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Glycosylated forms of nuclear lamins

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Chromatin and pore complex-lamina preparations were obtained from pig and chicken tissues, and their proteins were analysed by mono- and bidimensional electrophoresis. A glycosylated form of lamin A, recognized by concanavalin A, was shown to be present in at least 3 of the tissues examined. Glycosylation is suggested to be a further postsynthetic modification, besides phosphorylation and methylation, which can modify the properties of lamins.

Lamin; Glycoprotein; Concanavalin A

1. INTRODUCTION

Lamins are the prominent proteins which are found in the nuclear lamina; the latter is considered the framework of the nuclear envelope and the anchoring site for interphase chromatin. Lamins are well characterized proteins; post-translational modifications have been described, consisting of methylation [1] and phosphorylation [2,3]; hyperphosphorylated forms, which lead to a dissolution of the nuclear lamina, have been shown to occur during mitosis [2]. Lamins are considered to be devoid of bound carbohydrates, although in monodimensional gel electrophoresis they run close to some concanavalin A-binding proteins [4] and some indirect evidence has suggested the existence of a glycosylated form of lamin B [5].

In the course of a study on glycoproteins of chromatin from pig tissues [6], we noticed that one group of these glycoproteins has electrophoretic characteristics reminiscent of those of nuclear lamins. We demonstrate here that lamins can indeed exist as glycosylated proteins, so that glycosylation should be considered as a further way by which the properties and possibly the interactions of lamins between themselves and with other nuclear components can be modified.

2. EXPERIMENTAL

Nuclei were isolated from pig liver, kidney and heart and from chicken liver and heart according to Blobel and Potter [7] or, in some experiments, according to Simpson and Sober [8] in order to obtain a larger amount of material. The purity and integrity of the nuclei were checked by optical microscopy. Chromatin was prepared according to MacGillivray et al. [9]. Chromatin proteins were obtained by batchwise elution of chromatin adsorbed on hydroxyapatite (Bio Rad); two different protein fractions were dissociated from DNA, respectively in 3 M NaCl (fraction I) and in 2 M NaCl, 5 M urea and 2 M guanidine-HCl (fraction II) as previously described [6]. All solutions contained 0.1 M phenylmethane sulfonylfluoride (Sigma); during chromatin preparation the latter was 1 mM, and 100 units of aprotinin (Boehringer), 0.02 units of α_2-macroglobulin (Boehringer) per mg of DNA and 0.2 M paramercuribenzoate were also added.

The pore complex-lamina fraction was purified from nuclei essentially as described by Lehner et al. [10], or, in some experiments, as described by Gerace et al. [11]. Similar results were obtained in both cases. Proteins from chromatin and from lamina were analysed by SDS-PAGE according to Laemmli [12] and by two-dimensional electrophoresis according to O'Farrell [13]. Proteins were visualised by Coomassie blue staining; concanavalin A binding glycoproteins were visualised, after electroblotting on Immobilon membranes (Millipore), by incubation with concanavalin A followed by horseradish peroxidase according to Hawkes [14].

The markers of relative molecular mass for proteins to be stained with Coomassie blue were glycogen phosphorylase, bovine serum albumin, ovoalbumin and carbonic anhydrase. When blotting on membrane was required, followed by staining with concanavalin A-peroxidase, the markers were human transferrin and ovoalbumin.

3. RESULTS

The proteins extracted from chromatin of 3 pig tissues by the sequential use of 3 M NaCl (fraction I) and of 2 M NaCl, 5 M urea and 2 M guanidine (fraction II) were compared to the proteins of the corresponding nuclear lamina (fig.1A). For each tissue the patterns of proteins in fraction II are very similar to those of lamina proteins. Glycoproteins recognized by concanavalin A were detected after blotting and staining with concanavalin A-peroxidase (fig.1B). Very few glycoproteins are present in fraction I, where the main components are the histones. Again, the glycoprotein patterns obtained from fraction II and from lamina are quite similar for each tissue, while significant differences can be noticed among the 3 tissues examined.

