Nucleic Acids Research

Nonhistone proteins HMG$_1$ and HMG$_2$ unwind DNA double helix

Kashayar Javaherian and Mohamad Sadeghi
Institute of Biochemistry and Biophysics, University of Tehran, P.O. Box 314-1700, Tehran, Iran

Leroy F.Liu
Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138, USA

ABSTRACT

In a previous communication we have shown that both HMG$_1$ and HMG$_2$ non-histone proteins change the DNA helical structure and the binding of HMG$_1$ and HMG$_2$ to DNA induces a net unwinding equivalent of DNA double helix (Javaherian, K., Liu, L. F. and Wang, J. C. (1978) Science, 199, 1345-1346). Employing melting absorption technique, we now show that in the presence of salt HMG$_1$ and HMG$_2$ destabilize DNA whereas in the absence of salt, they both stabilize DNA molecules. Consequently the folded structure of HMG must play an important role in melting DNA. Furthermore, by measuring topological winding number using competition unwinding experiments, we conclude that HMG$_1$ has a higher affinity for a single-stranded DNA relative to double-stranded DNA. These results together suggest that HMG$_1$ and HMG$_2$ unwind DNA double helix by local denaturation of the DNA base pairs. The net unwinding angles have been measured to be 22° and 26° per molecule of HMG$_1$ and HMG$_2$ respectively.

INTRODUCTION

Extraction of calf thymus chromatin with 0.35 M Nacl results in separation of a number of nonhistone proteins. A group of these proteins have been termed HMG (High Mobility Group) by Johns and his collaborators (1). It now appears that there are four main proteins in HMG nonhistones, designated HMG$_1$, HMG$_2$, HMG$_{14}$ and HMG$_{17}$. They almost have equal amounts of acidic and basic amino acids and their molecular weights range from 9000-25000 (2, 3, 4, 5).

At the present time, the physiological roles of these HMG proteins are not known. However, two separate observations have been made which may have some significance with regards to their structural roles. It was found that pancreatic DNase 1 digestion of trout testis chromatin released the protein H$_6$ which is very similar to HMG$_{17}$. Staphylococcal nuclease digestion, on the other hand, led to the preferential solubilization of HMG-T which is similar to HMG$_1$ and HMG$_2$ in calf thymus (6). In another line of experiments,

we recently demonstrated that these proteins, when bound to an open circular DNA, resulted in a lower linking number upon closure of the DNA than is obtained upon closure of the circle in the absence of the proteins. The results were explained by either supercoiling or unwinding of DNA, induced by HMG_1 and HMG_2 proteins (7).

We now report experiments which clearly show that HMG_1 and HMG_2 are melting proteins and they unwind DNA molecule. To demonstrate this property, we have employed two different techniques. Through melting absorption technique, we have monitored the absorption of DNA-HMG complexes as a function of temperature. This method has been used in the past for investigating the unwinding proteins (8,9). We have also measured the changes in linking number of circular DNA introduced by HMG_1 and HMG_2 using nicked PM_2 DNA as substrate and single or double-stranded T7 DNA as competitor in the reaction mixture.

MATERIALS AND METHODS

HMG_1 and HMG_2 nonhistone proteins were isolated from calf thymus according to the procedure of Goodwin et al. (10). T7 DNA was a gift from Dr. T. Kovacic at Harvard University. PolydAt was purchased from Sigma. For melting absorption experiments, HMG and DNA were dialyzed separately against the proper buffer before being mixed together. A Gilford 2400-2 was used for thermal denaturation measurement. Temperature scanning rate was 1°C/min. The instrument was connected to a small computer which would convert the linear absorption data into a derivative melting curve and plot the data. DNA concentration was determined by using $E^{260}_{mg/ml} = 20$. Protein concentration was calculated by using $E^{280}_{mg/ml} = 0.82$.

For measuring linking number of circular DNA, nicked PM_2 DNA and HMG_1 protein were first mixed in a fixed ratio. Increasing amounts of double-stranded T7 DNA were then added to a series of samples containing nicked DNA and HMG_1. In a parallel experiment, single-stranded T7 DNA (by heating T7 DNA in low salt at 90°C for five minutes and cooling rapidly) was used instead of double-stranded T7 DNA. Following the procedure described before (7), PM_2 DNA was ligated and subjected to electrophoresis.

