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DNA helicase IV from HeLa cells

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ABSTRACT

Human DNA helicase IV, a novel enzyme, was purified to homogeneity from HeLa cells and characterized. The activity was measured by assaying the unwinding of ^{32}P labeled 17-mer annealed to M13 ss DNA . From 440g of HeLa cells we obtained 0.31mg of pure protein. Helicase IV was free of DNA topoisomerases, DNA ligase and nuclease activities. The apparent molecular weight is 100 kDa. It requires a divalent cation for activity ($Mg^{2+} = Mn^{2+} = Zn^{2+}$) and the hydrolysis of only ATP or dATP. The activity is destroyed by trypsin and is inhibited by 200mM KCl or NaCl, 100mM potassium phosphate, 45mM ammonium sulfate, 5 mM EDTA, 20μM ss M13 DNA or 20μM poly [G)] (as phosphate). The enzyme unwinds DNA by moving in the 5′ to 3′ direction along the bound strand, a polarity opposite to that of the previously described human DNA helicase I (Tuteja et al Nucleic Acids Res. 18, 6785–6792, 1990). It requires more than 84 bases of single-stranded DNA in order to exert its unwinding activity and does not require a replication fork-like structure. Like human DNA helicase I the enzyme can also unwind RNA-DNA hybrid.

INTRODUCTION

We have initiated a systematic study of the DNA helicases present in human cells with the objective of purifying them, describing their properties and eventually cloning their genes and defining their functions in different aspects of DNA metabolism (1). In connection with our studies on the initiation of DNA replication in human cells (2), we are particularly interested in identifying the helicase(s) involved in origin activation and possibly performing functions analogous to those of the helicase associated with the T antigen of SV40 (3,4) and polyoma (5). In this context we have recently reported (1) the existence of at least four different molecular species of human DNA helicases, namely HDH I, II, III (based on differential chromatographic fractionation) and HDH IV (present in the supernatant after precipitation with 35% [w/v] ammonium sulfate). We have already purified to homogeneity and characterized HDH I, a 65kDa protein which moves 3′ to 5′ along the bound DNA strand (1). Here we report the purification and characterization of HDH IV from HeLa cells.

MATERIALS AND METHODS

Reagents

All reagents used were as previously described (1) unless otherwise noted. Spermidine, poly [A], poly [C], poly [G] and poly [U] were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Sarkosyl was obtained from Fluka Chemie AG (Buchs/Switzerland). Yeast tRNA was obtained from Boehringer Mannheim Gmbh (Mannheim, FRG). The oligodeoxynucleotides used for making DNA helicase substrates, reported in table 1, were synthesised using an Applied Biosystems 380A DNA synthesiser.

Cell cultures

HeLa cells were grown as previously described (1).

Buffers

The following buffers were used: buffer A, 50mM Tris-HCl (pH 8.0), 50 mM KCl, 1mM DTT, 1mM EDTA, 10% (v/v) glycerol, 1mM sodium bisulfite and 1mM phenylmethanesulfonyl fluoride, buffer B was the same as buffer A except for the addition of 0.1M KCl, buffer C was buffer A but with the addition of 0.2M KCl and 1mM $MgCl_2$.

Preparation of DNA helicase substrates

The partial duplex consisting of ^{32}P labeled oligodeoxynucleotide 1 (17-mer, table 1), hybridised to M13mp19 single stranded (ss) DNA was mainly used for enzyme assay unless otherwise stated. The 17-mer was 5′ end labeled and annealed to ss DNA as described earlier (1). The substrates with 3′ tail, 5′ tail or both tails were also prepared in the same way. Blunt ended duplex substrate and small linear substrates were also prepared as described earlier (1) by using the oligodeoxynucleotides shown in table 1.

Preparation of direction specific substrates

The substrates consisting of long linear M13 ss DNA with short duplex ends for 3′ to 5′ and 5′ to 3′ unwinding were prepared as shown in fig. 6A and 6B respectively. The substrate for 3′ to 5′ unwinding was prepared by first 5′ end labeling of oligodeoxynucleotide 11 (32-mer, table 1) and then annealing with M13mp19 ss DNA as described earlier (1). The annealed substrate was digested with SmaI and purified by gel filtration through 1 ml of sepharose 4B. For 5′ to 3′ unwinding substrate the oligodeoxynucleotide 11 (32-mer, table 1) was first annealed to M13mp19 ss DNA and then labeled at 3′ end as described earlier (1). The annealed substrate was digested with SmaI and purified by gel filtration through 1 ml. sepharose 4B.

