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Human DNA helicase V, a novel DNA unwinding enzyme from HeLa cells

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ABSTRACT

Using a strand-displacement assay with ^{32}P labeled oligonucleotide annealed to M13 ssDNA we have purified to apparent homogeneity and characterized a novel DNA unwinding enzyme from HeLa cell nuclei, human DNA helicase V (HDH V). This is present in extremely low abundance in the cells and has the highest turnover rate among other human helicases. From 300 grams of cultured cells only 0.012 mg of pure protein was isolated which was free of DNA topoisomerase, ligase, nicking and nuclease activities. The enzyme also shows ATPase activity dependent on single-stranded DNA and has an apparent molecular weight of 92 kDa by SDS-polyacrylamide gel electrophoresis. Only ATP or dATP hydrolysis supports the unwinding activity. The helicase requires a divalent cation ($Mg^{2+} > Mn^{2+}$) at an optimum concentration of 1.0 mM for activity; it unwinds DNA duplexes less than 25 bp long and having a ssDNA stretch as short as 49 nucleotides. A replication fork-like structure is not required to perform DNA unwinding. HDH V cannot unwind either blunt-ended duplex DNA or DNA-RNA hybrids; it unwinds DNA unidirectionally by moving in the 3′ to 5′ direction along the bound strand, a polarity similar to the previously described human DNA helicases I and III (Tuteja et al. Nucleic Acids Res. 18, 6785–6792, 1990; Tuteja et al. Nucleic Acid Res. 20, 5329–5337, 1992) and opposite to that of human DNA helicase IV (Tuteja et al. Nucleic Acid Res. 19, 3613–3618, 1991).

INTRODUCTION

DNA helicases catalyze the unwinding of duplex DNA during DNA replication, repair, recombination and transcription (1, 2). In general helicases bind to single-strand (ss) DNA and translocate unidirectionally into the duplex region by utilizing mainly ATP or dATP as energy source to disrupt the double helix (1, 3). Intrinsic DNA-dependent ATPase activity is associated with all the known helicases (3, 4).

DNA helicases are ubiquitious in nature and have been isolated both from prokaryotes and eukaryotes (3, 4). Up to now eleven different DNA helicases from *Escherichia coli*, (4, 5), six from viruses, four from *Saccharomyces cerevisiae*, one each from lily, *Xenopus laevis*, mouse and nine from calf thymus have been described (for review see refs. 4–6). Whereas specific functions in different aspects of DNA metabolism have been ascribed to most of the prokaryotic, yeast and viral helicases, data on the *in vivo* function of animal cell helicases are missing, especially of human cells. A 100 kDa protein from human cells (RIP100) which binds to the dehydrofolate reductase origin of replication partially copurifies with a DNA helicase activity (7). Another DNA helicase from HeLa cells has been isolated which is dependent on the homologous ssDNA binding protein to unwind the DNA in the 3′ to 5′ direction (8). Recently, a mismatch-binding protein from HeLa cells has been purified which has DNA helicase activity associated with it (9).

We have previously reported the existence of at least four different molecular species of human DNA helicases from HeLa cells as well as the purification and characterization of three of them namely human DNA helicase (HDH) I, III and IV (10–12). In a continuation of our systematic study on human DNA helicases, we report here the isolation and characterization of another DNA helicase called HDH V which is present in very low abundance and shows the highest specific activity among the four enzymes isolated so far in our laboratory.

MATERIALS AND METHODS

Cell cultures and buffers

HeLa cells were grown as described earlier (10). All the buffers used during purification contained 1 mM PMSF, 1 mM sodium bisulfite, 1 μM pepstatin and 1 μM leupeptin. Buffer A contained 20 mM HEPES (pH 8.0), 0.1 M NaCl, 1 mM DTT, 1 mM EDTA and 20% glycerol. Buffer B contained 50 mM Tris–HCl (pH 8.0), 0.1 M KCl, 1 mM DTT, 1 mM EDTA and 10% glycerol.

DNA and RNA oligonucleotides

The DNA oligonucleotides used for making DNA helicase substrates were synthesised using an Applied Biosystems 380A DNA synthesizer. A total of 13 different oligonucleotides (17 to 101-mer) were used for constructing various DNA substrates.

