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Crosslinking of nuclear proteins to DNA by *cis*-diammminedichloroplatinum in intact cells

Involvement of nuclear matrix proteins

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In order to detect the nuclear matrix proteins involved in DNA binding, avoiding possible artifacts derived from the disruption of nuclei, proteins were crosslinked to DNA by the action of *cis*-diamminedichloroplatinum on intact chicken liver cells and analyzed by two-dimensional gel electrophoresis. At least eleven species of crosslinked proteins were found to derive from the nuclear matrix prepared from the same cell type, and five of these were found also among the proteins crosslinked to DNA in intact liver cells from ox and pig. This subset of common proteins, conserved in different animal species, is likely to have a fundamental role for the anchorage of DNA to the nuclear matrix.

DNA; Nuclear protein; Nuclear matrix; *cis*-Diamminedichloroplatinum

1. INTRODUCTION

DNA in eukaryotic chromatin is organized into large loops, which are associated with histones and non-histone proteins, and anchored to the nuclear periphery and to an internal proteinaceous structural meshwork called the nuclear matrix or scaffold [1–3]. This is thought to be involved in processes such as DNA replication, transcription, RNA splicing and transport (for reviews see Georgiev et al. [4] and Berezney [5]).

To better understand both the structural and functional aspects of loops organization it is necessary to identify the DNA–protein interactions which occur in the loops-matrix anchoring points. A number of authors have described the scaffold- or matrix-attachment regions (SAR or MAR) of DNA and some of the proteins which appear to be involved in these interactions (reviewed in [6–8]). A possible criticism of these results is that they have been obtained after introducing some modification in the native structure of the nuclei. Many different preparations of the nuclear matrix have been proposed, and possibly no one is immune from drawbacks [9]. Thus, for example, extraction of nuclei with high salt may cause a sliding of DNA over its attachment points or an exchange of the DNA-binding proteins, so that the original DNA–protein interactions are altered or lost.

Abbreviations: *cis*-DDP, *cis*-diamminedichloroplatinum (II); IEF, isoelectrofocusing

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Hnilica and coworkers [10, 11] have demonstrated that heavy metals or their complexes are able to diffuse inside intact cells and nuclei and to crosslink DNA to proteins located nearby. On the basis of an immunoblotting staining an indication was provided that nuclear matrix proteins could be crosslinked to DNA by the use of this technique. Considering the interest in determining the proximity of DNA to proteins without disruption of the nuclear structure, we analyzed in greater detail the proteins crosslinked to DNA by *cis*-diamminedichloroplatinum II (*cis*-DDP) in intact, viable cells from mammalian and avian liver, with the aim of identifying the nuclear matrix components among the crosslinked proteins.

2. EXPERIMENTAL

The hepatic parenchymal cells preparation from chicken, pig and ox, and DNA–protein crosslinking by *cis*-DDP were performed according to Ferraro at al. [12]. After the reaction the cells were treated with lysis buffer (5 M urea, 2 M guanidine-HCl, 2 M NaCl, 0.2 M potassium phosphate at pH 7.5) and mixed with hydroxyapatite (Bio-Rad), which, in these conditions, binds DNA and DNA–protein complexes. The hydroxyapatite was washed extensively with the same buffer, in order to remove completely RNA and free proteins; the DNA-bound proteins were released from DNA and eluted by incubation with the same buffer, in which 1 M thiourea was present instead of urea, as described before [12].

Two-dimensional IEF-SDS electrophoresis was performed according to O'Farrell [13]. Staining was carried out with Coomassie blue. Comparison of the patterns from different gels was made by the use of the densitometer 620 (Bio-Rad) and the program 2DAnalyst II (Bio-Rad). Proteins from crosslinked chicken liver cells and proteins from the nuclear matrix of the same cell type were also mixed and run together in two-dimensional electrophoresis.