RESULTS

Thermal Denaturation of T7 DNA-HMG_1:

During the course of our investigation using melting absorption method, we found out that the presence of sodium chloride in the medium has

a pronounced effect in the absorption derivative curve of DNA-HMG
complexes. In Fig (1), the results are shown for DNA-HMG$_1$ and DNA-HMG$_2$
complexes in the absence of salt. HMG$_1$ and HMG$_2$ stablize DNA, giving rise
to two peaks; the peak at 60°C is due to free DNA and the peak at higher
temperature corresponds to the complex. Addition of HMG$_1$ causes the Tm of
free DNA to shift to a higher temperature. The difference between Tm of
DNA and DNA-HMG complex is approximately 12°C. In the case of HMG$_2$ (Fig 1b),
both Tms show shifting toward higher temperature as more protein is added.
The above data are in general agreement with those of Yu et al. (11). One
can conclude that under these conditions T7 DNA is being stabilized by
HMG$_1$ and HMG$_2$.

In the presence of salt, melting behavior is quite different. In Fig (2),
the results are plotted for DNA-HMG$_1$ in the presence of sodium chloride. In
this case DNA is being destabilized by addition of HMG$_1$ molecule. Increasing
salt concentration appears to facilitate DNA melting by HMG$_1$. As sodium
chloride concentration changes from 0.025 to 0.075 M, a number of differences
take place. In the case of 0.025 M the destablization is observed only when
that ratio of protein to DNA is 2 (Fig. 2A). Further increase of salt makes
the denatured section of the melting absorption profile more pronounced.
At 0.075 M NaCl, the first Tm is approximately 27°C below that of control
DNA. Increasing protein concentration tends to decrease the lower Tm. One
should keep in mind that the protein denaturation by itself brings some
change in the absorption and this phenomenon explains the large area obtained
in the first part of the melting curve (the data have not been corrected
for protein denaturation). Negative derivatives shown in Fig(2) are the
result of downward curves of the protein denaturation, consistent with
earlier observations on DNA-Ribonuclease system (12).

It can be demonstrated that the first peak in Fig 2C is reversible
by simply cooling the sample gradually and monitoring absorption as a
function of temperature (data not shown). At higher ratios of HMG$_1$ to DNA
(larger than 2) the scattering increases considerably, making the analysis
more difficult. The results for DNA-HMG$_2$ are very similar to DNA-HMG$_1$.

PolydAt has been used in the past for observing the effect of the
unwinding proteins due to its low melting termperature (8, 9, 12). The data
for polydAt-HMG$_1$ are shown in Fig(3). In the absence of salt, stabilization
is noticed whereas in the presence of salt polydAT is being destabilized.

Measurements of Linking Number of Circular DNA:

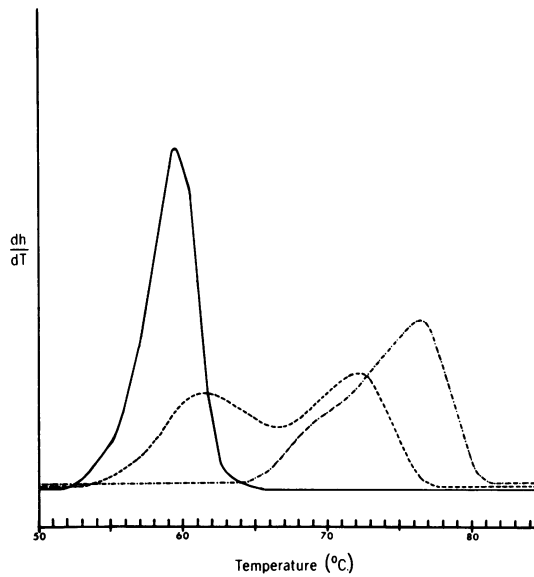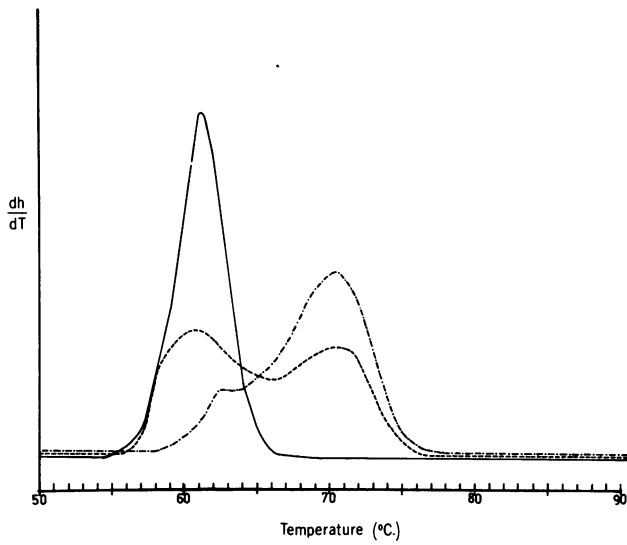In Fig 4A we have presented the results of HMG$_1$ binding to circular