Preparation of RNA-DNA substrate

The RNA-DNA substrate was prepared as described (1) by using oligodeoxynucleotide 12 (18-mer, table 1) and in vitro transcribed

Table 1. Oligodeoxynucleotides synthesized for the preparation of helicase substrates

Oligomer	Sequence	Note
1 (17-mer)	5'-GTTTTCCCAGTCACGAC-3'	Complementary to M13mp19 (+ strand) DNA. Used for 5'-end labelled substrate (17-mer/M13).
2 (17-mer)	5'-GTCGTGACTGGGAAAAC-3'	Complementary to oligodeoxynucleotide 1. Used for blunt-ended substrate.
3 (32-mer)	5'-$(T)_{15}$GTTTTCCCAGTCACGAC-3'	The last 17 nucleotides towards the 3' end are complementary to M13mp19 (+ strand) DNA. Used for 5' tail substrate.
4 (32-mer)	5'-GTTTTCCCAGTCACGAC$(T)_{15}$-3'	The first 17 nucleotides towards the 5' end are complementary to M13mp19 (+ strand) DNA. Used for 3' tail substrate.
5 (47-mer)	5'-$(T)_{15}$GTTTTCCCAGTCACGAC$(T)_{15}$-3'	The middle 17 nucleotides are complementary to M13mp19 (+ strand) DNA. Used for 5' and 3' tails substrate.
6 (101-mer)	5'-TTGAAAACGACGGCCAGTGAATTC-GAGTCGGTACCCGGGGATCCTCTAG-AGTCGACCTGCAGGCATGCAAGCTT-GGCGTAATCATGGTCATAGCTGTTT-3'	Complementary to M13mp19 (+ strand) DNA. Used for small linear substrate.
7 (17-mer)	5'-GGTCGACTCTAGAGGAT-3'	Complementary to the middle part of oligodeoxynucleotide 6. Used for small linear substrate.
8 (17-mer)	5'-CTGGCCGTCGTTTTCAA-3'	Complementary to the first 17 nucleotides towards 5' end of oligodeoxynucleotide 6. Used for small linear substrate.
9 (17-mer)	5'-AAACAGCTATGACCATG-3'	Complementary to the last 17 nucleotides towards 3' end of oligodeoxynucleotide 6. Used for small linear substrate.
10 (25-mer)	5'-TTCGAGCTCGGTACCCGGG-GATCCT-3'	Complementary to M13mp19 (+strand) DNA. Used for 25-mer/M13 substrate.
11 (32-mer)	5'-TTCGAGCTCGGTACCCGGGGATCCT-CTAGAGT-3'	Complementary to M13mp19 (+ strand) DNA. Used for direction substrate and 32-mer/M13 substrate.
12 (18-mer)	5'-GATGCCATATTGGGCCAG-3'	Complementary to a 1kb cDNA (position 241−258) of γ-subunit of human retinal cGMP phosphodiesterase (6). Used for RNA-DNA substrate.

RNA (1 Kb) from the plasmid Bluescript containing the DNA of the γ-subunit of retinal cGMP phosphodiesterase (6).

DNA helicase assay

The standard reaction mixture (10 μl) containing 20 mM Tris-HCl (pH 9.0), 8mM DTT, 0.5mM $MgCl_2$, 3mM ATP, 60 mM KCl, 4% (wt./vol) sucrose, 80 μg/ml BSA, 1.0 ng of ^{32}P labeled helicase substrate (1000 cpm) and the helicase fraction to be assayed was incubated at 37°C for 30 min. (unless otherwise indicated) and terminated by the addition of 0.3% SDS, 10mM EDTA, 5% glycerol and 0.03% bromophenol blue. After further incubation at 37°C for 5 min the substrate and product were separated by electrophoresis on a 15% non-denaturating polyacrylamide gel. After electrophoresis the gel was fixed in a 10% methanol and 10% acetic acid solution. The gel was dried and exposed to Amersham Hyperfilm with an intensifying screen for autoradiography. DNA unwinding was quantitated by excising the bands from the dried gel and counting in Beckman liquid scintillation fluid. One unit of DNA helicase activity is defined as the amount of enzyme unwinding 1% of the DNA helicase substrate in 1 min. at 37° (30% in a 30 min. reaction) as described (1).