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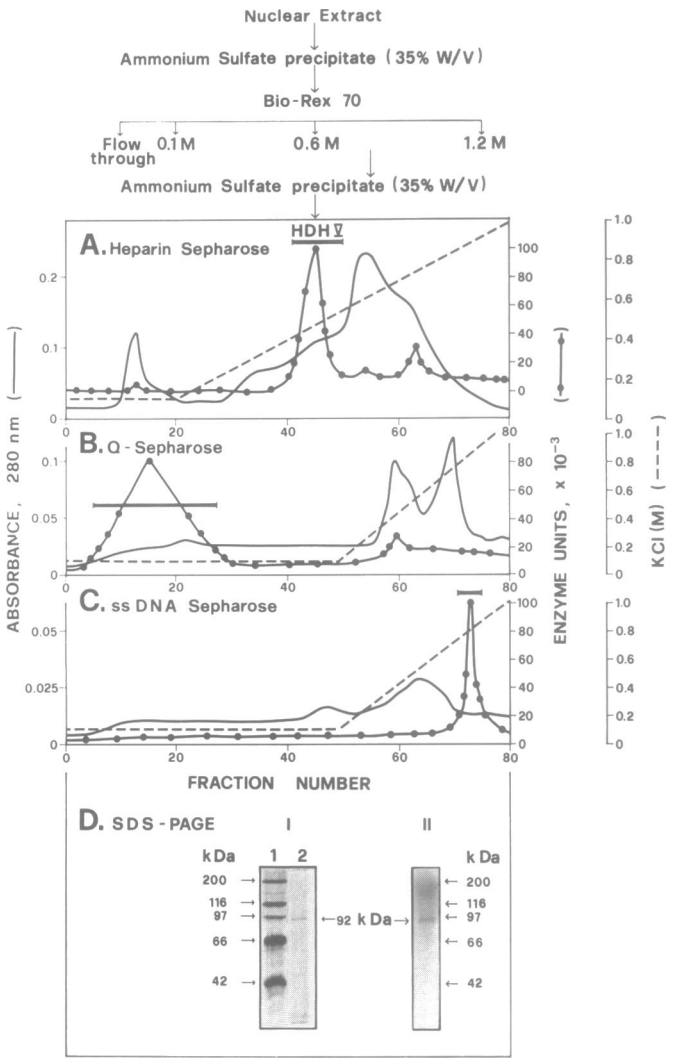

Figure 1. Purification scheme and elution profiles (panels A, B and C), of pure HDH V. The molar range of salt used to elute each column is indicated in the right ordinate axis. The detailed description of the chromatographic procedures is given in the text. The pooled fractions are indicated by the horizontal bar. SDS-polyacrylamide gel electrophoresis of HDH V (30 ng) is shown in panel D−I, lane 2. Autoradiogram of photoaffinity labeled HDH V (5 ng) with [α-^{32}P] ATP is shown in panel D−II.

The sequences of 10 of these DNA oligonucleotides were described previously (11). The sequences of the other three new ones used for constructing linear DNA substrates (Figure 4 panels, I and J), are as follows:

64mer: 5′−AATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACC-TGCAGGCATGCAAGCTTGGCGTAAT−3′
64mer: 5′−GTACCGAGCTCGAATTGCATGCATATGCATGTATGTATGT-ATGTATGTATGTATGCATGCATGC−3′
64mer: 5′−ATGCATGCATGCATGCATGCATGCATGCATGCACTG-ATGCATGCATTACGCCAAGCTTGC−3′

The sequence of an RNA oligonucleotide (17-mer) used for constructing a DNA-RNA substrate was also described previously (12).

Preparation of DNA helicase substrates

The structure of the DNA substrates (linear or circular) and of the DNA-RNA substrate employed in this study are shown in Figure 4. The oligonucleotides were 5′ phosphorylated using [γ^{32}P] ATP and T4 polynucleotide kinase and annealed to M13mp19 ssDNA or oligonucleotides as described by us previously (12). The fully duplex blunt-ended substrate (Fig. 4F) and the linear substrates (Fig. 4 I, J) were constructed by 5′ [^{32}P] end-labeling of the oligonucleotides with T4 polynucleotide kinase and annealing to an equal concentration of completely or partially complementary oligonucleotides by using the same conditions as described earlier (12). The direction-specific substrates (Fig. 4 K, L) were also prepared as described (11).

