Involvement of protein HMG1 in DNA replication

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Antibodies against HMG1 inhibit the incorporation of [^{3}H]thymidine in Ehrlich ascites cell nuclei. By the use of specific inhibitors it is shown that HMG1 is needed for the action of the replicative DNA polymerase and not for the reparative one. This is supported by the fact that the addition of exogenous HMG1 to the nuclei enhances the replication process.

High mobility group protein 1 *Antibody* *DNA replication* *Ehrlich ascites cell nucleus*

1. INTRODUCTION

The large high mobility group proteins HMG1 and HMG2 have long been suggested to take part in DNA replication. The reason for this is their increased amount in proliferating tissues [1] and the unwinding of the DNA double helix [2] which, similarly to the unwinding proteins from prokaryotes [3], may be necessary for the replication process. However, the opposite case has also been presented. HMG1 and 2 have been found in non-dividing chicken erythrocytes [4]. The reduction of the linking number of DNA may be explained by the observed supercoiling of DNA round these proteins to form nucleosome-like structures [5]. Recently we have shown that HMG1 and 2 cannot decrease the melting temperature of DNA [6].

2. MATERIALS AND METHODS

HMG1 was isolated in non-denaturing conditions as in [7]. Antisera against HMG1 were elicited in male white rabbits by injecting in multiple intradermal sites a total of 500 μg protein dissolved in 500 μl of 0.01 M Tris–HCl buffer (pH 8.0) and emulsified with an equal volume of Freund's adjuvant. The same dosage and procedure were repeated 28 and 42 days later and a booster was given 14 days after the third stimulus with 200 μg antigen. Bleedings were taken 8 and 15 days after the booster. Pure antibodies were affinity purified as follows: 14 mg chromatographically pure HMG1 were immobilised on 12 ml (wet) carboxymethylcellulose (Schleicher & Schull) with the water soluble *N*-cyclohexyl-*N'*-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate essentially as in [8]. Absorption of the antibodies from the serum, washing on a 1.5 × 6 cm column and elution of anti-HMG1 antibody were done exactly as in [9].

Immunodiffusion was performed on glass petri dishes containing 1% agarose. Plates were incubated for 8 h at room temperature and 48 h at 4°C. Enzyme-linked immunosorbent assay was performed according to [10].

Nuclei were isolated from Ehrlich ascites cells 7 days after tumour transplantation as in [11]. 0.5% Triton X-100 was included in the final wash to increase the permeability of the nuclear membrane to the antibodies. The nuclei were suspended in an incubation mixture for testing the DNA polymerase activity [12], containing 0.1 M NaCl, 0.03 M Hepes, pH 7.65, 0.008 M $MgCl_2$, 0.1 mM each of dATP, dGTP, dCTP, 0.5 mM ATP. After the ad-

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Abbreviations: HMG, high mobility group; NEM, *N*-ethylmaleimide; ddTTP, 2',3'-dideoxythymidine-5'-triphosphate

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dition of [^{3}H]TTP (Amersham, 40 Ci/mmol) to a final concentration of 0.001 mM the reaction mixture was kept at 37°C and aliquots were taken at different time intervals. The acid-insoluble radioactivity was determined after three washes of the nuclear pellet in 0.6 M CCl_3COOH, 0.01 M $Na_4P_2O_7$.

The activities of DNA polymerases α and β were separately determined by the use of the selective inhibitors NEM (0.5 mM) or ddTTP (0.05 mM) in the incubation mixture.

Anti-HMG1 antibody was added at 1.5 molar excess relative to HMG1 (assuming a HMG1/total histone ratio of 1:50). The nuclei were preincubated with the antibodies for 30 min at 25°C in 0.01 M NaCl, 0.005 M Hepes, pH 7.65; then the solution was brought to the concentrations necessary for the polymerase reaction and the activities of the polymerases were determined. Exogenous HMG1 from calf thymus was added and preincubated in the same way in a parallel experiment.

3. RESULTS AND DISCUSSION

For the purpose of antibody purification several methods for the immobilization of HMG1 were attempted. However, neither binding to CNBr-activated Sepharose [9], nor binding to an *N,N'*-carbonyldiimidazole activated agarose matrix [13] gave satisfactory yields. We were able to bind a significant (~80%) amount of the protein to a support by the method of [8]. The column thus prepared completely retained about 3 mg antibody from 25 ml serum; the yield was 0.12 mg/ml serum.

The antibody thus obtained gave a single precipitin band when tested against HMG1 (fig.1). The enzyme-linked immunosorbent assay did not reveal any cross-reactivity against histone H1 and about 60% against HMG2 (serum dilution 1:500).

The antibodies against calf thymus HMG1 were used in an in vitro replication system based on mouse Ehrlich ascites cell nuclei. While mouse and calf HMG1 have been shown to be immunologically and structurally related [14] this reduced the possibility that the observed effects could have been caused by antibodies against some undetectable contaminants in the antigen.