Other methods

DNA dependent ATPase was assayed as described by Hübscher and Stadler (7). DNA topoisomerases were assayed as described by Kaiserman et al (8) except that the plasmid DNA used was Bluescript containing a cDNA (1Kb) of human cGMP phosphodiesterase (6) and the assay was also done in presence of 1mM ATP for Topoisomerase II. DNA ligase activity was checked as described earlier (1). DNA nicking activity was assayed as described by Hughes et al (9). SDS polyacrylamide gel electrophoresis and protein determination were performed according to Laemmli (10) and Bradford (11) respectively. Silver staining was done using a Bio-Rad silver staining kit.

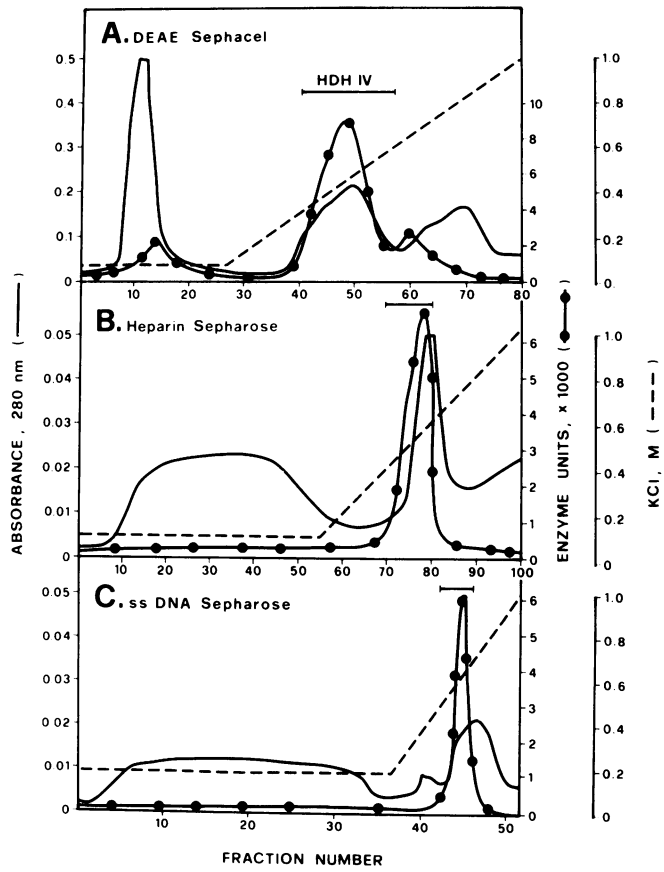

Figure 1. Elution profile of proteins and helicase activity on DEAE-Sephacel (A), Heparine-Sepharose (B) and ssDNA-Sepharose (C) columns. The detailed description of the chromatographic procedures is given in the text. The pooled fractions are indicated by the horizontal bars.

Table 2. Purification of human DNA helicase IV (HDH IV)

Fraction	Step	Volume (ml)	Total Protein (mg)	Total activity (units)	Specific activity units/mg
	Nuclear Extract	760	1950	N.D.	
I	Amm. Sulfate Sup. (after 35%, w/v)	950	96	N.D.	
II	Amm. Sulfate ppt. (35% to 55%, w/v)	40	70	N.D.	
III	DEAE Sephacel	72	12.5	29,400	2,352
IV	Heparin Sepharose	22	4.40	21,333	4,848
V	ssDNA Sepharose	5	0.31	15,833	51,074

N.D. = Not Determined

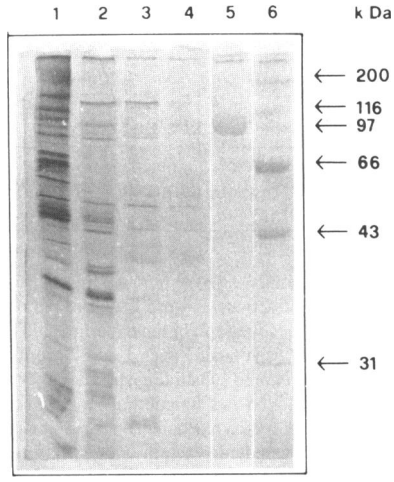

Figure 2. SDS-polyacrylamide electrophoresis of the fractions at various stages of purification of HDH IV. Fractions were separated in a 10% gel according to Laemmli (9) and stained with silver using a Bio-Rad silver stain kit. Lane 1: 10μg protein of fraction I. Lane 2: 10μg protein of fraction II. Lane 3: 5μg protein of fraction III. Lane 4: 3μg protein of fraction IV. Lane 5: 3.5μg protein of fraction V. Lane 6: size markers, indicated in Kilodaltons (kDa).