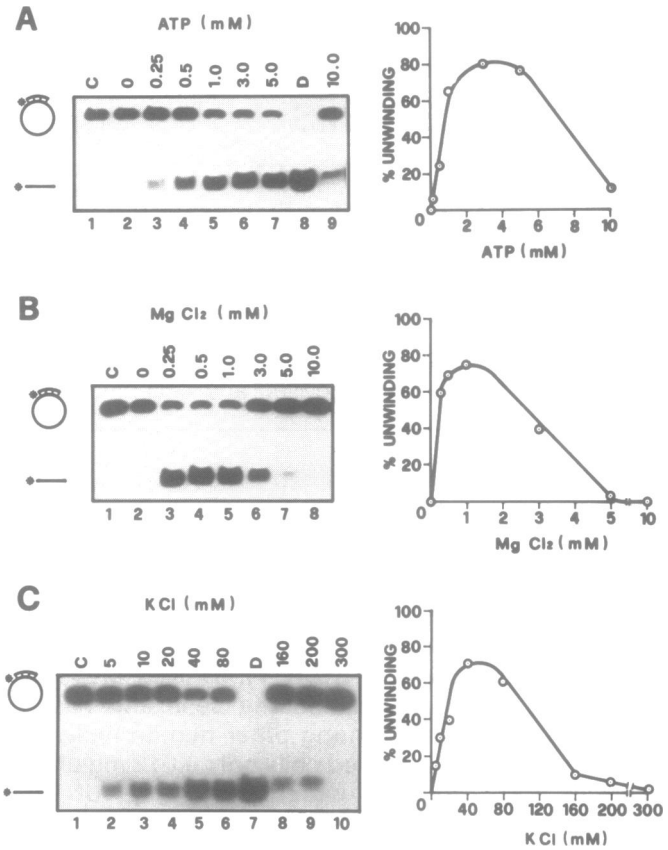

Figure 2. Effect of ATP, $MgCl_2$ and KCl concentrations on the activity of 300 pg of fraction VI. The structure of the substrate used to measure the helicase activity is shown at the extreme left of each panel. On the left side of each panel the autoradiogram of the gel is shown, whereas the concentration of each reagent is indicated at the top of each lane; the 'C' lane corresponds to a reaction without enzyme and the 'D' lane to the denatured substrate (heated 2 min. at 95°C). On the right side of each panel, the amount of unwound DNA, is quantitated as described in Material and Methods. The activity is shown as % unwinding.

Enzyme assays

DNA helicase assays were performed in 20 mM Tris−HCl (pH 8.5), 8 mM DTT, 1 mM $MgCl_2$, 3 mM ATP, 40 mM KCl, 4% (w/v) sucrose, 80 μg/ml BSA, 1.0 ng of ^{32}P labeled helicase substrate (~ 1000 cpm) and the helicase fraction in a final volume of 10 μl. The reaction mixture was incubated for 15 min at 37°C unless otherwise stated and stopped by addition of 0.3% SDS, 10 mM EDTA, 5% glycerol and 0.3% bromophenol blue. Products were analyzed by 12% native polyacrylamide gel electrophoresis, visualized by autoradiography and quantitated as previously described (12). One unit of helicase activity is defined as the amount of enzyme that unwinds 15% of the DNA

Table I. Purification of human DNA helicase V (HDH V)

Fraction	Step	Volume (ml)	Protein (mg)	DNA helicase activity Total (units)	Specific (units/mg)
	Nuclear Extract	480	2100	N.D.	
I	Amm. Sulfate ppt.	455	910	N.D.	
II	Bio-Rex 70	150	111	N.D.	
III	Amm. Sulfate ppt.	25	50	N.D.	
IV	Heparin Sepharose	17.7	5.4	360,000	67,000
V	Q-sepharose	80	1.1	288,000	262,000
VI	ssDNA sepharose	6.3	0.012	245,000	20,416,000

N.D. = Not Determined

helicase substrate at 37°C in 15 min. (1% in one min.) in the linear range of enzyme concentration dependence. The helicase activity using DNA-RNA substrate was also determined as described above but including 1 unit of RNase Block.

Assay for DNA dependent ATPase was performed with 300 pg of pure HDH V using the method described earlier(12). The ATP concentration was 3 mM instead of 1 mM.

Affinity labeling with [α-^{32}P] ATP

The reaction was performed in 1.5 ml eppendorf tube in ice with 5 ng of pure HDH V in 10 μl buffer containing 12 mM HEPES pH 7.9, 60 mM KCl, 1 mM $MgCl_2$, 6 mM DTT and 0.5 μl [α-^{32}P] ATP (specific activity 15 TBq mmol^{-1}, Amersham) in the presence of 3 μg ml^{-1} unlabeled DNA helicase substrate (17 mer annealed to M13 ssDNA). The mixture was irradiated with a 4 watt, 254 nm UV light at a distance of 5 cm for 30 min. in ice and then incubated for 10 min. without irradiation at room temperature. The products were separated by 10% SDS-PAGE and radiolabeled polypeptide was visualized by autoradiography.

Miscellaneous

DNA topoisomerases were assayed according to Kaiserman et al. (13), except that the plasmid DNA used was Bluescript containing a cDNA insert (1kb) of human cGMP phosphodiesterase (14). DNA ligase activity was measured as previously described (10) and nicking activity was assayed as described by Hughes et al. (15). Protein concentration was determined using protein assay kit of Bio Rad. SDS-PAGE was performed by the method of Laemmli (16) followed by silver staining with the Bio-Rad kit.