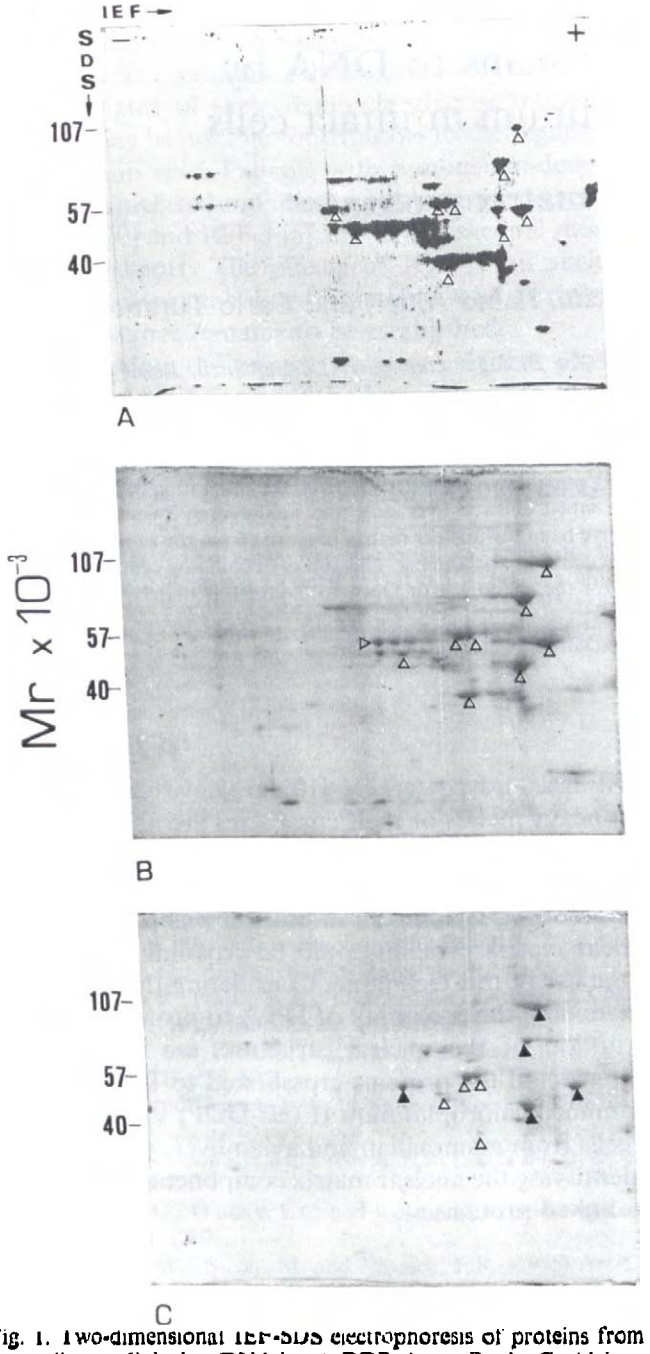

Fig. 1. Two-dimensional IEF-SDS electrophoresis of proteins from liver cells crosslinked to DNA by *cis*-DDP. A, ox; B, pig; C, chicken. Arrowheads indicate proteins common to the three animal species. Closed arrowheads in panel C indicate proteins common to the three species and found also in nuclear matrix from chicken liver cells (see Fig. 3).

