested that the ssDNA region exposed upon enzyme binding generates positive superturns elsewhere; strand passage in the unwound region would result in net Lk increase [20]. However, the role of ATP hydrolysis in this process remains to be clarified. In the second model, strand passage in the DNA bubble produced by enzyme binding is followed by an ATP hydrolysis-driven switch of the enzyme affinity for dsDNA, that induces reannealing of the denatured region [21]. However, at odds with this model, binding to both ssDNA and dsDNA was shown to be independent of nucleotide binding/hydrolysis [19]. Finally, the last model proposes that reverse gyrase induces a unidirectional strand passage towards Lk increase through some undetermined mechanism [13].

**A role in genome protection and repair?**

Several *in vivo* and *in vitro* studies addressed the function of reverse gyrase, which, unfortunately, remains elusive. In the simplest view, reverse gyrase-induced DNA overlinking could compensate for the destabilizing effect of high temperature on DNA structure. Accordingly, the plasmidic form of SSV1 (*Sulfolobus* spindle-shaped virus 1) and several plasmids from different hyperthermophilic strains were found in relaxed or positively supercoiled form [22]. In addition, reverse gyrase might protect DNA against heat-induced depurination and degradation, as shown in *in vitro* experiments [23].

However, reverse gyrase was shown to be not essential in *T. kodakaraensis*, although growth of the knockout strain was retarded at high temperature, thus confirming a specific role of the enzyme in thermostability [11].

If overwinding could contribute to DNA protection and stabilization against the harmful effects of heat, it is also true that unwinding is needed in several essential DNA activities, and Lk variations are associated with response to temperature...
Ferrara and M. Ciaramella, unpublished work). Binding and genetic elements (promoters, coding and intergenic content co-purified with the chromatin fraction [27]. Analysis to DNA under normal growth conditions; however, after reasonable, considering the structural similarity and evolution, modulate the enzyme activity under different circumstances.

SSB was shown to recognize DNA lesions [28] and stimulate enzyme, rather than an inactivation mechanism. Interestingly, growth resumes, suggesting a functional recruitment of the lethal UV doses, recruitment is transient and lasts until cell fraction of DNA-bound enzyme is active and that, at non-lethal DNA damage has not been shown to date.

Recent evidence suggested that reverse gyrase might be involved in the cell response to DNA damage. This might be reasonable, considering the structural similarity and evolutionary relatedness with helicase-topoisomerase complexes. In S. solfataricus, the enzyme was found not tightly associated to DNA under normal growth conditions; however, after UV irradiation more than 80% of the total reverse gyrase content co-purified with the chromatin fraction [27]. Analysis by chromatin immunoprecipitation of several different loci and genetic elements (promoters, coding and intergenic sequences) could not show any preference for reverse gyrase binding in vivo, either before or after UV irradiation, thus suggesting that it is recruited to DNA randomly (M.C. Ferrara and M. Ciaramella, unpublished work). In vitro, reverse gyrase was blocked after the cleavage step by the presence of UV-induced lesions, suggesting that UV-induced lesions trap reverse gyrase on to DNA, stabilizing the covalent enzyme-DNA complexes [27]. Whether reverse gyrase recruitment to DNA is a passive consequence of UV-induced lesions, inflicting further damage, or has a positive role in DNA repair, signalling or recalling repair factors, remains to be clarified. It might be noted, however, that the fraction of DNA-bound enzyme is active and that, at non-lethal UV doses, recruitment is transient and lasts until cell growth resumes, suggesting a functional recruitment of the enzyme, rather than an inactivation mechanism. Interestingly, SSB was shown to recognize DNA lesions [28] and stimulate reverse gyrase binding to UV-irradiated DNA [26], thus raising the possibility that the reverse gyrase-SSB complex might be involved in damage recognition.

Reverse gyrase was shown to be degraded after treatment of S. solfataricus cells with the alkylating agent MMS (methyl methanesulfonate) [29]. This phenomenon is highly selective, since other proteins, including topoisomerase VI and several DNA-binding proteins, were not involved. Experiments in vitro suggested that reverse gyrase is selectively degraded by a yet unidentified metal-dependent protease activated in MMS-treated cells through a post-translational mechanism. Genomic DNA was shown to be extensively degraded after MMS treatment, with striking coincidence with reverse gyrase degradation. Both reverse gyrase and DNA were irreversibly degraded at lethal MMS concentrations, but only transiently when cells could recover from the damage. Reverse gyrase might protect DNA from degradation by the combined effect of MMS and high temperature. In this view, DNA degradation is a secondary event of MMS-induced degradation of the enzyme [29]. Although this model is at the moment only speculative, it is intriguing to note that this phenomenon is characterized by cell death, DNA fragmentation and activation of proteases, all hallmarks of apoptosis in higher eukaryotes. However, the analogy is limited, as no homologues of eukaryotic caspases are present in archaea, and degradation of other proteins in response to DNA damage has not been shown to date.

Whereas the reported evidence supports the hypothesis that reverse gyrase is involved in maintenance of genome stability, its exact role in physiological growth and in the cell response to DNA damage is not understood. Clearly, further biochemical and functional studies are needed to shed more light on the activity and function of this mysterious enzyme.

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### References


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