RESULTS

Purification of human DNA helicase IV (HDH IV)

All purification steps were carried out at 0–4°C. The purification was started from 440g of frozen HeLa cells. The cells were thawed on ice and a nuclear extract was prepared by the method of Dignam et al (12). Most of the proteins were precipitated by slowly adding solid ammonium sulfate (0.35g/ml) with constant stirring. The mixture was stirred for an additional hour and the precipitate containing HDH I, II and III (1) was removed by centrifugation at 20000 rpm for 20 min at 4°C in a SW28Ti rotor. The supernatant (fraction I: 950 ml.) contained HDH IV. Additional 0.15mg/ml solid ammonium sulfate was added to fraction I and the precipitate was collected as described above. The pellet was resuspended and dialysed in buffer A (fraction II: 40 ml). The amount of helicase activity present in fraction I and II could not be determined precisely due to the presence of nucleases. Fraction II was adsorbed onto a 70 ml DEAE sephacel column (2.5×14.4cm) previously equilibrated with buffer A. The column was washed with buffer A and eluted with 4 column volumes of a linear gradient ranging from 0.05M to 1.0M KCl in buffer A. The elution profile of proteins is shown in Fig.1A. The gradient fractions containing helicase activity were eluted around 0.37M KCl and pooled (fraction III; 72ml, 29,400 units). Fraction III was diluted to 0.1M KCl with buffer A (without KCl) and adsorbed onto a 19 ml heparin sepharose column (1.5×10cm) previously equilibrated with buffer B. The column was washed with buffer B and HDH IV was eluted with a gradient of 4 column volumes containing 0.1M KCl to 1.0M KCl in buffer B. The elution profile of the proteins is shown in Fig. 1B. The active fractions were eluted around 0.55M KCl and pooled (fraction IV; 22ml, 21,333 units). Fraction IV was diluted with buffer A having no KCl until the KCl concentration became 0.2M. After adding $MgCl_2$ to a final concentration of 1mM this fraction was loaded onto a 3 ml ss DNA sepharose 4B column (1.5×1.75 cm) previously equilibrated with buffer C. The ss DNA sepharose 4B was prepared by coupling HeLa cell ss DNA to CNBr activated sepharose 4B as described (1). After loading, the column was washed with buffer C and subsequently eluted with a 22 ml gradient from 0.2M to 1.0M KCl in buffer C. The elution profile of proteins is shown in Fig. 1C. The activity eluted at about 0.6M KCl (fraction V; 5ml 15,833 units).

Table 2 reports a summary of the purification. The HDH IV activity eluted at 0.6M KCl from a ss DNA sepharose 4B column showed only one band on SDS-PAGE of about 100kDa (Fig 2, lane 5). Overall, from 440g of HeLa cells we were able to purify 0.31 mg of HDH IV with the specific activity of 51,074 units/mg. The pure HDH IV fraction did not contain any detectable level

Table 3. Reaction requirements of the purified HDH IV activity[a]

Reaction Conditions	% Unwinding
Complete	75
− Enzyme	< 2
+ heated enzyme (56°×5min)	< 2
− ATP	< 2
+ dATP (3mM)	45
+ 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
+ $MgSO_4$ (1mM)	74
+ $Mg(C_2H_3O_2)_2$ (1mM)	75
+ $CaCl_2$ (1mM)	< 2
+ $MnCl_2$ (1mM)	72
+ $ZnSo_4$ (1mM)	71
+ $CuCl_2$ (1mM)	< 2
+ $NiCl_2$ (1mM)	< 2
+ $AgNO_3$ (1mM)	< 2
Complete	
+ $(NH_4)_2SO_4$ (45mM)	11
+ KCl or NaCl (200mM)	< 2
+ KPO_4 (pH8, 100mM)	< 2
+ EDTA (5mM)	< 2
+ M13 ss DNA (20μM as P)	< 2
+ HeLa cell total RNA (40μM as P)	22
+ M13 RFI DNA (40μM as P)	45
+ Yeast t RNA (40μM as P)	56
+ Trypsin (1 unit)	< 2
+ Poly [A],[C] or [U](20μM as P)	76
+ Poly [G] (20μM as P)	5
+ Sarcosyl (0.04%)	< 2
+ Spermidine (0.1mM)	75

[a]Helicase reaction was carried out with 50ng of pure protein using 17-mer/M13 substrate as described under 'Materials and methods'.

