Coupling ATP hydrolysis to DNA strand passage in type IIA DNA topoisomerases

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Abstract
Type IIA topos (topoisomerases) catalyse topological conversions of DNA through the passage of one double strand through a transient break in another. In the case of the archetypal enzyme, DNA gyrase, it has always been apparent that the enzyme couples the free energy of ATP hydrolysis to the introduction of negative supercoiling, and the structural details of this process are now becoming clearer. The homologous type IIA enzymes such as topo IV and eukaryotic topo II also require ATP and it has more recently been shown that the energy of hydrolysis is coupled to a reduction of supercoiling or catenation (linking) beyond equilibrium. The mechanism behind this effect is less clear. We review the energy coupling process in both classes of enzyme and describe recent mechanistic and structural work on gyrase that addresses the mechanism of energy coupling.

Introduction
DNA topos (topoisomerases) control the topological state of DNA and are of fundamental importance in cells, supporting DNA replication and transcription [1,2]. Among their key functions are the relaxation of positive supercoils that form ahead of replication forks (and transcription complexes) and unlinking (decatenation) of inter-linked daughter chromosomes following replication. The importance of topoisomers is underlined by the fact that they have become key drug targets [3–5]. The enzymes can be classified into two basic types, I and II; they cleave one (type I) or both (type II) strands of DNA, forming a phosphodiester bond between an active-site tyrosine residue and the phosphate group of DNA. This phosphodiester exchange reaction is readily reversible, allowing the rescaling of the break at the end of the reaction. Most topoisomers operate by a strand-passage mechanism in which one segment of DNA is passed through a transient break in another. This process can account for all the known reactions of topoisomers, i.e. relaxation/supercoiling, catenation/decatenation and knotting/unknotting of DNA. Mechanistic and structural differences between topoisomers have led them to be further subdivided into subtypes: IA and IB, IIA and IIB [2].

Type II topos perform vital roles in all organisms. Type IIA enzymes (e.g. topo II, DNA gyrase) are the best characterized and are found in both prokaryotes and eukaryotes. Type IIB enzymes (e.g. topo VI) were discovered in archaea and more recently in plants [6–8]; these are less well characterized but appear to share the principal mechanistic features of the type IIA enzymes. The type II strand-passage mechanism involves the cleavage of a segment of DNA (the gate or ‘G’ segment) in both strands and passage of another double-stranded segment (the transported or ‘T’ segment) through the break, which is then rescaled. In an intramolecular reaction, where the G and T segments are part of the same molecule, strand passage leads to a change in linking number of the DNA, resulting in the introduction or removal of supercoils. Alternatively, this mechanism can lead to knotting/unknotting of DNA, or catenation/decatenation of two DNA circles when strand passage is intermolecular. The involvement of transient DNA cleavage in the mechanism is a particular vulnerability for cells, and is frequently exploited in chemotherapy. Indeed, type II topos have become important drug targets for antimicrobial and anticancer therapy. For example, bacterial gyrase and topo IV are targets for a range of clinical antibacterial agents, such as the quinolone drug ciprofloxacin, and human topo IIα is the target for many anti-tumour agents, e.g. etoposide [3,4]. These drugs trap the covalent enzyme–DNA complex, which can lead to the subsequent creation of a double-stranded DNA break and cell death.

In the present paper, we briefly review recent advances in our understanding of the mechanism of type II topos.

Energy coupling in topo IIA enzymes
The DNA strand-passage reaction of the type II enzymes is coupled to the hydrolysis of ATP, but exactly how the free energy of hydrolysis is used in the strand-passage process is not clear. The best-studied type IIA topo is bacterial DNA gyrase, which is the only topo that can actively introduce negative supercoils into DNA, using ATP binding and hydrolysis to drive the energetically unfavourable supercoiling process. All other type IIA enzymes can catalyse only the reverse reaction, DNA relaxation, as well as the
Figure 1 | Models for topo II action

