Abstract

RecQ helicases are Superfamily 2 (SF2) DNA helicases that unwind a wide spectrum of complex DNA structures in 3' to 5' direction and are involved in maintaining genome stability. RecQ helicases from protozoan parasites have gained significant interest in recent times because of their involvement in cellular DNA repair pathways, making them important targets for drug development. The present study describes biophysical and biochemical characterization of the catalytic core of a RecQ helicase from hemoflagellate protozoan parasite Leishmania donovani. Among the two putative RecQ helicases identified in L. donovani, the catalytic core of LdRECQb was cloned, over-expressed and purified. The catalytic core was found to be very efficient in unwinding a wide variety of DNA substrates like forked duplex, 3' tailed duplex and Holliday Junction DNA. Interestingly, the helicase core also unwound blunt-ended duplex with slightly less efficiency. The enzyme exhibited high level of DNA stimulated ATPase activity with preferential stimulation by forked duplex, Holliday Junction and 3' tailed duplex. Walker A motif lysine mutation severely affected the ATPase activity and significantly affected unwinding activity. Like many other RecO helicases, L. donovani RECQb also possesses strand annealing activity. Unwinding of longer DNA substrates by LdRECQb catalytic core was found to be stimulated in the presence of replication Protein A 1 (LdRPA-1) from L. donovani. Detailed biochemical studies of L. donovani RECQb identify it as a typical RecQ helicase with characteristic functions.

Keywords: RecQ helicase, unwinding, strand annealing, replication protein A, FRET