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Magnesium influences the discrimination and release of ADP by human RAD51

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ATPase, ATP hydrolysis activity; bp, base pair; ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; ϕX174, bacteriophage ϕX174; ADP, adenosine diphosphate; ATP, adenosine triphosphate; ATPγS, adenosine-5′-*O*-thio triphosphate; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; HEPES, *N*-[2-hydroxyethyl]-piperazine-*N*′-[2-ethanesulfonic acid]; hRAD51,

ABSTRACT

hRAD51 lacks cooperative DNA-dependent ATPase activity and appears to function with 5–10-fold less Mg^{2+} compared to RecA. We have further explored the effect of Mg^{2+} on adenosine nucleotide binding, ATPase, and DNA strand exchange activities. hRAD51 was saturated with the poorly hydrolyzable analog of ATP, ATPγS, at approximately 0.08 mM Mg^{2+}. In contrast, >0.5 mM Mg^{2+} was required to saturate hRAD51 with ADP. We found ADP to be a significantly less effective competitive inhibitor of the hRAD51 ATPase at low Mg^{2+} concentrations (0.08 mM). Mg^{2+} did not appear to affect the ability of ATPγS to competitively inhibit the hRAD51 ATPase. Low Mg^{2+} (0.08–0.12 mM) enhanced the steady-state ATPase of hRAD51 while higher Mg^{2+} concentration (>0.3 mM) was inhibitory. At low Mg^{2+}, hRAD51 appeared capable of nearly complete hydrolysis of available ATP, suggesting a lack of ADP product inhibition. There was a strong correlation between the amount of Mg^{2+} required for stable ADP binding and the inhibition of hRad51 strand exchange activity. Simultaneous inclusion of exogenous ATP and chelation of Mg^{2+} with EDTA significantly enhanced ADP → ATP exchange by hRAD51. These studies are consistent with the hypothesis that Mg^{2+} influences the discrimination and release of ADP, which may sequentially impose an important regulatory step in the hRAD51 ATPase cycle.

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human RAD51; Mg^{2+}, magnesium ion; NPF, nucleoprotein filament; nt, nucleotide; RFI/RFII, replicative form I/II; TLC, thin layer chromatography

1. Introduction

Like its prototype, *Escherichia coli* RecA, the human RAD51 (hRAD51) forms a nucleoprotein filament (NPF) that promotes pairing and strand exchange between homologous duplex DNAs [1–3]. Although hRAD51 and RecA have many structural similarities [4–6], there are significant differences in the processing of ATP [7]. The consequences of these differences on their role in homologous recombination are poorly understood.

RecA displays a substantial DNA-stimulated ATPase [8–10] as well as preferential binding and stability on ssDNA that is dependent on adenosine nucleotide(s) [11–15]. DNA binding by RecA correlates with an increased affinity for ATP and a decreased affinity for ADP [8,16]. Accumulating results are consistent with the hypothesis that DNA facilitates cooperative ATP hydrolysis within a RecA NPF by maximizing its affinity for ATP while minimizing its affinity for ADP ([13]; references therein).

In contrast, ssDNA binding by hRAD51 appeared to be very stable in the presence of adenosine nucleotides [17]. Moreover, the protein retains a high intrinsic affinity for ADP, ATP, and ATPγS regardless of the DNA cofactors [17]. The hRAD51 DNA-stimulated ATPase is approximate 50-fold less active than RecA [5,7] and lacks cooperative ATP hydrolysis [7]. One possible explanation for the differences in ATP processing is that hRAD51 does not enjoy extensive coordinated hydrolysis along the entire NPF [18]. In this scenario, conformational changes driven by ATP processing of individual protein monomers within the NPF are both necessary and sufficient for hRAD51 function. Alternatively, hRAD51 may only require regulated and/or cooperative ATP hydrolysis within the limited portion of the NPF performing localized recombination functions. In this latter case, additional cofactors or special conditions may be required to facilitate cooperativity. Understanding the hRAD51 catalytic mechanism may help to differentiate between these possibilities.

The coordination of ATP/GTP by Magnesium ion (Mg^{2+}) is a key mechanistic feature of enzymes that catalyze phosphoryl transfer reactions [11,18,19]. It has been suggested that Mg^{2+} may neutralize a local buildup of negative charges near the β–γ-phosphate and/or correctly position the nucleotide within the active site [20]. Stabilization of this localized phosphate charge may aid in the formation and collapse of a pentacovalent transition state in the hydrolysis reaction pathway [20,21]. Appropriate coordination of Mg^{2+} appears to be essential for cooperative ATP binding and hydrolysis in the F1 ATPase [22].

Mg^{2+} is required by RecA/RAD51 family members to form the active ATP-bound NPF required for strand exchange [23]. Distinct Mg^{2+} conditions are required to stimulate RecA and hRAD51 activities. For example, hRAD51 mediated strand exchange was observed the presence of 1–2 mM Mg^{2+} but was completely absent in 6–10 mM Mg^{2+} [24]. In contrast, RecA requires at least 6–10 mM Mg^{2+} for optimal strand exchange [25]. It has been suggested that Mg^{2+} may be required for charge neutralization between pairing DNAs [26,27] and/or proper coordination of ATP binding/hydrolysis [18]. Structural analysis of RAD51 paralogs suggests that two Mg^{2+} ions are bound per monomeric unit: one involved in coordinating the ATP β–γ phosphates and one near the interface between monomers [20,28].

Here we examine the role of Mg^{2+} in hRAD51 ATP processing and recombination activities compared to bacterial RecA. We find that significant discrimination between ATP and ADP by hRAD51 only occurs at low Mg^{2+} concentrations (<250 μM) and correlates with enhanced hRAD51 ATPase activity as well as that ability of ADP to function as an effective competitive inhibitor of the hRAD51 ATPase. A similar pattern for the Mg^{2+}-dependent activation of strand exchange by hRAD51 was observed. Mg^{2+} does not appear to alter binding and/or discrimination of ssDNA or dsDNA by hRAD51. Simultaneous chelation of Mg^{2+} and addition of ATP were found to provoke efficient release of ADP (ADP → ATP exchange), which appears to be a rate-limiting step in the hRAD51 ATPase. Our results are consistent with the hypothesis that Mg^{2+} dissociation is important for hRAD51 ADP/ATP processing and subsequent recombination, which is substantially different from bacterial RecA.

2. Materials and methods

2.1. Enzymes and biochemicals

ATP and ADP were purchased from Pharmacia. ATPγS was purchased from Roche Molecular Biochemicals. Adenosine nucleotides were dissolved in water and adjusted to pH 7.5. The molar concentrations (M) were determined by spectrophotometry and the equation $M = A/\varepsilon$, where A is the absorbance at 259 nm and ε is the extinction coefficient $1.54 \times 10^4\ M^{-1}\ cm^{-1}$. [$\gamma^{32}$P]-ATP was purchased from NEN Dupont. [γ^{35}S]-ATP and [^{3}H]-ADP were purchased from Pharmacia. All adenosine nucleotides were examined for their constituent phosphate form(s) by silica gel TLC in 0.5 M $LiCl_2$ and judged to be >95% diphosphate (ADP) or triphosphate (ATP; ATPγS). T4 polynucleotide kinase (PNK) and ApaL1 were purchased from Promega. Other restriction enzymes, ϕX174 dsDNA (RFI and RFII) and circular ssDNA were purchased from New England Biolabs. Chemicals of the highest grade such as HEPES, Tris, and magnesium acetate were purchased from Amresco, Fisher and Sigma. EDTA was purchased from GIBCO BRL. Activated charcoal was purchased from Sigma.