Volume 336, number 2, 340–342 FEBS 13433 December 1993

Identification of DNA binding proteins in vaccinia virus by DNA–protein crosslinking

A.N. Chkheidze[a], G.G. Prikhod'ko[b], V.L. Karpov[a], S.K. Vassilenko[b], A.D. Mirzabekov[a,*]

[a]*W.A. Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Vavilov St. 32, Moscow 117984, Russian Federation*
[b]*All-Union Institute of Molecular Biology, Scientific-Industrial Association 'Vector', Koltzovo, Novosibirsk Region 633159, Russian Federation*

Received 4 November 1993

DNA binding proteins of vaccinia virus (VV) virions, strain LIVP, have been studied by their covalent crosslinking to DNA, using two-dimensional gel retardation electrophoresis of crosslinked DNA and proteins as well as the 'protein image' hybridization assay. Five proteins with molecular masses of 16, 25, 27, 41 and 54 kDa, respectively, associated with all analysed DNA sequences, including early and late genes and their promoters, have been identified.

Vaccinia virus (VV); Strain LIVP; DNA binding protein; DNA–protein crosslinking; Dimethyl sulfate (DMS)

1. INTRODUCTION

DNA in eukaryotic nuclei is compacted by histones [1]. DNA of some viruses, such as SV 40, is also organized with host histones as 'minichromosomes' [2].

If the mature virion core, vaccinia virus (VV) DNA is covered by virus-specific structural proteins rather than with cell histones. It was shown earlier that there are at least four proteins in the virus nucleoprotein complex [3], moreover one of these protein is similar to histone H1 with respect to its DNA-binding properties [4].

Several methods were used to study the rather complicated structural organization of VV. These methods are mostly based on the controlled disruption of purified VV, first described by Easterbrook [5]. However, the use of ionic or non-ionic detergents and strong reducing agents in these methods could interfere with DNA–protein interactions, and consequently cause some re-distribution of proteins on DNA and the loss of some proteins.

To identify virus proteins bound to DNA in VV virions we applied another approach based on fixation of DNA–protein contacts directly in the whole virus by their chemical crosslinking [6] and measuring the protein size by 'protein image' hybridization [7].

2. EXPERIMENTAL

VV virions, strain LIVP, were grown in suspension cultures of BHK-21 with multiplicity of 30 PFU/cell and isolated from infected cells by centrifugation in sucrose density gradient [8]. Purified virus particles were suspended in 30 mM potassium phosphate buffer (pH 7.6) and used for chemical fixation of DNA–protein contacts. The conditions for crosslinking virus proteins to partially depurinated DNA after DNA methylation were as described earlier for histones [9]. VV DNA methylation was performed in 3 mM dimethyl sulphate (DMS).

VV virions were lysed by adding EDTA to 1 mM, NP-40 to 0.1%, lauryl sarcosinate to 2%, and sonicated four times at 44 kHz for 15 s. The removal of most of the uncrosslinked proteins by centrifugation in pre-formed CsCl gradients [10] and phenol enrichment [9], two-dimensional gel retardation electrophoresis of the protein–DNA complexes to obtain 'protein images' [9], as well as DNA labeling, hybridization and filter washing [11], were carried out as described earlier.

The molecular masses M_r of proteins crosslinked to DNA were measured by two-dimensional gel electrophoresis of crosslinked proteins [12,13] following their labeling with ^{125}I by Bolton-Hunter Reagent [14]. Taking into account the possibility that DNA–protein complexes could be formed within a wide range of M_r we used a 5–15% linear gradient of acrylamide in the resolving gel of the first dimension. In the second dimension, 12.5% polyacrylamide was used. The M_r standards from a Sigma standard kit (MW-SDS-70) were loaded near to the polymerized strip onto the second dimension gel. After the second dimension run, the gel was fixed in 10% TCA, dried and autoradiographed.

All the procedures described above, with the exception of electrophoresis, were carried out in the presence of 0.5 mM diisopropylfluorophosphate (DFP).

3. RESULTS

An apparent M_r of proteins crosslinked to different VV DNA regions was determined by two-dimensional diagonal gel retardation electrophoresis. After electrophoresis of DNA–protein crosslinked complexes in the first direction, DNA was released from protein by their digestion, and electrophoresed in the second direction. Then DNA was transferred onto a Hybond membrane

*Corresponding author. Fax: (7) (095) 135 1405.