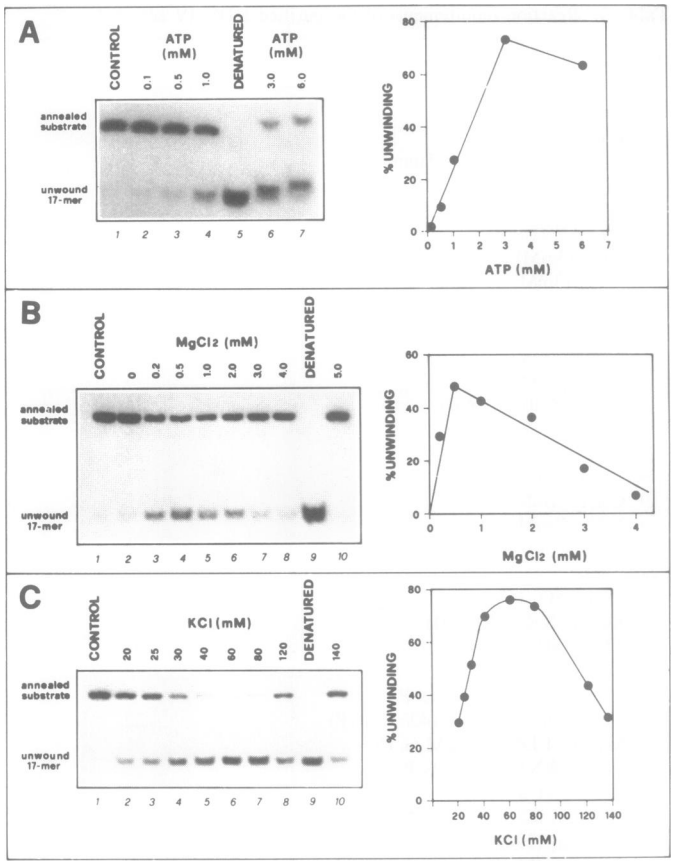

Figure 3. Effect of ATP (A), $MgCl_2$ (B) and KCl (C) on the DNA helicase activity. Helicase reactions were carried out for 30 min. at 37°C with 50ng of pure HDH IV (fraction V) and ^{32}P labeled 17-mer/M13 DNA substrate as described in the Materials and Methods. On the left side of each panel the autoradiograph of the gel is shown; the amounts used are indicated at the top of each lane. The 'control' lane corresponds to a reaction without enzyme and the 'denatured' lane to that with a substrate heated at 95°C for 5 min. On the right side of each panel, the unwound DNA was quantitated as described in the Materials and Methods.

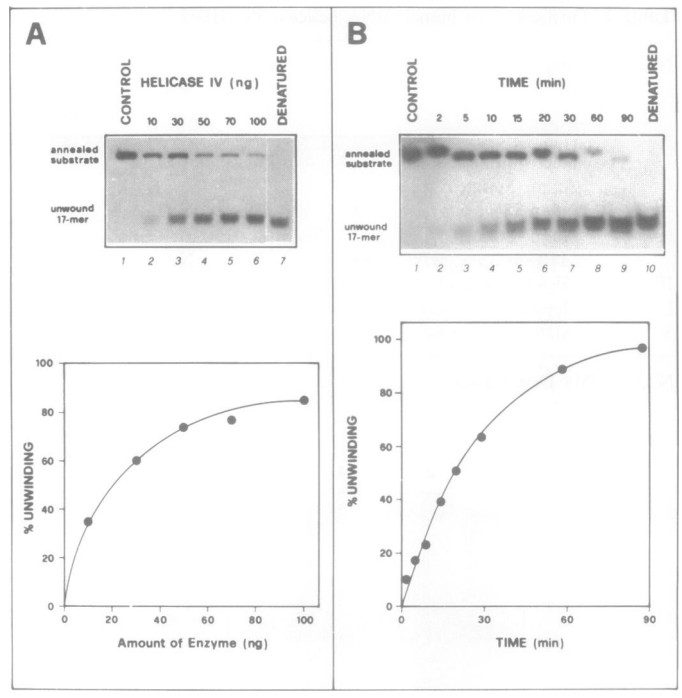

Figure 4. Titration (A) and Kinetics (B) of human DNA helicase IV. A, different amounts of DNA helicase (fraction V) were added in the unwinding reaction. The top panel shows the autoradiograph of the experiment. The amount of HDH IV is indicated at the top of each lane. Lanes 1 and 7 are the control reactions without enzyme and heat denatured substrate respectively. Bottom, the unwound DNA was quantitated and plotted as a function of the amount of HDH IV used. B, 50ng of HDH IV was used in each reaction. Top panel shows the autoradiograph of the experiment. Lane 1, standard reaction without enzyme. Lanes 2–9 are helicase reactions at different time intervals. The time of reaction is indicated on top of each lane. Lane 10 is a heat-denatured substrate. The bottom panel shows the quantitative data.

of nucleases, topoisomerases, DNA ligase or DNA nicking activities. DNA dependant ATPase activity was present at a level of 47 pmoles of ATP hydrolysed in 20 min by 50 ng of protein, which corresponds approximately to the splitting of one ATP molecule per unwound base pair.