Fig.2 shows the curves of incorporation of labeled thymidine in the nuclei. In freshly isolated nuclei most of the polymerase activity is inhibited by NEM, i.e., it is the replicative (alpha) polymerase that is active. One should also note that the sum of the separate α and β polymerase activities

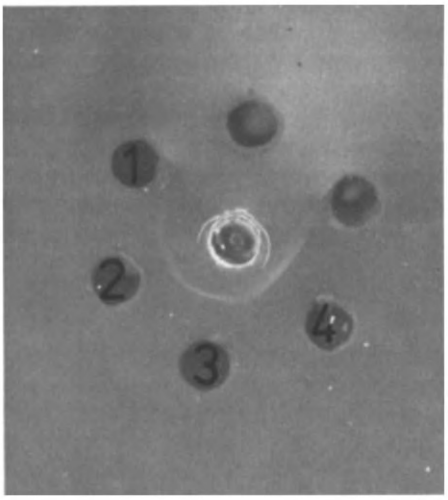

Fig.1. Immunodiffusion with purified anti-HMG1 (center well) – 30 μg in 20 μl. Wells 1–4 contain 5, 10, 15 and 30 μg pure HMG1.

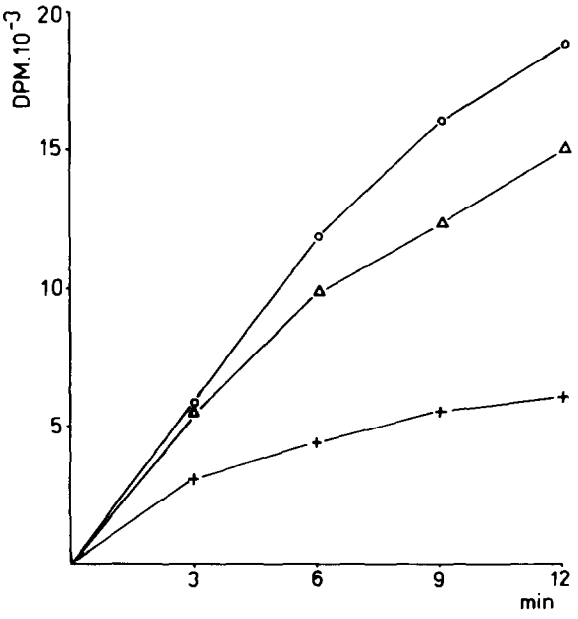

Fig.2. Incorporation of [^{3}H]TTP in freshly isolated nuclei. (—○—) Without inhibitors; (—+—) with 0.5 mM NEM; (—△—) with 0.05 mM ddTTP.

exceeds the control. This may be explained by the incomplete efficiency of inhibition.

In the presence of the anti-HMG1 antibody the replication process was significantly inhibited. The effect could not be due to some unspecific action of the IgG molecule; IgG isolated from the animals before the immunization did not produce any effect (fig.3).

It was interesting to determine which polymerase activity was enhanced by HMG1. For this purpose the reaction with the antibodies was repeated in the presence of NEM or ddTTP. The results are also presented on fig.3. The combined action of NEM and the antibody did not bring about a further decrease in the values of the acid-insoluble radioactivity compared to the cases of NEM or anti-HMG1 alone. This could be if the target of biological action of HMG1 is the function of the replicative polymerase. This was further supported by the fact that the lowest values of incorporation were observed when HMG1 was blocked by the antibody and the inhibitor of the reparative polymerase was present.

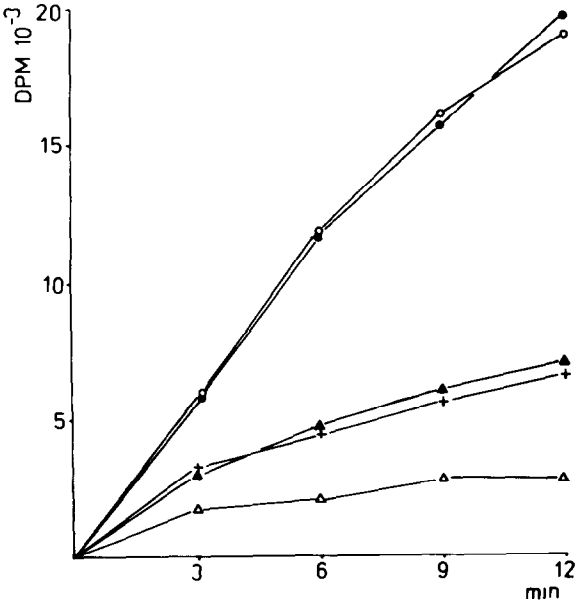

Fig.3. Incorporation of [^{3}H]TTP in nuclei. (—●—) Without inhibitors; (—○—) with non-immune serum; (—▲—) with anti-HMG1; (—+—) with anti-HMG1 and NEM; (—△—) with anti-HMG1 and ddTTP.

Two additional experiments were performed to rule out the possibility that the inhibitory effect was due to simple steric hindrance by the bound antibody. First, the addition of anti-histone H2a antibody did not produce any effect on the system. Second, the addition of exogenous HMG1 from calf thymus to the replication system already containing the anti-HMG1 antibody accelerated the rate of incorporation 1.5-fold.

These data clearly suggest that HMG1 is involved in the process of DNA replication. However, it is not possible to specify exactly the site of its action. Two possibilities seem most likely: (i) HMG1 (and 2) have been shown to enhance the reconstitution process and the proper deposition of histones along DNA [15]. While in isolated nuclei there is no supply of newly synthesized histones and this seems unlikely, HMG1 (or 2) may equally well act in the reverse way – to complex histones and remove them, so that DNA polymerase can pass along DNA. (ii) Due to their higher affinity to single stranded DNA they may bind to such regions in the vicinity of the replication or bind to origins of replication and enhance the initiation process. We feel that at present there is no experimental basis to speculate further about this and more data are needed to elucidate the problem.

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