Fig 1. Thermal denaturation of T7 DNA-HMG complexes in the absence of salt.
(A) T7 DNA-HMG$_1$ complex, (B) T7 DNA-HMG$_2$ complex. The buffer is 2.5 x 10^{-4}M
EDTA, 10^{-3}M Dithiotreitol, PH = 8.0. All ratios are by weights. (————) T7
DNA control, (-----) ratio of HMG to DNA = 1, (-.-.-.-.) ratio of HMG to
DNA = 2.

DNA in the presence of linear double-stranded or single-stranded T7 DNA. Two
control experiments, one without HMG$_1$ and the other with HMG$_1$ but without
competitive T7 DNA are shown in Fig 4A (a) and (b) respectively. In view of
the fact that HMG proteins bind to DNA nonspecifically (2, 3, 4), the
competition experiments in Fig 4A demonstrate that both double-stranded and
single-stranded T7 DNA bind some of HMG$_1$ which is already present on nicked
PM$_2$ DNA. However, the single-stranded T7 DNA causes a more drastic change in
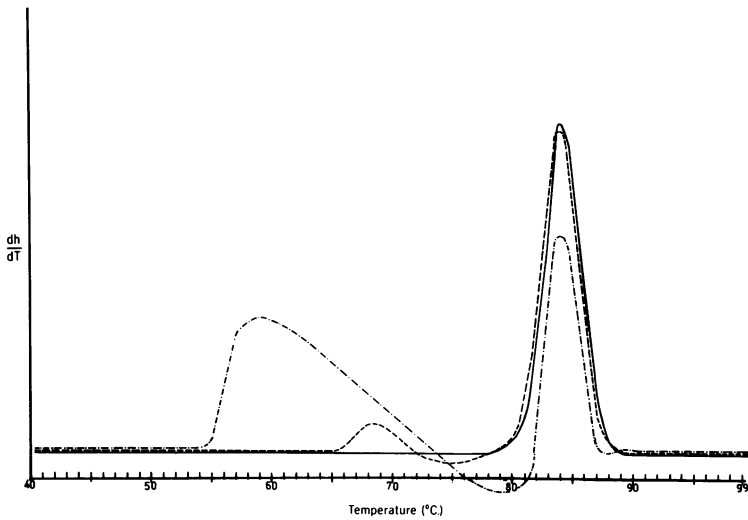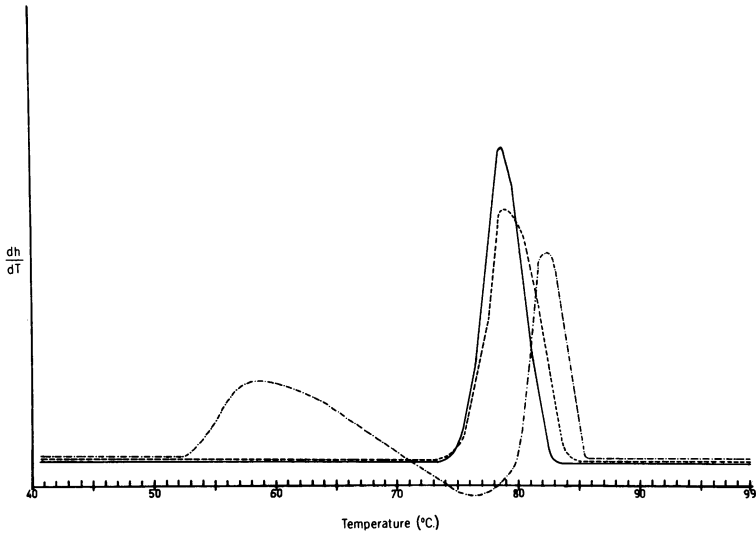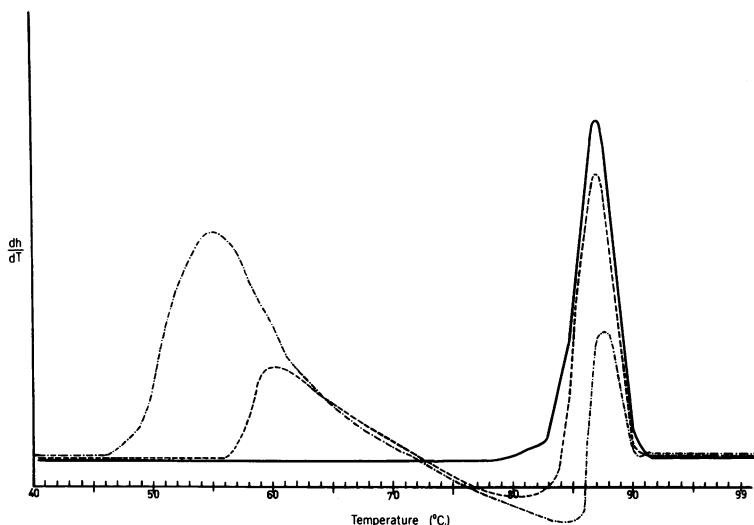
Fig 2. Thermal denaturation of DNA-HMG complex in the presence of salt. The buffer is 10^{-3}M Tris-HCl, 2.5×10^{-4}M EDTA, 10^{-3}M Dithiotreitol, PH = 8 and varying amounts of NaCl. (A) 0.025M NaCl. (B) 0.05M NaCl. (C) 0.075M NaCl. The curves are being described by the same symbols as in Fig 1.