RESULTS

Purification of human DNA helicase V (HDH V)

The substrate used to measure the unwinding activity during the purification procedure and for most of the characterization consisted of a [^{32}P] labeled 17-mer completely annealed to M13mp19 ssDNA. A summary of the procedure and the protein elution profiles are shown in Fig. 1; all operations were carried out at 0–4°C. Nuclear extract was prepared from about 300 grams of frozen HeLa cells pellet as described by Dignam et al (17). The proteins of the nuclear extract were precipitated with ammonium sulfate (0.35 g/ml), dialyzed in buffer A (fraction I, 455ml) and passed through 169 ml of Bio-Rex 70 column (2.3×32 cm) exactly as previously described (12). The active fractions eluted at around 0.8M NaCl in buffer A were pooled (fraction II, 150 ml). The proteins of fraction II were precipitated by ammonium sulfate (0.35 g/ml) and dialyzed in buffer B (fraction III, 25 ml). The amount of helicase activity up to this step could not be quantitated precisely due to the contamination of nucleases. Fraction III was passed through a 19 ml heparin sepharose column (1.6×9 cm) as previously described (12). The active fractions eluted at about 0.45 M KCl in buffer B were pooled (fraction IV, 17.7 ml, 360,000 units) for further purification. The fractions eluted at about 0.57 M KCl in buffer B from the same column showed activity only with substrate containing hanging tails and were further purified by us recently as HDH III (12). Fraction IV was diluted to 80 ml with buffer B and loaded onto a 6.0 ml Q-sepharose fast flow column (1.6×3 cm) equilibrated with buffer B. The column was washed with 10 column volumes of buffer B and eluted with 10 column volumes of a linear gradient from 0.1 M to 1.0 M KCl in buffer B. The protein and activity profile are shown in Fig. 1 B. The activity eluted in the flow-through fractions (fraction V, 80 ml, 288,000 units). Fraction V was made 1 mM in $MgCl_2$ and

Table II. Reaction requirements of the purified HDH V activity

Reaction Conditions	% Unwinding *
Complete	90
− Enzyme	< 2
+ heated enzyme (56°C×5min)	< 2
− ATP	< 2
" + dATP (3mM)	82
" + ATP γS (3mM)	< 2
" + ADP (3mM)	< 2
" + AMP (3mM)	< 2
" + C, G or UTP (3mM)	< 2
" + dC, dG or dTTP (3mM)	< 2
− $MgCl_2$	< 2
" + $CaCl_2$ (1mM)	< 2
" + $ZnSO_4$ (1mM)	< 2
" + $MnCl_2$ (1mM)	78
" + $CdCl_2$ (1mM)	< 2
" + $CuCl_2$ (1mM)	< 2
" + $NiCl_2$ (1mM)	< 2
" + $AgNO_3$ (1mM)	< 2
" + $CoCl_2$ (1mM)	< 2
+ KCl or NaCl (200mM)	5
+ EDTA (5mM)	< 2
+ M13 ssDNA (30μM as P)	< 2
+ M13 RFI DNA (30μM as P)	71
+ *E.coli* t-RNA (30μM as P)	75
+ Trypsin (1 unit)	< 2
+ Poly [A], [C] or [U](30μM as P)	86
+ Poly [G] (30μM as P)	17

* Helicase reaction was carried out with 300 pg of pure protein (fraction VI) as described under 'Materials and Methods'.

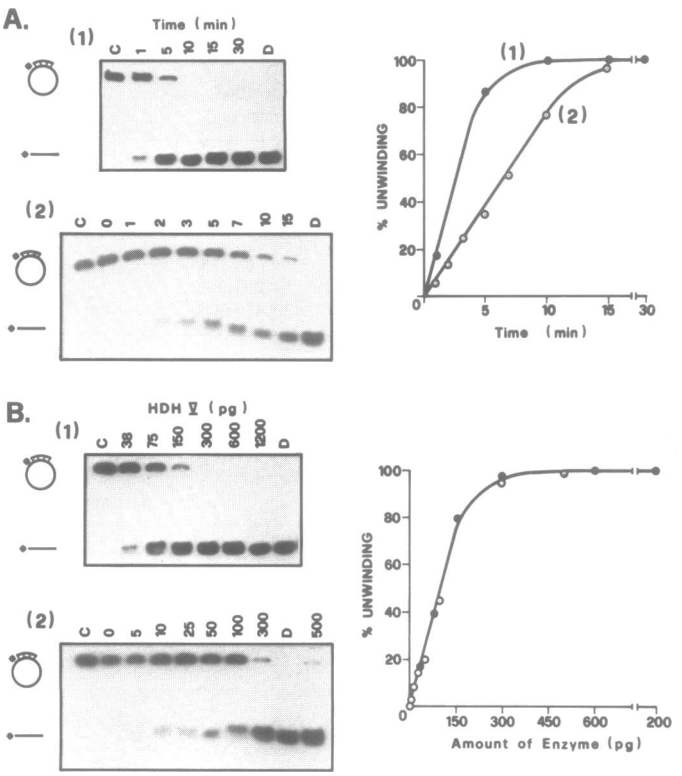

Figure 3. Time and concentration dependence of HDH V activity. **A:** 1200 pg (1) or 300 pg (2) of HDH V were used in each standard 10 μl reaction mixture and incubated for different time intervals. The left panel shows the autoradiagram of the gels. The time of reaction is indicated on the top of each lane. The right panel shows the quantitative data. **B:** increasing amounts of HDH V (fraction VI) were incubated in the standard 10 μl reaction mixture for 15 min. The left panel shows the autoradiogram of the gels. Autoradiograms (1) and (2) are form two different experiments with the amount of enzyme indicated at the top of each lane. The right panel shows the quantitative data of (1) and (2). Lanes 'C' and 'D' of each autoradiogram of panels A and B are control assays without enzyme and heat-denatured substrate respectively.

