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Kinetic mechanism of DNA translocation by the RSC molecular motor ☆

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ABSTRACT

ATP-dependent nucleosome repositioning by chromatin remodeling enzymes requires the translocation of these enzymes along the nucleosomal DNA. Using a fluorescence stopped-flow assay we monitored DNA translocation by a minimal RSC motor and through global analysis of these time courses we have determined that this motor has a macroscopic translocation rate of 2.9 bp/s with a step size of 1.24 bp. From the complementary quantitative analysis of the associated time courses of ATP consumption during DNA translocation we have determined that this motor has an efficiency of 3.0 ATP/bp, which is slightly less that the efficiency observed for several genetically related DNA helicases and which likely results from random pausing by the motor during translocation. Nevertheless, this motor is able to exert enough force during translocation to displace streptavidin from biotinylated DNA. Taken together these results are the necessary first step for quantifying both the role of DNA translocation in nucleosome repositioning by RSC and the efficiency at which RSC couples ATP binding and hydrolysis to nucleosome repositioning.

Introduction

Eukaryotic DNA is packaged into chromatin that renders these sequences largely inaccessible for transcription or repair [1,2]. The basic packaging unit of chromatin is the nucleosome which consists of a central histone octamer (two molecules of each of the four core histones: H2A, H2B, H3 and H4) about which approximately 147 base pairs (bp) of DNA is wrapped in 1.67 turns [3,4]. Chromatin structure and by extension gene expression can be regulated either through covalent modification of histone proteins [5,6] or through an ATP-dependent mechanism of nucleosome repositioning carried out by a family of molecular motors, termed chromatin remodelers [7–9]; in fact, these two processes are intimately linked as modifications of histones can affect chromatin remodeler function [10–12]. Chromatin remodelers have been grouped into four functionally and structurally similar families: SWI/SNF, ISWI, NURD/Mi-2/CHD, and INO80 [13,14]; members of each family share a conserved ATPase domain, but are differentiated based upon the additional domains within their catalytic subunits [12,15]. There are currently two general models proposed for the process of ATP-dependent nucleosome repositioning [13,16–18]. In one model, translocation of the chromatin remodeler along nucleosomal DNA, or nucleosome flanking DNA, results in the formation of small DNA loops that propagate around the histone octamer giving rise to synchronized DNA translocation and nucleosome repositioning. In the other model, DNA translocation by the chromatin remodeling complex results in the formation of large DNA loops which accumulate on the nucleosome surface creating tension that is eventually released through periodic nucleosome "jumping". Thus, a mechanistic description of DNA translocation by remodelers is required for further modeling of the mechanism of their ATP-dependent nucleosome repositioning activity.

Saccharomyces cerevisiae RSC (Remodels the Structure of Chromatin) is an essential SWI/SNF-family chromatin remodeling enzyme capable of repositioning nucleosomes from the center of DNA fragments toward the ends without disrupting the integrity of the nucleosomes [19,20]; RSC under high concentrations has also been shown to be capable of ejecting nucleosomes from the DNA [21]. RSC complex consists of 15 proteins, five of which are highly similar to the subunits of the SWI/SNF complex, and has a molecular weight of approximately 1 MDa [19]. The ability of RSC to translocate along DNA is essential for its nucleosome repositioning activity [13,22] and estimates of 1 bp or 2 bp have been determined for the kinetic step size of DNA translocation by RSC [23]. The results of recent studies have also suggested a two-step mechanism for the repositioning of nucleosomes by RSC in which the formation of a stable complex of DNA, RSC, and the histone oct-

Abbreviations used: RSC, remodel the structure of chromatin; RSCt, remodel the structure of chromatin trimer (used in this study); NLLS, non-linear least squares; bp, basepair.

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amer serves as an intermediate for the repositioning reaction [24–26]. According to this model, after the initial binding of RSC to the nucleosome, competition between RSC and the histones for binding to the nucleosomal DNA leads to formation of the intermediate complex. This intermediate is characterized by the nucleosomal DNA being associated primarily with RSC and largely dissociated from the histones [25,26]. This (at least partial) unwrapping of the DNA from the histones provides an environment in which the small loops of DNA formed by the DNA translocation of RSC can more easily propagate around the surface of the octamer and subsequently reposition the nucleosome. However, despite the significance of DNA translocation to the nucleosome repositioning activity of RSC and other remodelers [27], details about the mechanism that couples the binding and hydrolysis of ATP to mechanical work remains unclear.

In this study we utilize a truncated construct of RSC, termed 'trimeric minimal RSC' (RSCt), which only contains three of the original 15 subunits of full length RSC [23,28]; the subunits are ARP7, ARP9, and a truncated version of STH1. STH1 is the DNA-binding ATPase and translocation motor [28,29] of the construct and ARP7 and ARP9 are nuclear actin related proteins whose interaction with STH1 greatly improves the stability and solubility of the complex [19,29,30]. The truncated construct was utilized because it can be over expressed in *Escherichia coli*, unlike the full complex. This allows for increased yields during the purification process. This construct has been used previously by Sirinakis et al. to characterize the translocation properties of the RSC motor domain as well as Malik et al. to study DNA binding [23,28]. In our experiments we monitored the translocation of RSCt along double-stranded DNA using a stopped-flow assay that monitors changes in fluorescence intensity associated with the translocation of RSCt along fluorophore labeled DNA [31–33]. Through global analysis of associated time courses of translocation we were able to determine the macroscopic rate of DNA translocation and associated kinetic step size. Through further analysis of the ATPase activity associated with DNA translocation we were also able to determine the efficiency at which RSCt couples the binding and hydrolysis of ATP to its physical movement along the DNA. This in turn provides a limit on the efficiency at which RSC could couple ATP binding and hydrolysis to nucleosome repositioning. Finally, in a separate assay we monitored the ability of the RSC trimer to displace streptavidin from biotinylated DNA [31,34]. The ability of RSC to actively displace the streptavidin demonstrates the large forces that this molecular motor is capable of exerting during translocation and thus, by extension, during nucleosome repositioning.