 Published by Elsevier Science Publishers B.V.

and hybridized with different probes ('protein image' hybridization) [7,9].

The hybridization with total nick-translated virus DNA in Fig. 1 revealed the crosslinked proteins in the VV core. As well as the uncrosslinked DNA diagonal 1, some additional diagonals were observed which corresponded to DNA bound to the proteins with approximate M_r's of 15, 30, 40 and 50 kDa (diagonals 2, 3, 4 and 5, respectively). The gel was calibrated by electrophoresis of DNA-crosslinked standard proteins of known size [15]: the σ-subunit of RNA polymerase from *E. coli* (90 kDa), bovine serum albumin (67 kDa), histones H1 (37 kDa) and core histones (14 kDa). The protein image pattern did not depend on the applied method of crosslinked complex reduction that attached proteins to DNA either through lysine (sodium borohydride) or through histidine residues (sodium cyanoborohydride) [9] (data not shown).

In order to compare the protein content of functionally different regions of early and late genes, the same filter was successively hybridized with various cloned probes (Fig. 2). The clones were obtained from *Hin*dIII-F (nomenclature given in [16]) genome fragments of VV DNA and corresponded to the promoter and structural regions of the early genes, F1, F2, F4, and the late genes, FE1, F5. Fig. 2 shows the protein image hybridizations for F1 and F5 only. The number of diagonals, their approximate intensity and disposition appear to be very similar upon hybridization of the blot with total genome DNA, *Hin*dIII-F fragment (not shown) or its subfragments (compare Fig. 1 and Fig. 2). Therefore, at least four polypeptides with M_r's of approximately 15, 30, 40 and 50 kDa, correspondingly, are bound to DNA of VV, including the promoters and structural regions of early and late genes. The hybridization pattern was the same upon successive hybridizations of the filter with either of the two complementary strands of the DNA probes (data not shown) which suggests that the proteins bind similarly with the two viral DNA strands.

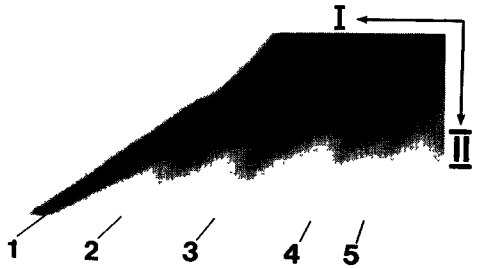

Fig. 1. 'Protein image' hybridization with nick-translated VV total DNA probe. Electrophoresis of DNA–protein crosslinked complexes was carried out in the 1st dimension from right to left, and of released DNA in the 2nd dimension from top to bottom. The gels of the first and second dimension contained 7% acrylamide. Diagonal lines under the autoradiographs indicate positions of uncrosslinked DNA-1, and protein image diagonals of crosslinked proteins having approximate M_r's of 15 kDa (2), 30 kDa (3), 40 kDa (4), 50 kDa (5).