linking number implying that HMG_1 has higher affinity for single-stranded DNA than double-stranded DNA. The results of Fig 4A is also plotted and shown in Fig 4B. $\Delta\bar{\alpha}$ represents the change in linking number of the DNA.

As an extension of our earlier work (7), we can now determine the unwinding angles introduced by HMG_1 and HMG_2. Increasing amounts of HMG_1 and HMG_2 proteins were added to nicked PM_2 DNA. After ligation by E. coli ligase, the products were examined by gel electrophoresis. The results are shown in Fig 5A ((a) - (m)). The changes in linking number is plotted and shown in Fig 5B. The curves are linear indicating that the unwinding is noncooperative. Since a change of one unit in α corresponds to $360°$, one can estimate the unwinding angles introduced by HMG_1 or HMG_2. The values are $22°$ and $26°$ per molecule of HMG_1 and HMG_2 respectively using the molecular weight given before (7), and assuming that all the protein molecules are bound to DNA (3).

We have also looked into the question of reversible binding of HMG proteins to DNA. Two different samples were prepared. In one sample, HMG_1 and HMG_2 were added to DNA as usual. In the other sample, half of the DNA was added after HMG protein was introduced into the sample. Both
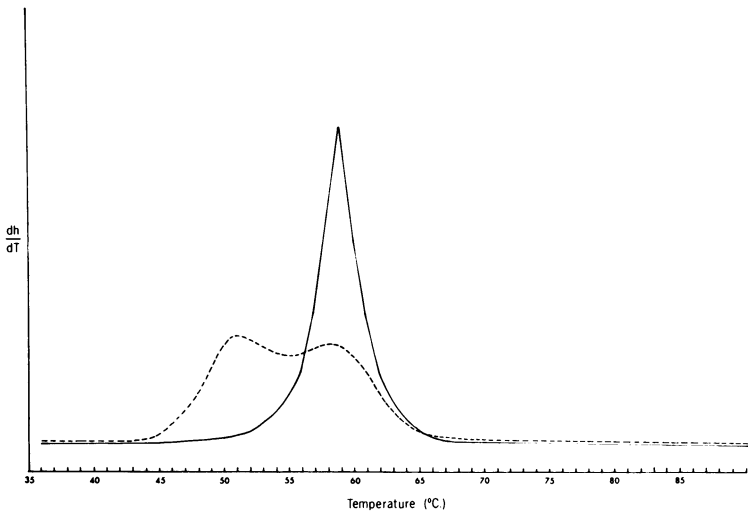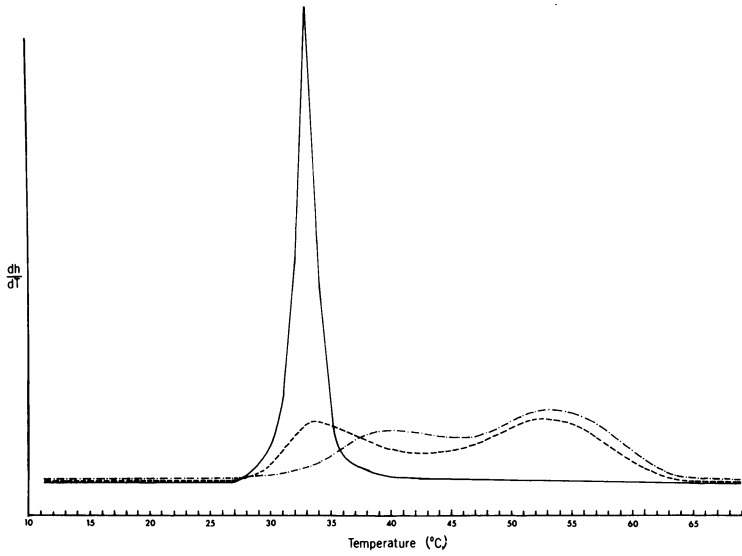
Fig 3. Melting absorption of Poly dAT-HMG$_1$ complex (A) no salt, (B) 0.05M
NaCl. The buffer is the same as in Fig 2. The curves are described as in
Fig (1) and Fig (2) except the Poly dAT has replaced T7 DNA.

samples had equal amounts of DNA and HMG at the end. The data in Fig 5
(slots o-r) show that the change in linking number is approximately the
same in both cases. It therefore suggests that HMG proteins bind to DNA

reversibly consistent with the previous reports (2).