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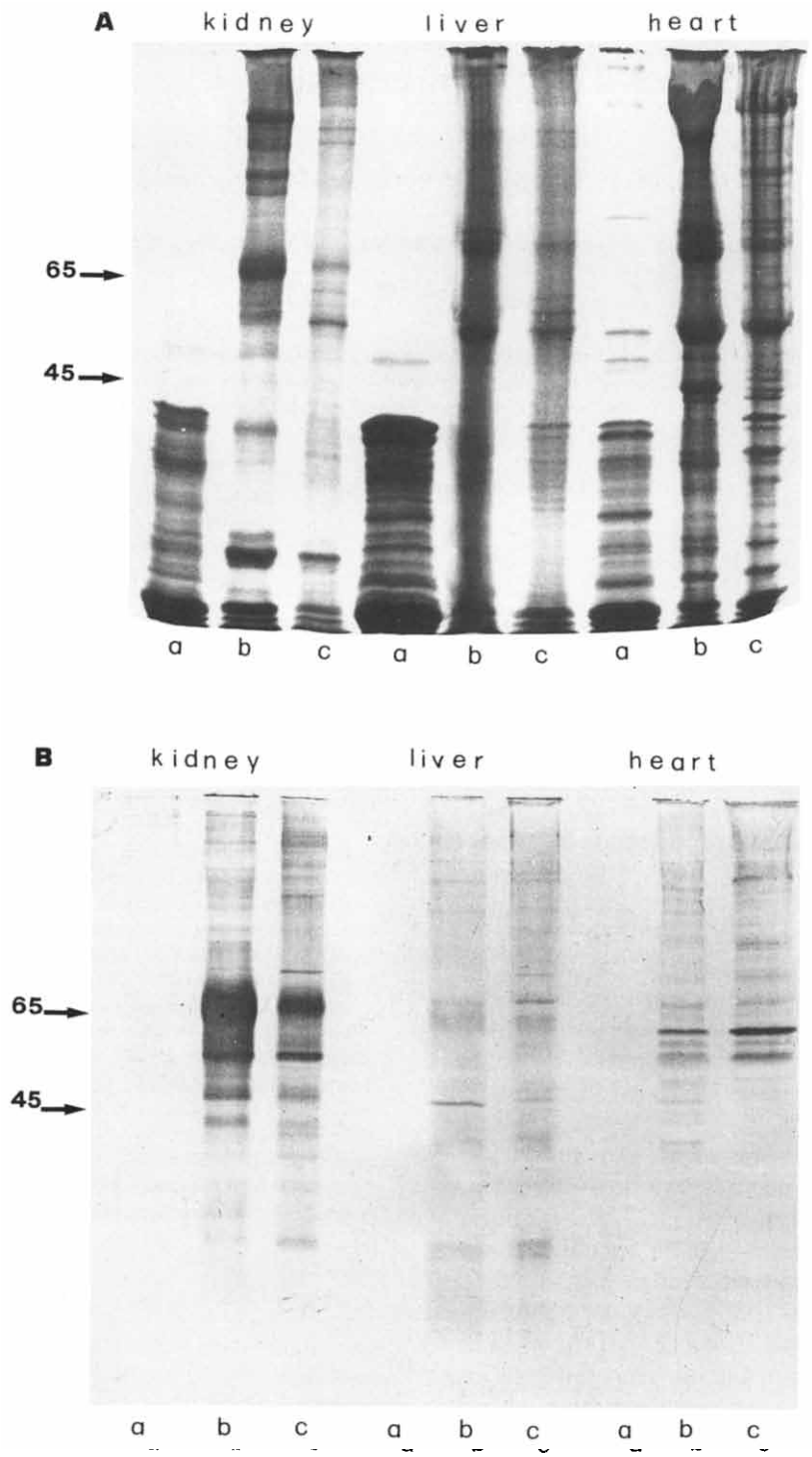

Fig.1. SDS-gel electrophoresis of nuclear proteins from pig tissues, stained with Coomassie blue (A) or with concanavalin A-peroxidase (B). (a) Chromatin proteins, salt extracted (fraction I); (b) chromatin proteins, extracted with urea and guanidine (fraction II); (c) proteins from pore complex-lamina fraction.

However, at least two glycoprotein bands in the region of 65–75 kDa can be detected in liver and heart; one of these bands is visible also in kidney, while the other is probably hidden by a strong 60 kDa band. It should be noticed that these 65–75 kDa glycoproteins are more intense among the lamina proteins than in fraction II.

A glycoprotein of 70 kDa appears also among the lamina proteins from chicken liver nuclei (data not shown).