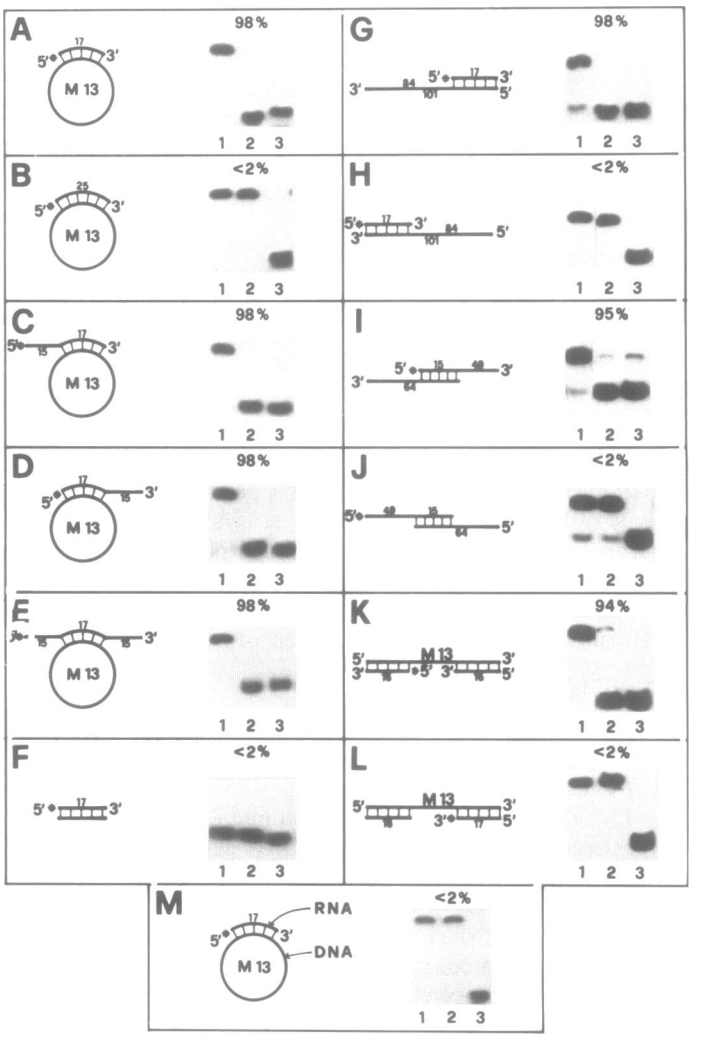

Figure 4. Helicase activity with various substrates. The helicase reaction was performed with 300 pg of pure HDH V (fraction VI) as described in Materials and Methods. Each panel shows the structure of the substrate used and the autoradiogram of the gel. Asterisks denote the ^{32}P labeled end. Percent unwinding is shown on top of each autoradiogram. Lanes 1 and 3 correspond to control reactions without enzyme and heat-denatured substrate respectively. Lane 2 corresponds to the reaction with pure HDH V. Direction of unwinding is analyzed in panels G to L. The substrates of the reactions reported in panels G, I and K detect 3′ to 5′ direction while those of panels H, J and L show the 5′ to 3′ direction unwinding. Panel M shows the lack of unwinding of a DNA-RNA substrate.

loaded onto a 2.5 ml ssDNA sepharose 4B column (1.6×1.3cm) equilibrated with buffer B containing 1mM $MgCl_2$. The ssDNA sepharose 4B was prepared by coupling ssDNA from HeLa cells to cyanogen bromide activated sepharose 4B as described by us previously (12). The column was washed with 5 column volumes of buffer B containing 1 mM $MgCl_2$ and eluted with a gradient from 0.1 M to 1.0 M KCl in buffer B containing 1 mM $MgCl_2$. The elution profile is shown in Fig. 1C.

A summary of the purification is shown in Table I. From 300 grams of HeLa cells we were able to recover only 0.012 mg of apparently pure enzyme with specific activity of 2×10^7 units/mg. Purified HDH V (fraction VI) was free of nucleases, topoisomerases, DNA ligase or DNA nicking activities. Fraction VI showed one band of about 92 kDa on SDS-polyacrylamide gel electrophoresis with silver stain (Fig 1D−I, lane 2). Photoaffinity cross-linking of pure HDH V with [$\alpha-^{32}P$] ATP also showed one radioactive band of about 92 kDa (Fig. 1D−II).

DNA-dependent ATPase activity was present at a level of approximately 40 pmol of ATP hydrolysed in 15 min by 0.3 ng of pure HDH V.