Reaction requirements and characterization of HDH IV

Table 3 shows the reaction requirements of HDH IV. For maximum activity it required 3mM ATP (Fig. 3A), 0.5mM $MgCl_2$ (Fig. 3B) and 60mM KCl (Fig. 3C). The reaction required the hydrolysis of the gamma phosphate of ATP, since the non hydrolysable analog ATPγS was inactive as a cofactor. dATP supported 60% of the activity while other NTPs, as well as ADP and AMP were inactive. Mg^{2+}, Mn^{2+} and Zn^{2+} were almost equally active at 1mM concentrations but Ca^{2+}, Cu^{2+}, Ni^{2+} and Ag^{2+} were not active. KCl and NaCl at concentrations of 200mM, potassium phosphate at 100mM and EDTA at 5mM abolished the activity. Ammonium sulfate at 45mM reduced the activity to 15%. The helicase reaction was inhibited in presence of 20μM M13 ss DNA (as phosphate), while in presence of M13 RFI DNA (40μM), HeLa cell total RNA (40μM) and yeast tRNA (40μM) showed 60%, 30% and 75% residual activity. Trypsin (1 unit) or sarkosyl at 0.04% totally destroyed the enzyme activity. Spermidine (upto 0.1mM) had no effect on HDH IV. Poly [G] (20μM) showed inhibition of activity while poly [A], [C] and [U] at 20μM had no effect on helicase activity (Table 3). The optimum temperature for the helicase activity was 37°C. No detectable unwinding was observed at 0°C or when the enzyme fraction was preheated at 56°C for 5min. HDH IV had a broad pH range between pH 7.5 to 9.5 with an optimum near 9.0.

Titration of DNA helicase under optimal assay conditions showed a maximum value of unwinding at 85% with 100ng of the enzyme (Fig. 4A). In the presence of 50ng (2.5 units) of enzyme (Fig. 4B) the unwinding was linear up to 30 min. and saturated at 90 min.

DNA helicase activity on various substrates

Fig. 5 shows the HDH IV activity with different types of substrates. HDH IV can easily unwind a 17-mer duplex (Fig. 5A), even if it has a hanging tail at either the 5′ end, the 3′ end or both (Fig. 5D, E and F) but it cannot unwind a 25-mer or 32-mer duplex (Fig. 5B and C). HDH IV failed to unwind the blunt-ended 17-mer duplex DNA. Also, HDH IV cannot unwind the small linear substrate if this has little (84 bases or less) ss DNA free as shown in Fig. 5 H, I and J.

Direction of unwinding by HDH IV

In order to determine the direction of unwinding, two substrates were constructed with long linear ss DNA bearing short stretches

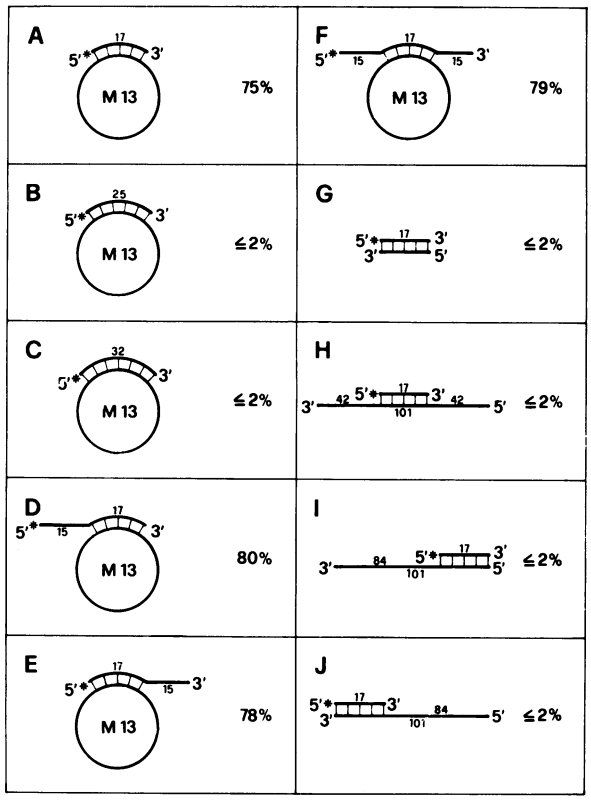

Figure 5. Helicase activity with various substrates. The helicase reaction was performed with 50ng of pure enzyme (fraction V) as described in the Materials and Methods. Each panel shows the structure of the substrate used and the% of unwinding. Asterisks denote the ^{32}P labeled end.