DISCUSSION

In 1963, Felsenfeld et al. (12) observed that bovine pancreatic rib-
onuclease can destabilize the native DNA conformation. Few years later a
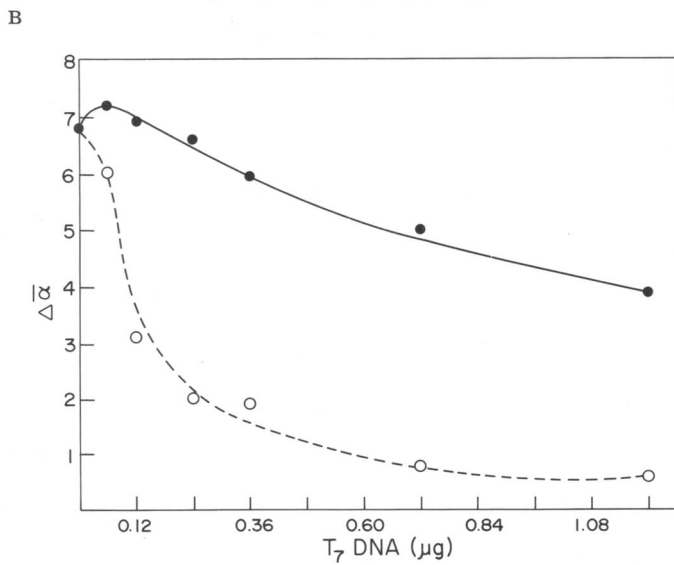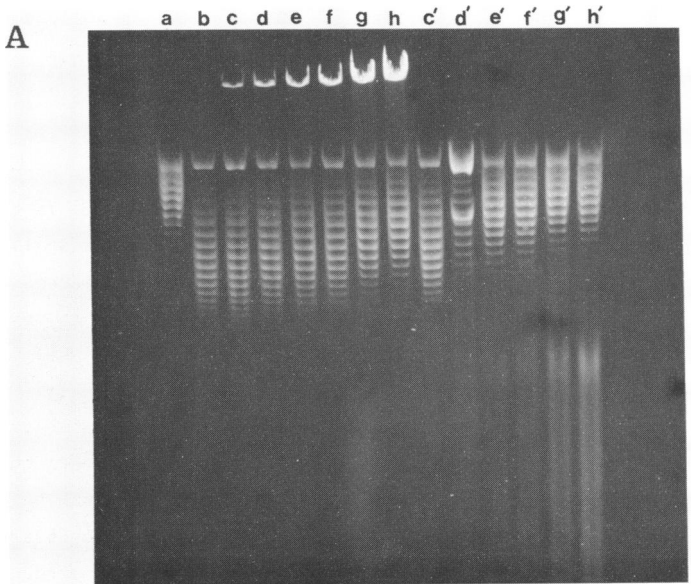
series of HD proteins (helix destabilizing proteins) were isolated from prokaryotic and eukaryotic systems and were shown to be involved in DNA replication (8, 9). HMG_1 and HMG_2 proteins share a number of unwinding characteristics with the above proteins. However, they have certain novel features which distinguish them from ribonuclease and HD proteins. Our preliminary observation on melting behavior of DNA-HMG_1 complex revealed destabilization (15) of DNA molecule by HMG_1 protein. Later measurements of linking number of circular DNA in the presence of HMG_1 and HMG_2 led us to believe that these proteins were very likely unwinding proteins (7). In this report, we present evidence which demonstrates that the proteins unwind DNA molecules. There are two modes of interactions between the proteins and DNA. In the absence of salt HMG_1 and HMG_2 proteins are not in their native conformations and behave as polycations which in turn stabilize DNA. In the presence of salt, the HMG proteins are highly structured and can cause destabilization. However, raising the temperature gives rise to denaturation of the protein molecule, changing the protein into a polycation, resulting in DNA stabilization. The situation is analogous to