Reaction requirements and characterization of HDH V

The reaction requirements of HDH V are shown in Table II. Enzyme activity is totally lost after heating at 56°C for 5 min. The maximum activity was found at pH 8.5 and at 37°C (data not shown). Trypsin (1 unit) destroyed the activity completely. The reaction was inhibited by 200 mM KCl or NaCl or by EDTA (5 mM), as well as by M13 ssDNA (30 μM as P) whereas M13 RF1 DNA and *E.coli* tRNA at same concentration had little effect (Table II).

Hydrolysis of ATP is required to support the unwinding activity, a requirement that could not be replaced by ATPγS, ADP, AMP and other nucleoside 5-triphoshate, with the exception of dATP which supported more than 90% of the activity (Table II). For optimal activity HDH V required 3 mM ATP, 1 mM $MgCl_2$ and 40 mM KCl (Fig. 2). Without ATP or $MgCl_2$ the activity was not detectable and at 10 mM ATP or 5 mM $MgCl_2$ or 300 mM KCl the activity was completely inhibited (Fig. 2). Mg^{2+} is required for maximum activity in

Table III. Summary of properties of HDH I, III, IV and V

Property	HDH I	HDH III	HDH IV	HDH V
Apparent adundance * (μg/100 g of cells)	110	85	70	3.9
Size kDa (by SDS PAGE)	65	46	100	92
Direction of unwinding	3′ to 5′	3′ to 5′	5′ to 3′	3′ to 5′
DNA-RNA unwinding **	ND	−	+	−
RNA-DNA unwinding **	+	ND	+	ND
Blunt end duplex unwinding	−	−	−	−
Preference of fork-like structure of substrate	−	+	−	−
Requirement of free ssDNA on substrate bases	>11	>84	>84	<49
Maximum length unwound (bp)	>40	>40	<40	<40
Cation requirements	$Mg^{2+}>Mn^{2+}>Ca^{2+}$	$Mg^{2+}>Mn^{2+}$	$Mg^{2+}=Mn^{2+}=Zn^{2+}$	$Mg^{2+}>Mn^{2+}$
Nucleotides unwound per molecule of enzyme per sec ***	9.4×10^{-5}	2.8×10^{-6}	1.4×10^{-5}	3.3×10^{-3}
Enzyme/Substrate molecular ratio at saturating enzyme concentration	240	4000	2000	6.5
Molecules of ATP hydrolyzed per molecule of enzyme per sec ***	1.5×10^{-4}	5.0×10^{-3}	7.8×10^{-2}	14
Nucleotides unwound per ATP molecule split ***	6.3×10^{-1}	5.6×10^{-4}	1.8×10^{-4}	2.4×10^{-4}

* Values are calculated from the final recovery of pure protein
** DNA-RNA is a short RNA stretch annealed to a long DNA strand, RNA-DNA is the opposite
*** with 1 ng of helicase substrate and 3 mM ATP
ND: Not Determined

preference to Mn^{2+}, which supported 80% of maximal activity. All other tested cations such as Ca^{2+}, Zn^{2+},Cu^{2+}, Ni^{2+}, Ag^{2+}, Cd^{2+} and Co^{2+} were not active at same concentration. Poly [G] (30 μM as P) caused more than 80% inhibition while Poly [A], [C] and [U] at same concentration had no effect on helicase activity (Table II).

Titration of HDH V under optimal assay conditions showed a maximum value of unwinding of 98% in 15 min with 500 pg of enzyme (Fig. 3A). In the presence of 300 pg (6 units) of enzyme the unwinding rate was linear up to 15 min (Fig. 3B) and deviated from linearity with longer incubation.

HDH V activity on various substrates

The helicase activity on different model substrates is shown in Fig. 4. HDH V can unwind a 17-mer annealed to M13 ssDNA (Fig. 4A) regardless of the presence or absence of mismatched hanging tails at either the 5′ end, the 3′ end or both (Fig. 4C, D, E). However, HDH V is unable to unwind if the 17-bp-long duplex portion of the substrate is increased to 25-bp (Fig. 3B) and also fails to unwind the blunt-ended 17-mer duplex DNA (Fig. 3F), even if it has the same nucleotide sequence of duplex region as the substrate of Fig. 3A.

In order to determine the direction of unwinding of HDH V we have constructed six different substrates, three for the 3′ to 5′ and three for the 5′ to 3′ direction (Fig. 4 G to L). The substrates shown in Fig. 4I and 4J are substrates for which helicase can use both strands for moving in the same direction. The results show that HDH V moves in 3′ to 5′ direction along the bound strand as it unwinds the 3′ to 5′ direction-specific substrates (Fig. 4G, I and K) whereas it cannot unwind substrates specific for the 5′ to 3′ direction (Fig. 4 H, J and L).