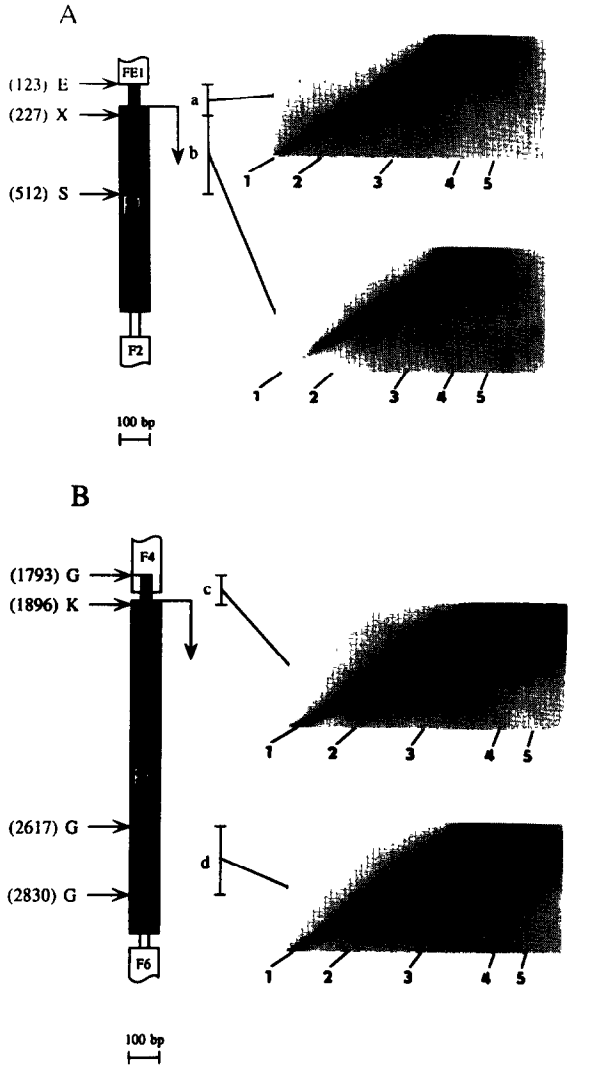

Fig. 2. 'Protein image' hybridizations of different regions in early (A, F1) and late (B, F5) genes of VV. A map of the early (F1) and late (F5) genes is shown to the left of each panel. Also shown here are the hybridization probes (a and c, promoter regions of corresponding genes; b and d, structural regions of corresponding genes). Restriction sites used for obtaining hybridization probes are designated by the capital letters: E, *Eco*RI; X, *Xba*I; S, *Sau*3A; G, *Sal*GI; K, *Kpn*I. Filters in A and B were obtained in two different experiments. For other details see Fig. 1.

We also identified proteins which are crosslinked to DNA using two-dimensional 'protein' gel electrophoresis [12,13]. After electrophoresis of crosslinked DNA–protein complexes in the first direction, DNA was hydrolysed and the released ^{125}I-labeled proteins were electrophoresed in the second direction (Fig. 3). One can see five strong spots on the diagonal of uncrosslinked proteins. Five horizontal lines (1, 2a, 2b, 3 and 4) which shifted from this diagonal to the left correspond to the crosslinked proteins of apparent M_r's of about 16, 25, 27, 41 and 54 kDa, respectively. It appears that the 16 kDa protein corresponds to the diagonal 2, the 25 and 27 kDa proteins to diagonal 3, and the 41 and 54 kDa

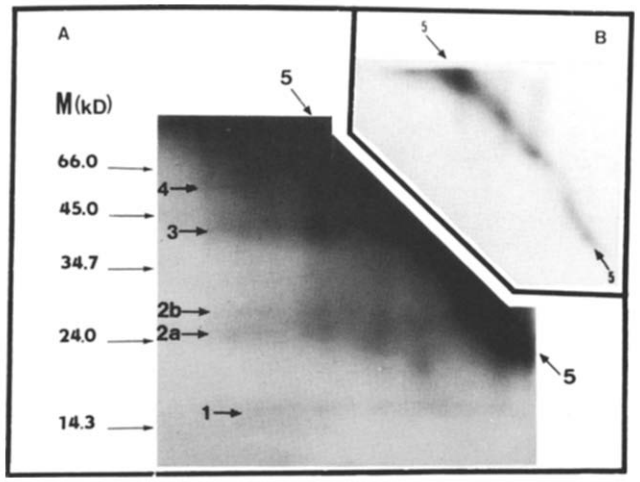

Fig. 3. Two-dimensional gel electrophoresis of proteins crosslinked to DNA in VV virions. (B) Short exposure of gel shown in A. The crosslinked DNA–protein complexes were separated in the first dimension (from left to right) in a 5–15% linear gradient polyacrylamide slab gel (200 × 200 × 0.6 mm) containing 7 M urea. After hydrolysis of DNA in the gel, electrophoresis in the second dimension of ^{125}I-labeled proteins (from top to bottom) was performed in a 12.5% polyacrylamide slab gel (200 × 200 × 1 mm). The spots of uncrosslinked proteins are indicated by arrow 5 in the extreme right top corner of the gel. Arrows 1, 2a, 2b, 3 and 4 indicate the bands corresponding to the crosslinked proteins. M, numbers on the left correspond to the M_r's of protein markers.

proteins to diagonals 4 and 5, respectively, in Figs. 1 and 2.

4. DISCUSSION

Earlier we developed a general approach to identify and measure the relative content of proteins bound to DNA in different regions of eukaryotic chromatin [6,7,9,13]. In the present study, the proteins were crosslinked to DNA directly in purified mature native virions of VV. This method allowed us to investigate the native structure of virions without its partial destruction, to determine and compare the protein content in different functional genome regions.