RNase (12, 16). This phenomenon is observed in Fig 2A where stabilization follows destabilization due to the protein denaturation. At higher salt concentration (Fig 2B and 2C), the polycation property exerts little influence on the denaturation of DNA at high temperature. It is interesting to observe that in the case polydAT due to the fact that the protein does not denature, a simple biphasic pattern is obtained (Fig 3B).

It seems plausible to assume that the globular structure of HMG proteins must play an important role in the destabilization process. Both HMG_1 and

Fig 4. Binding affinity of HMG_1 protein to double-stranded and single-stranded DNA. In Fig 4A, gel electrophoresis results of nicked PM_2 and HMG_1 in the presence of increasing amounts of T7 DNA are shown. Each sample (86 µl) contained 0.4 µg of nicked PM_2. The reaction mixture was 20 mM Tris, PH = 8, 3 mM $MgCl_2$, 50 µg of bovine serum albumin per millimeter, 33 µg of NAD per millimeter, 0.5 mM dithiotreitol. After addition of T7 DNA, ligation and electrophoresis were carried out as described before (7). (a) no HMG_1 (b) 0.32 µg of HMG_1 protein. (c) through (h) are the same as (b) except T7 DNA has been added. (c) 0.06 µg T7 DNA, (d) 0.12 µg, (e) 0.24 µg (f) 0.36 µg (g) 0.72 µg (h) 1.2 µg. Single-strand results are shown in slots (c)'' - (h)'. Slots (c)' - (h)' correspond exactly to (c) - (h) respectively except single-stranded DNA has been added instead of double-stranded DNA.