The DNA-RNA substrate consisted of a [^{32}P] labeled RNA 17-mer annealed to M13 ssDNA. The results show that HDH V is unable to unwind a DNA-RNA duplex (Fig. 4M).

DISCUSSION

Whereas for most of the DNA helicases isolated from *E.coli* a reasonable attribution of function in DNA metabolism has been obtained, in eukaryotes this objective has not yet been obtained with clear-cut evidence for anyone of the different enzymes already isolated. Even in a relatively simple prokaryote, such as *S.cerevisiae*, no helicase essential for DNA replication process has been unambiguously recognized, and only in one case of radiation-deficient strains (the Rad 3 gene) a function in excision repair has been recognized for a helicase moving in 5′ to 3′ direction (18).

The situation is certainly more difficult for the helicases isolated from plants, *Xenopus* or mammalian organs and cells; over seventeen such enzymes have been purified so far (4), but their function has still eluded the investigators and no obvious similarity among the different mammalian helicases has been observed, such as could allow a classification analogous to the one which was successfully made for the different eukaryotic DNA polymerases (19). Among animal virus-encoded DNA helicases, the T-antigen associated helicase activity stands out insofar that its role in DNA replication initiation and fork advancement is well proven (20−22). On the other hand, the variety of viral strategies evolved to reproduce inside the infected cells are such that one cannot expect any obvious similarity between the molecules involved in similar processes on the viral and mammalian chromosomes. Similarly, from the properties of prokaryotic helicases, whose function in different aspects of DNA metabolism has been well established, no reasonable inference is possible as regards the properties of the comparable enzymes in eukaryotes.

In our laboratory we have initiated a systematic survey and isolation of the DNA helicases present in HeLa cells with the programme of eventually isolating their genes and probing their *in vivo* function by a combination of gene inactivation and enzyme

inhibition through micro-injection of specific neutralizing antibodies. In the present paper we report the existence of another enzyme from HeLa cells named human DNA helicase V (HDHV). We have purified this enzyme to apparent homogeneity and found that it is present in very low abundance: from about 300 grams of HeLa cells we were able to recover only 12 micrograms. Earlier we have reported the existence in HeLa cells nuclei of at least four different DNA helicases and the purification of HDH I, (10), HDH IV (11) and HDH III (12); purification of HDH II is still in process; the main properties of the human helicases are summarized in Table III. Photaffinity cross-linking of the purified HDH V with [α-^{32}P] ATP resulted in labeling of a polypeptide of approximately 92 kDa showing that the molecule visible with silver stain in the experiment of Fig. 1 D–I corresponds to the catalytically active protein.

HDH V is present in the 35% (w/v) ammonium sulfate precipitate of the nuclear extract like HDH I, II and III (10, 12) and unlike HDH IV which is present in the supernatant (11). The fractionation of HDH I, II, III and V depends on their behaviour on Bio-Rex 70 column which is a weak acidic cation exchanger. HDH V binds to this column like HDH II and III and unlike HDH I which elutes in the flow-through (10). By following a procedure very similar to ours two distinct DNA helicases from nuclei of calf thymus were isolated by Zhang and Grosse 1991 (23). HDH V eluted from Bio-Rex 70 column at 0.8 M NaCl in buffer A together with HDH III (12) whereas these calf thymus helicases eluted at 0.15 M NaCl from the same column, indicating that the two calf thymus helicases are probably different from HDH V. HDH V and HDH III separate from each other on heparin sepharose column (12). HDH III is different from HDH V in that it requires a fork-like structure of substrate and has different molecular weight (12). Also, HDH V does not bind to Q-sepharose column and elutes in the flow through (Fig. 1) whereas HDH III binds to this column and elutes at 0.4 M KCl (12).

In the standard assay conditions HDH V does not need a fork-like structure of the substrate but unwinds only short duplexes (17-mer/M13) and cannot unwind 25-mer or longer duplexes. As far as the direction of unwinding is concerned HDH V moves in a 3′ to 5′ direction along the bound strand, i.e. in the same direction as HDH I (10), HDH III (12), SV40 T-antigen (20), Polyoma T-antigen (21), 47 kDa calf thymus DNA helicase (24), calf thymus DNA helicase E (25) and I (23), as well as of the HSSB- dependent DNA helicase from HeLa cells (8).

The most distinctive features of HDH V from other human helicases are: a) that it is present in extremely low abundance in the cells; b) that it is highly active, so that it can be detected even in picogram quantities; c) that it can use small linear DNA substrates, contrary to other HDHs. In fact HDH V is the most active one among the helicases we have described so far: from the data of Table III one can see how the unwinding rate per enzyme molecule is from 200 to 3000 fold higher than that of the other previously described human helicases. This may be a reflection of the very high affinity of the enzyme for single-stranded DNA, a property proven also by the purification procedure, (see figure 1) since the enzyme elutes only at 0.8 M KCl from the single-stranded DNA sepharose column. Also, the more modest requirement, with respect to that of the other enzymes, for a single-stranded DNA portion to bind in order to act, (see also Table III) may be related to the high affinity for ssDNA. In agreement with this is the fact, also shown in Table III, that at saturating enzyme concentration the ratio of enzyme molecules per substrate molecules is of the order of only 6 for HDH V, whereas it varies from 200 to 4000 for the other helicases.