(A) DNA sliding [9]. The enzyme (grey sphere with notch) binds a G segment and a second site elsewhere on the DNA, and can then constrain a catenated or knotted strand or supercoiled region within a shorter loop by moving along the DNA. This makes the capture of a T segment more probable, favouring product simplification. (B) DNA kinking [13]. The enzyme sharply bends the G segment into a ‘hairpin’ such that the entrance gate for the T segment is only on the inside and it can only pass from inside to outside. (C) Kinetic proofreading [14]. The enzyme binds DNA (1) then transiently binds a second strand (synapse) (2). This activates the enzyme (3) such that upon achieving a second synapse (4), strand passage can occur (5).

catenation/decatenation and knotting/unknotting reactions. These reactions are not obviously energy requiring; indeed relaxation of DNA is clearly energetically favourable. It has therefore been puzzling that all type II enzymes require ATP hydrolysis to carry out their topo reactions.

In 1997, Rybenkov et al. [9] showed that the type IIA enzymes reduce the topological complexity of DNA, relative to that at equilibrium. Relaxed DNA circles at equilibrium consist of a distribution of topos centred on the most relaxed topoisomer. In the presence of a type IIA topo and ATP, the steady-state distribution became narrower (less supercoiled) than that produced by an ATP-independent type I enzyme. Similarly, steady-state mixtures of knotted and unknotted, and catenated and decatennated, DNA molecules, in the presence of type IIA enzymes, had a lower proportion of knotted or catenated DNA circles compared with that at equilibrium [9]. In other words, the free energy of ATP hydrolysis is used to drive the reaction away from equilibrium.

There have been various mechanistic explanations for this effect. In the original paper, it was proposed that the enzyme binds to a third site on the DNA (in addition to the T and G segments) and that ATP-dependent sliding of the enzyme along the DNA traps the T segment in a small loop, promoting reduction in complexity (Figure 1A). However, there is only indirect evidence for this third DNA-binding site [10,11]. This mechanism also envisages a dual role for ATP hydrolysis in driving both strand passage and sliding of the enzyme along the DNA, which seems implausible [12], although a variant involving the initial binding of two T segments that does not require sliding has been suggested [10].

Alternatively, Vologodskii et al. [13] suggested that type II topoisomers bend the G segment such that the T segment becomes trapped inside a hairpin, with strand passage then occurring preferentially from inside to outside the hairpin (Figure 1B). There is evidence for the kinking of the G segment by Escherichia coli topo IV and yeast topo II [13], but other work shows the opposite result for topo II, i.e. absence of bending [11]. Moreover, calculations suggest that this mechanism cannot account for the product distributions found experimentally [14]. More recently, it has been proposed that type II topos act at hooked juxtapositions of DNA strands [15] and that this can account for the observed preference for unlinking and unknotting by these enzymes, since such hooked crossovers are characteristic of entangled long strands.

The most efficient type II enzyme known, when judged by its ability to perturb the equilibria, is E. coli topo IV. This enzyme also demonstrates high selectivity for decatenation reactions, and for the relaxation of positive supercoils over negative [16–18]. An explanation for the latter selectivity has been proposed, involving the preferential action of the enzyme on DNA crossovers of the particular geometry encountered in positively supercoiled DNA. The recent
structures of the C-terminal domains of the gyrase A subunit and the ParC subunit of topo IV provide a molecular explanation for the preference for positively supercoiled substrates [19–21].

The foregoing explanations are essentially geometric, explaining how the enzyme may be able to select T segments that lead to product simplification. A kinetic proofreading scheme has also been suggested to explain this effect. This is a well-established principle, explaining how enzymes can use an energy source to act with greater specificity than expected at thermal equilibrium [22]. Yan et al. [14] have applied this to type II topos and suggested a scheme involving two collisions of the enzyme with DNA (Figure 1C). The requirement for two collisions can enable ‘proofreading’ of the first reaction by the second. Kinetic proofreading is actually a general kinetic scheme rather than a specific mechanism, and although the proposed model is clumsy and has no real supporting evidence, it could be that proofreading forms part of a kinetic explanation for a geometrical model such as those described above, or some derivatives of them.