In order to rule out the possibility that the proteins obtained as described before derive from aspecific and meaningless aggregates, the DNA–protein complexes formed by *cis*-DDP were isolated, run in SDS-gel electrophoresis, subjected to nuclease treatment, and run in a second dimension again in SDS-gel electrophoresis. By this technique, while aggregates should be incapable of entering the gel, the individual proteins, each crosslinked to a family of DNA fragments of various lengths, should migrate as streaks in the first dimension. After hydrolesis of DNA, i.e. in the second dimension, they should migrate according to their M_r, as in the usual SDS-electrophoresis, generating a pattern of horizontal lines. This technique is similar to the two-dimensional 'protein' electrophoresis employed by Mirzabekov and coworkers to characterize the products of crosslinkage [14]. For these experiments, protein–DNA complexes formed by *cis*-DDP were isolated essentially as described by Mirzabekov et al. [14], employing a Cetavlon (cetyltrimethyl ammonium bromide) precipation, followed by a SDS-KCl precipitation. The DNA moieties were sonicated to fragments less than 800 base pairs long. The complexes were then redissolved in 2% SDS, 10% glycerol and 60 mM Tris-HCl buffer at pH 6.8 and run in a 10% polyacrylamide gel. A strip of the gel was cut, equilibrated with a 50 mM Tris-HCl, pH 7.5, buffer containing 0.3 mM $CaCl_2$ and 50 U of micrococcal nuclease (Boehringer) and incubated for 18 h at 37°C. The strip was then equilibrated in 2% SDS, 10% glycerol, 20 mM dithiothreitol and 60 mM Tris-HCl buffer at pH 6.8, run in the second dimension and silver stained. [15].

Internal and peripheral nuclear matrix were isolated according to Kaufmann [16].

3. RESULTS

Intact liver cells from chicken, ox and pig were treated with *cis*-DDP and the proteins crosslinked to DNA were isolates and analyzed by two-dimensional electrophoresis. The resulting three patterns, shown in Fig. 1, have at least nine major components in common, judging both from the isoelectric points and from the molecular masses.

In order to check the validity of the procedure used by us for the crosslinkage by *cis*-DDP, the protein–DNA complexes were run in SDS-gel electrophoresis, treated with a nuclease and run in a second dimension. The resulting gel, stained with silver, is shown in Fig. 2B. The pattern of horizontal lines would not have been expected (see section 2) from aspecific aggregates or protein- protein complexes. The separation in the second dimension corresponded to that obtained with the crosslinked proteins isolated by the hydroxyapatite procedure (panel A in Fig. 2).

In order to identify among the proteins crosslinked to DNA those deriving from the nuclear matrix, the

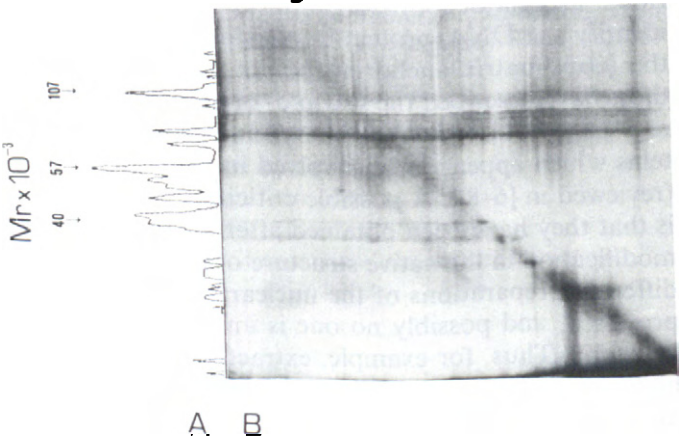

Fig. 2. Two-dimensional SDS-gel electrophoresis: DNA–protein complexes formed by *cis*-DDP in chicken liver cells were run in the first (horizontal) dimension. DNA was digested, and the proteins were run in the second dimension (panel B). Panel A shows a densitometric scan of a SDS-gel electrophoresis of the proteins from the same cells crosslinked to DNA by *cis*-DDP and isolated by hydroxyapatite. This scan was obtained by compacting the pattern of a two-dimensional IEF-SDS separation on the vertical axis by means of the 2DAnalyst progam by Bio-Rad.