In Fig 4B, results of Fig 4A are plotted. α signifies the average change in linking number. (———) double-stranded T7 DNA, (-----) single-stranded T7 DNA.
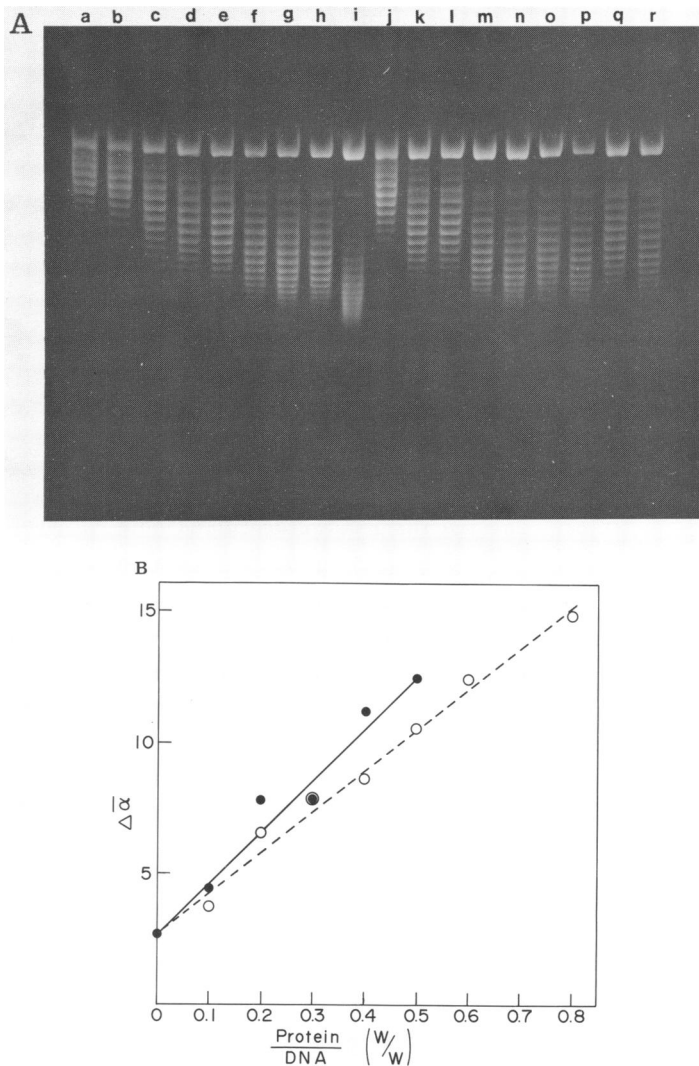
Fig. 5. Changes in linking number of circular DNA as a function of increasing amounts of HMG_1 and HMG_2 proteins. The reaction mixture was the same as in Fig 4 except the Tris concentration was 30 mM. Each sample (58µl) contained 0.5 µg nicked PM_2 DNA. In Fig 5A the electrophoresis results are shown. (a) no HMG (b) 0.05 µg HMG_1 ($\frac{HMG1}{DNA}$ $(\frac{w}{w})$ = 0.1). (c) 0.1 µg (d) 0.15 µg (e) 0.2 µg (f) 0.25 µg (g) 0.3 µg (h) 0.35 µg (i) 0.4 µg. Results for HMG_2 protein are shown in slots (J)-(n). (J) 0.05 µg HMG_2, (k) 0.1 µg, (l) 0.15 µg, (m) 0.2 µg, (n) 0.25 µg. Slots (0)-(r) are related to reversible binding of HMG_1 and HMG_2 proteins to DNA. (0) the same as (g) except half of DNA was added after HMG_1 addition to DNA. (p) the same as (g). (q) the same as (l) except half of DNA was added after HMG_2 addition. (r) the same as (l).

HMG_2 are known to have approximately 45%α-helix at neutral PH under moderate salt conditions (13, 14). The absence of salt appears to have a small effect on α-helix content of HMG_1 and HMG_2 (13). However, the structure of HMG_1 as probed by circular dichroism in near ultraviolet region shows a conformational change as a result of salt introduction into the medium (14). HMG_1 and HMG_2 proteins bind to DNA noncooperatively similar to RNase. This property is not shared by HD proteins of Alberts and coworkers as described by Jensen and Von Hippel (16). Furthermore, like ribonuclease, HMG_1 and HMG_2 do not change the circular dichroism spectrum of DNA in near ultraviolet (14).

Recently, by using sequential chromatography on double-stranded DNA and single-stranded DNA columns, it was shown that two proteins from HTC cells were bound preferably to single-stranded DNA. A number of criteria established them similar to HMG_1 and HMG_2 of calf thymus (17).

ACKNOWLEDGEMENTS

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