The use of DNA–protein crosslinking through histidine and lysine amino acid residues did not reveal any difference in the two-dimensional DNA patterns [9]. It appears that both lysine and histidine take part in the DNA–protein crosslinking.

We observed four DNA diagonals on the two-dimensional patterns of DNA electrophoresis (Figs. 1 and 2) corresponding to DNA-crosslinked proteins with M_r's of 15, 30, 40 and 50 kDa. Higher resolution two-dimensional protein electrophoresis revealed five proteins, and their corresponding M_r's were determined more precisely as 16, 25, 27, 41 and 54 kDa. The DNA diagonal 3 in Figs. 1 and 2 was obviously formed by the two merged diagonals corresponding to proteins with M_r's of 25 and 27 kDa (see bands 2a and 2b in Fig. 3).

Hybridization patterns of two-dimensional gels do not depend on the chosen probes (a, b, c and d hybridization probes in Fig. 2), which means that these proteins interact with all investigated genome regions of VV and both DNA strands taking part in this interaction. These data suggest that the DNA, including early and late genes and their promoters, is organized in mature VV with the same proteins as a regular structure over the whole genome or at least over the regions studied.

It is difficult to correlate our results directly with those of other researchers since different electrophoretic systems and lysis buffers were used for the identification of M_r's of VV proteins in previous studies. This approach of 'zero length' DNA–protein crosslinking [9] revealed a minimum of five proteins directly in contact with DNA and participating in VV nucleoprotein organization. It was reported earlier that some proteins are associated with the core [3,17]. The absence of these proteins in crosslinked DNA–protein complexes could result from different factors, e.g. these proteins do not interact with DNA, they cannot be fixed on virus DNA by the chosen method of crosslinking, etc. Further characterization of the crosslinked proteins will need the application of other methods.

REFERENCES

[1] McGhee, J.D. and Felsenfeld, G. (1980) Annu. Rev. Biochem. 49, 1115–1156.
[2] Griffith, J.D. (1975) Science 187, 1202–1203.
[3] Soloski, M.J. and Holowczak, J.A. (1981) J. Virol. 37, 770–783.
[4] Kao, Sh.Y., Ressner, E., Kates, J. and Bauer, W.R. (1981) Virology 111, 500–508.
[5] Easterbrook, K.D. (1966) J. Ultrastruct. Res. 14, 484–496.
[6] Levina, E.S., Bavykin, S.G., Shick, V.V. and Mirzabekov, A.D. (1981) Anal. Biochem. 110, 93–101.
[7] Karpov, V.L., Preobrazhenskaya, O.V. and Mirzabekov, A.D. (1984) Cell 36, 423–431.
[8] Joklik, W.K. (1962) Biochim. Biophys. Acta 61, 290–301.
[9] Nacheva, G.A., Guschin, D.Y., Preobrazhenskaya, O.V., Karpov, V.L., Ebralidse, K.K. and Mirzabekov, A.D. (1989) Cell 58, 27–36.
[10] Gilmoure, D.S. and Lis, J.T. (1985) Mol. Cell. Biol. 5, 2009–2018.
[11] Church, G.M. and Gilbert, W. (1984) Proc. Natl. Acad. Sci. USA 81, 1991–1995.
[12] Mirzabekov, A.D., Shick, V.V., Belyavsky, A.V. and Bavykin, S.G. (1978) Proc. Natl. Acad. Sci. USA 75, 4184–4188.
[13] Mirzabekov, A.D., Bavykin, S.G., Belyavsky, A.V., Karpov, V.L., Preobrazhenskaya, O.V., Shick, V.V. and Ebralidse, K.K. (1989) Methods Enzymol. 170, 386–408.
[14] Bolton, A.E. and Hunter, W.M. (1973) Biochem. J. 133, 529–539.
[15] Belikov, S.V., Belgovsky, A.I., Preobrazhenskaya, O.V., Karpov, V.L. and Mirzabekov, A.D. (1993) Nucleic Acids Res. 21, 1031–1034.
[16] Mackett, M. and Archard, L.C. (1979) J. Gen. Virol. 45, 683–701.
[17] Oie, M. and Ichihashi, Y. (1981) Virology 113, 263–276.