HDH V also shows the highest turnover number among all the other described ones, as far as ATPase activity is concerned. A comment on this hydrolysing activity is of the order: in all human helicases we have studied, when the two assays are made in exactly the same condition, the ratio of ATP molecules split is outrageously higher than the number of unwound basepairs. In fact, as Table III shows, the nucleotides unwound per ATP hydrolysed range from approximately 0.6 for HDH I, to values of the order of 2×10^{-4} for the other helicases. In all probability, much of this apparent waste is due to the fact that the helicases find a great excess of single-stranded DNA in the assay conditions and operate therefore what may be considered futile hydrolysis of ATP. Furthermore, it is likely that, *in vitro*, the unwinding activity may be modulated by the interaction with other specific proteins.

We are in the process of isolating the DNA coding for the five HDHs identified so far and of raising neutralizing antibodies for them. The study of the effect of activity disruption of the helicases on DNA metabolism will hopefully give us clues as to their *in vivo* function.

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REFERENCES

1. Geider, K. and Hoffmann-Berling, H. (1981) Ann. Rev. Biochem. **50**, 233–260.
2. Kornberg, A. and Baker, T. A. (1991) in DNA Replication 2nd edition (W.H. Freeman & company, New York) pp. 355–378.
3. Matson, S.W. and Kaiser-Rogers, K.A. (1990) Annu. Rev. Biochem.**59**, 289–329.
4. Thommes, P. and Hubscher, U. (1992) Chromosoma **101**, 467–473
5. Lohman, T. M. (1992) Molecular Microbiology **6**, 5–14.
6. Siegal, G., Turchi, J.J., Bambara, R.A., Myers, Y.W. and Jessee, C.B. (1992) J. Biol. Chem. **267**, 13629–13635.
7. Dailey, L., Caddle, M. S., Heintz, N. and Heintz, N. H. (1990) Mol. Cell. Biol. **10**, 6225–6235.
8. Seo, Y-S., Lee, S-H and Hurwitz, J. (1991) J. Biol. Chem. **266**, 13161–13170.
9. Hughes, M.J. and Jiricny, J. (1992) J. Biol. Chem. **267**, 23876–23882.
10. Tuteja, N., Tuteja, R., Rahman, K., Kang, L. and Falaschi, A. (1990) Nucleic Acids Res. **18**, 6785–6792.
11. Tuteja, N. Rahman, K., Tuteja, R. and Falaschi, A. (1991) Nucleic Acid Res. **19**, 3613–3618.
12. Tuteja, N., Rahman, K., Tuteja, R., Ochem, A., Skopac, D. and Falaschi, A. (1992) Nucleic Acids Res. **20**, 5329–5337.
13. Kaiserman, H.B., Ingebristen, T.S. and Benbow, R.M. (1988) Biochemistry **27**, 3216–3222.
14. Tuteja, N., Danciger, M., Klisak,I., Tuteja, R., Inana, G., Mohandas, T., Sparkes, R.S. and Farber, D.B. (1990) Gene **88**, 227–232.
15. Hughes, M.J., Liang, H., Jiricny, J. and Jost, J. P. (1989) Biochemistry **28**, 9137–9142.
16. Laemmli, U.K. (1970) Nature **227**, 680–685.
17. Dignam, J.B., Lebrovitz, R.M. and Raeder, R.G. (1983) Nucleic Acids Res. **11**, 1475–1489.

18. Harosh,I., Naumovski, L. and Friedberg E. C. (1989) J. Biol. Chem. **264**, 20532–20539.
19. Burgers, P. M. J., Bambara, R. A., Campbell, J. L., Chang, L. M. S., Downey, K. M., Hubscher, U., Lee, M. Y. W. T., Linn, S. M., So, A. G. and Spadari, S. (1990) Eur. J. Biochem. **191**, 617–618.
20. Stahl, H. A. and Knippers, R. (1987) Biochem. Biophys. Acta. **910**, 1–10.
21. Seki, M., Enomotto, T., Hanaoka, F. and Yamada, M. (1987) Biochemistry **26**, 2924–2928.
22. Goetz, G.S., Dean, F.B., Hurwitz, J. and Matson, S.W. (1988) J. Biol. Chem. **263**, 383–392.
23. Zhang, S. and Grosse, F. (1991) J. Biol. Chem. **266**, 20483–20490.
24. Thommes, P. and Hubscher, U. (1990) J. Biol. Chem. **265**, 14347- 14354.
25. Turchi, J.J., Siegal. G. and Bambara R.A. (1992) Biochemistry **31**, 9008–9015.