Materials and methods

Buffers

All buffers were prepared with reagent grade chemicals using twice-distilled water that was deionized with a Milli-Q purification system (Millipore Corp., Bedford, MA). The RSCt storage buffer used is 25 mM Tris HCl (pH 7.5 @ 25 °C), 150 mM KOAC, 10% (v/v) glycerol, and 0.5 mM 2-mercaptoethanol. All experiments were conducted in the reaction buffer consisting of 10 mM HEPES (pH 7.0 @ 25 °C), 5 mM $MgCl_2$, 100 mM KOAC, 4% (v/v) Glycerol, 2.5 mM DTT, and 0.1 mg/mL BSA.

Oligonucleotide substrates

All labeled and unlabeled oligonucleotides were purchased from IDT Technologies (Coralville, IA) and were HPLC purified. Each oligonucleotide was extensively dialyzed against Milli-Q water. Double-stranded DNA substrates were annealed by mixing together equal concentrations of complimentary strands in a DNA annealing buffer consisting of 10 mM HEPES (pH 7.0) and 40 mM KAC. The mixture is then heated to 95 °C and allowed to cool to room temperature over the course of 12 h. For fluorophore-labeled DNA, a 5% overabundance of unlabeled DNA was included to ensure complete annealing of all fluorescein-labeled DNA strands. For all fluorophore-labeled substrates, the fluorophore was attached only at the 5′ end of a single strand of the duplex, leaving the opposing single-stranded mated substrate unlabeled. This allows sampling of only the population of protein translocating along one backbone.

Protein purification

RSCt was over-expressed and purified from an *E. coli* bacterial over-expression system. The CDF Duet-1 vector (Novagen) bearing a Sth1 construct (301–1097aa) with a 10X histidine tag at the N-terminus was transformed into BL21 (DE3) codon plus strain along with the RSF Duet vector (Novagen) containing Arp9 and Arp7 constructs. These were selected on streptomycin and kanamycin plates. The cells were grown in nutrient-rich auto-inducible media at 37 °C for 4 h, 30 °C for 12 h and at 22 °C for 24 h, harvested by centrifuging at 6000*g* at 4 °C, resuspended in lysis buffer (50 mM phosphate buffer pH 7.5, 300 mM NaCl, 10% glycerol, 0.5 mM β-mercaptoethanol and 1× protease inhibitors), sonicated at 30% duty cycle for 30 s 10–15 times. The cells were kept on ice at all times. The lysate was then centrifuged at 20,000*g* for 30 min at 4 °C. The supernatant was run on a pre-packed Ni-NTA column pre-equilibrated with 20 mM Tris pH 7.5, 100 mM NaCl, 10% glycerol, 0.5 mM β-mercaptoethanol and 30 mM imidazole. The protein was eluted in a gradient using buffer containing 20 mM Tris pH 7.5, 100 mM NaCl, 10% glycerol, 0.5 mM β-mercaptoethanol and 500 mM imidazole. The protein started eluting at 50% of the gradient. The purified protein was run on 12% polyacrylamide gel containing SDS. The purified fractions were pooled, concentrated and then run through a gel filtration column equilibrated with a sizing buffer (20 mM Tris pH 7.5, 200 mM NaCl, 10% glycerol, 0.5 mM β-mercaptoethanol and 1× PMSF). The fractions that eluted corresponding to RSCt were pooled and concentrated further and then flash frozen in liquid nitrogen to be stored at −80 °C. Protein concentration was determined using a Bradford assay comparison to a BSA standard at 595 nM.

ATPase assay

ATPase activity was measured at 30 °C by monitoring the hydrolysis of [α-^{32}P] ATP. The reaction was carried out in a reaction buffer containing 10 mM HEPES pH 7.0, 1 mM ATP, 5 mM $MgCl_2$, 20 mM potassium acetate, 4% glycerol, 0.5 mM DTT and 0.1 mg/ml BSA. RSCt at 50 nM concentration was pre-incubated with a series of concentrations of double-stranded DNA, ranging from 10 to 1000 nM, and the reaction was initiated by the addition of ATP. Aliquots of the reaction samples were removed at fixed time points and quenched with an equal volume of 0.5 M EDTA to stop the reaction. The reaction products were separated by TLC on PEI Cellulose F sheets in 0.6 M potassium phosphate buffer, pH 3.4 and quantized with a PhosphorImager (GE healthcare). The ATPase time course was analyzed using ImageJ to determine the amount of ATP hydrolyzed.

Streptavidin displacement assay

Hundred picomole of each DNA length was radio-labeled using 5 units polynucleotide kinase from phage T4 infected *E. coli* (T4 PK), and 50 uCi ^{32}P γ-labeled ATP. DNA labeling buffer is 40 mM Tris HCl (pH 7.5 at 25 °C), 10 mM $MgCl_2$, and 0.5 mM DTT. The