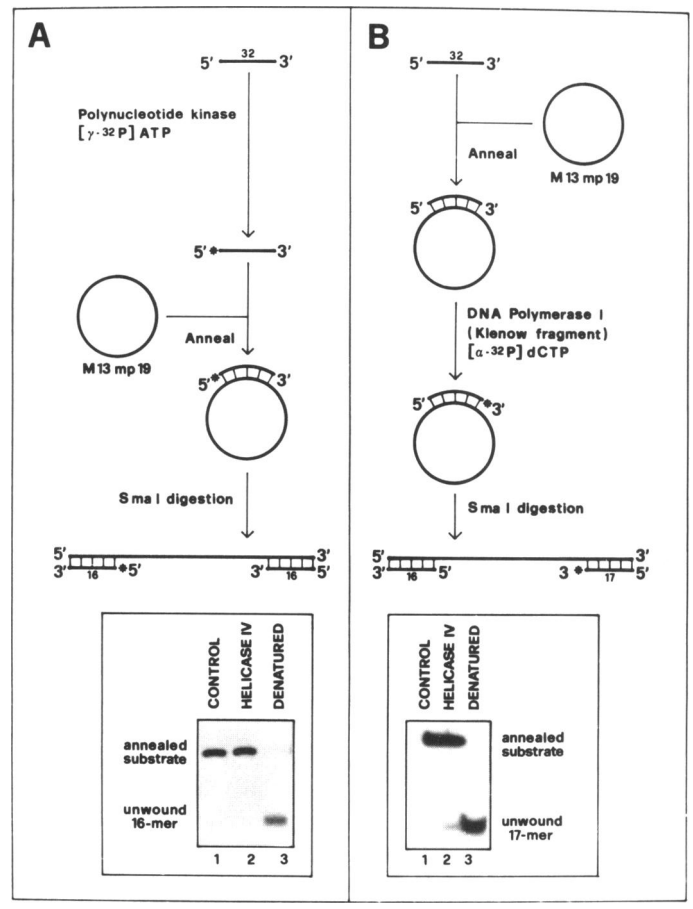

Figure 6. The direction of unwinding by HDH IV. Schematic representation of the construction of the linear substrates used to detect 3′ to 5′ (panel A) and 5′ to 3′ (panel B) unwinding directions is shown on the top. The bottom of each panel shows the autoradiograph of the gel. In each gel, lane 1 corresponds to a control (reaction without enzyme), lane 2 to a reaction with 50ng of pure HDH IV fraction and lane 3 with a heat-denatured substrate.

of duplex DNA at both ends as shown in Fig. 6A and B respectively. The results show that HDH IV moves unidirectionally in a 5′ to 3′ direction, along the DNA strand to which it binds, since it unwinds only the substrate with a duplex at the 3′ end of the molecule (Fig. 6B).

RNA-DNA unwinding by HDH IV

A RNA-DNA hybrid substrate consisting of 1Kb RNA annealed in the middle with a radioactively labeled 18-mer oligodeoxynucleotide was used with a 50 ng HDH IV fraction under the conditions used for the DNA helicase assay. The result shows that HDH IV can also unwind RNA-DNA hybrids (Fig. 7).

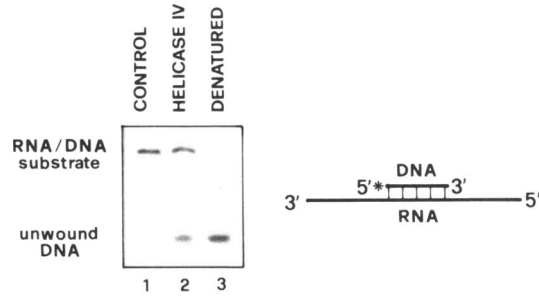

Figure 7. RNA-DNA unwinding by HDH IV. The structure of the substrate used to determine the unwinding of a RNA-DNA hybrid is shown on right side. Lane 1 is the control (reaction without enzyme). Lane 2 is the reaction with pure HDH IV fraction (50ng). Lane 3 is the heat denatured substrate.