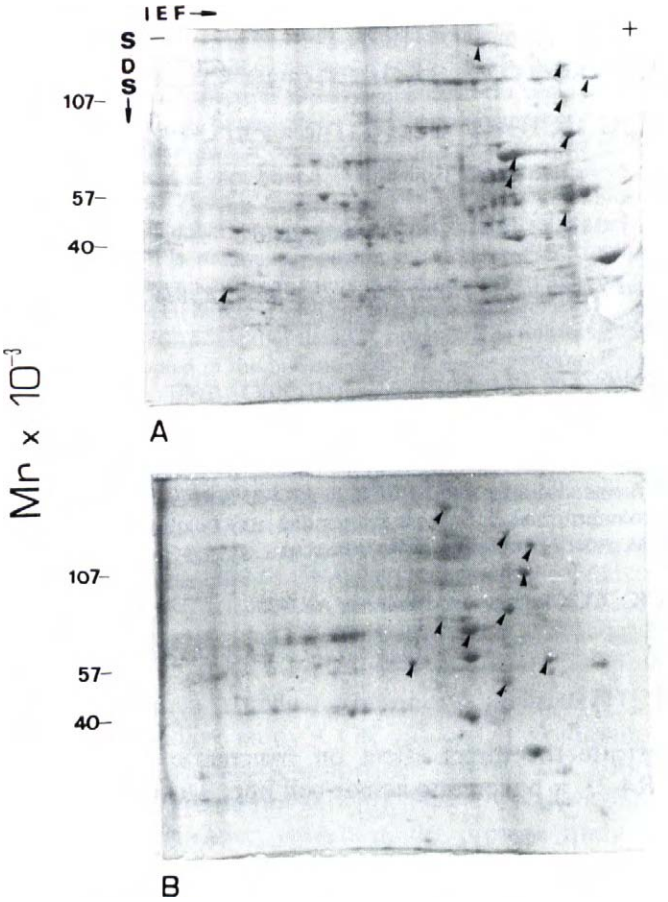

Fig. 3. Two-dimensional IEF-SDS electrophoresis of the proteins from the nuclear matrix prepared from chicken hepatocytes. A, peripheral matrix; B, internal matrix. Arrowheads indicate the eleven protein species crosslinked to DNA by *cis*-DDP in the same cell type (Fig. 1C). Seven of these proteins are present in both matrix fractions, and are probably proteins which can be only partially solubilized by reduction [16].

latter was prepared from chicken hepatocyte nuclei and fractionated in internal and peripheral matrix according to Kaufmann [16]. Proteins from the two preparations were analyzed by two-dimensional electrophoresis, and the results are shown in Fig. 3.

A comparison of the pattern of Fig. 1C with those of Fig. 3 reveals that eleven polypeptides, crosslinked to DNA in chicken liver cells, derive from the nuclear matrix. Five of these matrix polypeptides, crosslinked to DNA by *cis*-DDP, are common to all the animal species investigated (closed arrowheads, in Fig. 1C).

4. DISCUSSION

The reported results show that *cis*-DDP is capable of crosslinking a variety of nuclear proteins to DNA in intact hepatocytes. A comparison of the various electrophoretic patterns obtained, taking into account also the patterns from nuclear matrix preparations, has allowed the detection of several similarities. In particular, it is noteworthy that at least eleven polypeptides crosslinked to DNA in chicken liver cells were also found among the proteins of the nuclear matrix from the same cell type.

Five of the proteins crosslinked to DNA in chicken liver cells and identified as nuclear matrix proteins can be found also among the crosslinked proteins from ox and pig liver cells, as shown in Fig. 1. The finding of these highly conserved matrix proteins, presumably involved in the DNA binding, suggests that they have a unique role in the anchorage of chromatin DNA.

At least four other DNA-crosslinked proteins, not identified as matrix proteins, are common to the three animal species investigated. This classification as non-matrix proteins, however, should be taken with caution considering the variations in composition of nuclear matrices prepared by different methods.

It appears therefore that some nuclear proteins, either of matrix origin or not, constitute a definite subset of nuclear components, characterized by a relative abundance in nuclei, a location within a crosslinking distance from DNA and a conserved structure in animal species ranging from birds to mammals. These features suggest that they play an essential structural role in the intranuclear packing of interphase chromatin.

These results, and particularly the finding of a subset of DNA-interacting non-histone proteins common to many animal species, point out the potentiality of the crosslinking method to identify the scaffold attachment sites of DNA in intact cells.

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