DNA wrapping by gyrase

DNA gyrase is the only type II topo able to introduce negative supercoils into DNA. Although gyrase shares a lot of structural similarities with other type IIA enzymes, it possesses a DNA-wrapping domain at the C-terminus of GyrA (DNA gyrase A protein) that is responsible for ensuring that the G and T segments are closely spaced on the same DNA, thus favouring intramolecular strand passage. Catalytic supercoiling by gyrase requires the hydrolysis of ATP, but limited supercoiling can be achieved in the presence of the non-hydrolysable ATP analogue p[NH]ppA (adenosine 5′-[β,γ-imido]triphosphate), suggesting that nucleotide binding can support one round of strand passage [23,24].

In the absence of nucleotide, various lines of evidence suggest that a segment of DNA (100–150 bp) is wrapped around the gyrase complex in a positively supercoiled sense [25]. In particular, hydroxyl-radical footprinting experiments suggested that 128 bp of DNA is wrapped around the enzyme; addition of p[NH]ppA led to an apparent increase in the length of the wrapped segment (to 142 bp) and a more symmetrical pattern of protection from hydroxyl radicals [26]. Probing the gyrase–DNA complex with topo I suggested that the wrap is abolished in the presence of p[NH]ppA [27,28]. These results have been interpreted as reflecting a DNA–protein complex with an increased length of DNA associated with the enzyme but with a conformation resulting in a net or average wrap of zero [27]. Specifically, a model was proposed in which the addition of nucleotides (ATP or p[NH]ppA) leads to capture of a T segment (contiguous with the bound G segment) with high probability, irrespective of the superhelical density of the substrate DNA. This leads to the formation of a complex in which the T segment is trapped in the central cavity of the enzyme, but can equilibrate across the break in the G segment [27]. The overall efficiency of strand passage is determined by the position of this equilibrium, which will depend on the superhelical density of the DNA substrate; positively supercoiled DNAs lead to high efficiency strand passage, whereas negatively supercoiled DNAs lead to low efficiency [24].

One prediction of this model is that, although the DNA within the p[NH]ppA-bound gyrase complex has no net wrap, there should be an increased length of DNA associated with the complex in the presence of nucleotide compared with the nucleotide-free complex. However, experiments probing the gyrase–DNA complex using atomic-force microscopy suggest that the presence of p[NH]ppA leads to a shorter length of DNA associated with the complex, i.e. that the wrap is lost in the presence of the nucleotide [29]. These observations have led to a re-examination of the hydroxyl-radical data to try to reconcile these differences. This analysis suggests that in the absence of nucleotide, DNA is wrapped around the gyrase complex in a manner that positions a potential T segment above the G segment (Figure 2, stage 2), forming a positive node or crossover. This is an asymmetric wrap and accounts for the features observed in the hydroxyl-radical experiments. Addition of p[NH]ppA promotes strand passage and results in a more symmetrical DNA–protein complex (Figure 2, stage 4). However, the exact path of the DNA around the gyrase complex and how this changes during the supercoiling cycle is not yet clear.
Figure 3 Structure of the GyrA dimer

The GyrA59 (N-terminal) domain is shown in dark grey and the C-terminal domain in light grey; the structure is based on ab initio modelling from SAXS experiments [34]. The surface represents the X-ray crystallographic structures of the E. coli GyrA59 [32] and the structure of the C-terminal domain modelled from the homologous structure from B. burgdorferi [19].

Conclusions and perspectives

In the case of DNA gyrase, the overall coupling between the binding of ATP and the energy-requiring introduction of negative supercoils is fairly clear. The wrapping of the DNA in a positive sense positions a T segment over the G segment at the site of DNA cleavage. Subsequent transfer of the T segment through the cleaved G segment is driven by the binding of ATP, and the strand-passage event converts a positive to a negative node, corresponding to the introduction of two negative supercoils. The precise details of the conformational changes required to drive strand passage, and the subsequent ATP hydrolysis and product release, remain to be elucidated.

In the case of the other type II enzymes, the mechanism of the product simplification effect described by Rybenkov et al. [9] is more obscure. The methods used by the enzymes to control both the selectivity of their reactions and the coupling of hydrolysis to the perturbations of equilibria seem contradictory, and further work will be required to produce a physical and kinetic explanation for this effect.

References


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