DISCUSSION

Following the previously reported purification of DNA helicase I from human cells, as part of our systematic survey of the DNA helicases present in human cells, we have now brought our attention to a novel molecule that we previously defined as human DNA helicase IV (1). This enzyme, contrary to the helicases I, II and III, was found in the supernatant after 35% (w/v) ammonium sulfate precipitation. The decision to concentrate our effort on HDH IV after HDH I was due to the consideration of the obvious difference in the catalytic properties of the two enzymes: in fact, as mentioned in our previous paper (1) and as shown in the results herein reported, human DNA helicase IV moves with a polarity opposite to that of HDH I as well as that of HDH II and III. Like HDH I, HDH IV appears to be rather scarce in the nuclear extracts: from 440 g of cultured HeLa cells we could isolate only 0.31mg of homogeneous enzyme. The purified HDH IV has a molecular weight of 100kDa as compared with HDH I which is a molecule of 65kDa (1) and calf thymus DNA helicase, which has a native molecular weight of 47kDa

(13), the latter enzyme also moves with the same polarity (3′ to 5′) as HDH I.

HDH IV can utilize ATP or dATP for its unwinding activity like HDH I (1), whereas mouse helicase can use all NTP's (14), SV40 T-antigen also uses dTTP (4) and calf thymus helicase also uses CTP and dCTP (12). Mg^{2+} is essential for the activity of HDH IV and Mn^{2+} can also replace it as reported for SV40 T-antigen (4), calf thymus (13), mouse (14), *X.laevis* (15) and HDH I of HeLa cells (1). It is interesting to observe that HDH IV can also utilize Zn^{2+} instead of Mg^{2+} while HDH I cannot (1). On the other hand HDH IV cannot utilize Ca^{2+} while HDH I can do so (1). HDH IV activity is inhibited by 200mM KCl or NaCl like that of DNA helicase of calf thymus (13) and T-antigen (4), but unlike HeLa cell HDH I (1). HDH IV was completely inhibited by 100mM potassium phosphate whereas HDH I was not (1). Phosphate ions were also inhibitory to SV40 T-antigen (4) but not for *X.laevis* helicase (15). Ammonium sulfate at 45mM inhibits HDH IV activity as well as that of calf thymus (7) whereas HDH I of HeLa cells was not inhibited (1).

HDH IV unwinds only short DNA duplexes (17-mer/M13), and cannot unwind a 25-mer or longer. It does not need a fork-like structure for its unwinding activity, being in this sense similar to HDH I (1), and different from the DNA helicase from herpes simplex virus type I (HSV-I) which needs a 3′ single-stranded tail on its duplex DNA substrate (16). HDH IV can not unwind a 17bp blunt-ended duplex DNA, nor can it unwind a duplex having up to 84 bases of ss DNA on either side. This indicates that, in order to perform its unwinding action HDH IV needs more than 84 bases of ss DNA to sit on and then move along the strand. The polarity of movement of HDH IV is 5′ to 3′ along the ss DNA to which it binds, that is opposite to that of HDH I of HeLa cells (1), SV40 T-antigen (3), Polyoma T-antigen (5) and calf thymus helicase (13).

It is worthwhile to point out that the HDH IV probably corresponds to the DNA-dependant ATPase described by our group (17–19) which had an apparent native molecular weight of approximately 100 kDa and showed a limited unwinding capacity with a 5′ to 3′ polarity, whereas HDH I (1) probably corresponds to the 68 kDa DNA-dependent ATPase described later (20).

As already commented in a previous publication (1), all prokaryotic DNA helicases which are known to be involved in growing fork advancement and origin activation move with the same 5′ to 3′ polarity with respect to the strand to which they are bound (21), i.e. like HDH IV. On the other hand the SV40 (3) and Polyoma T antigen (5), which are proven to be essential for origin activation in these mammalian viruses, as well as for fork advancement, both move with a 3′ to 5′ polarity. Only future work will tell whether either of the two enzymes described by us is involved in DNA replication, whether at the origin, the growing fork, or both.

HDH IV also unwinds a RNA-DNA hybrid consisting of a short DNA (18-mer) annealed to a long RNA. The unwinding of RNA-DNA hybrids has also been reported for HDH I of HeLa cells (1), human p68 protein (22), *E.coli* Helicase II (23), *E.coli* Rho protein (24) and SV40 T-antigen (25). HDH IV may also conceiveably play a role in RNA metabolism. Further studies of the different human DNA helicases and their genes will attempt at assign them a function in the different facets of nucleic acid metabolism of human cells.

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