Proteins in the 65–75 kDa range, found preferentially among the lamina proteins and also in chromatin proteins solubilised by urea and guanidine could be

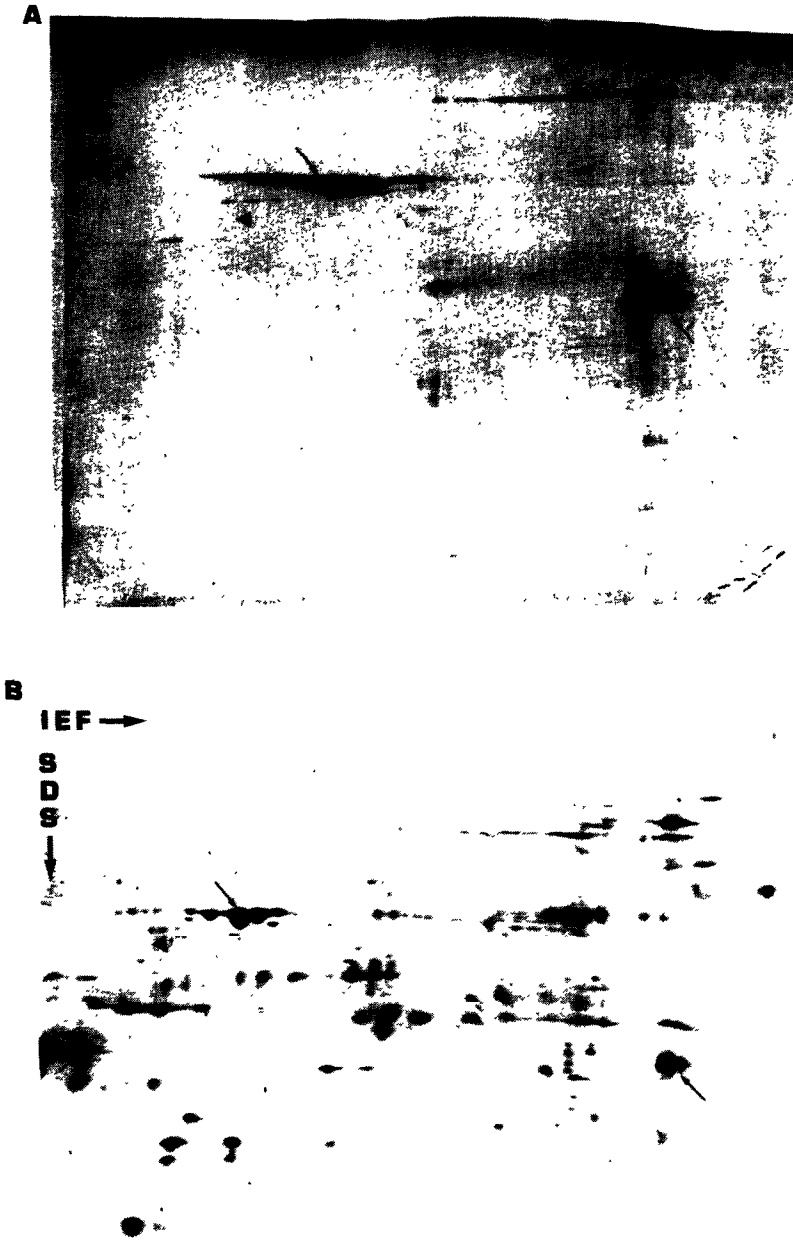

Fig.2. Bidimensional electrophoresis of nuclear proteins from pig liver, stained with concanavalin A-peroxidase. The pH range of the isoelectrofocusing was approximately 7.5 to 5 (from left to right). The two arrows indicate the internal standard proteins, human transferrin (upper left arrow) and ovoalbumin (lower right arrow). The arrowhead indicates lamin A. (A) Glycoproteins from pore complex-lamina fraction. (B) Glycoproteins from chromatin, fraction II.

identified with lamins. For a safer identification, the same protein fractions were examined in bidimensional electrophoresis.

Fig.2 shows the patterns of glycoproteins of a pore complex-lamina preparation (A) and of chromatin fraction II (B) from pig liver. A weak spot can be seen in both patterns with a M_r of about 70000, in the pH region and with the multiple spot appearance characteristic of lamin A. The pattern of lamina glycoproteins shows the expected 175 kDa glycoprotein [15] and a component of about 50 kDa [4], which could represent a cytoskeleton protein. Fig.3 shows the glycoprotein patterns of a pore complex-lamina preparation (A) and of chromatin fraction II (B) from chicken liver. Fig.4 shows the glycoprotein pattern of chromatin fraction II from chicken heart. In all 3 patterns a protein band with the electrophoretic characteristics of lamin A is present. In liver the

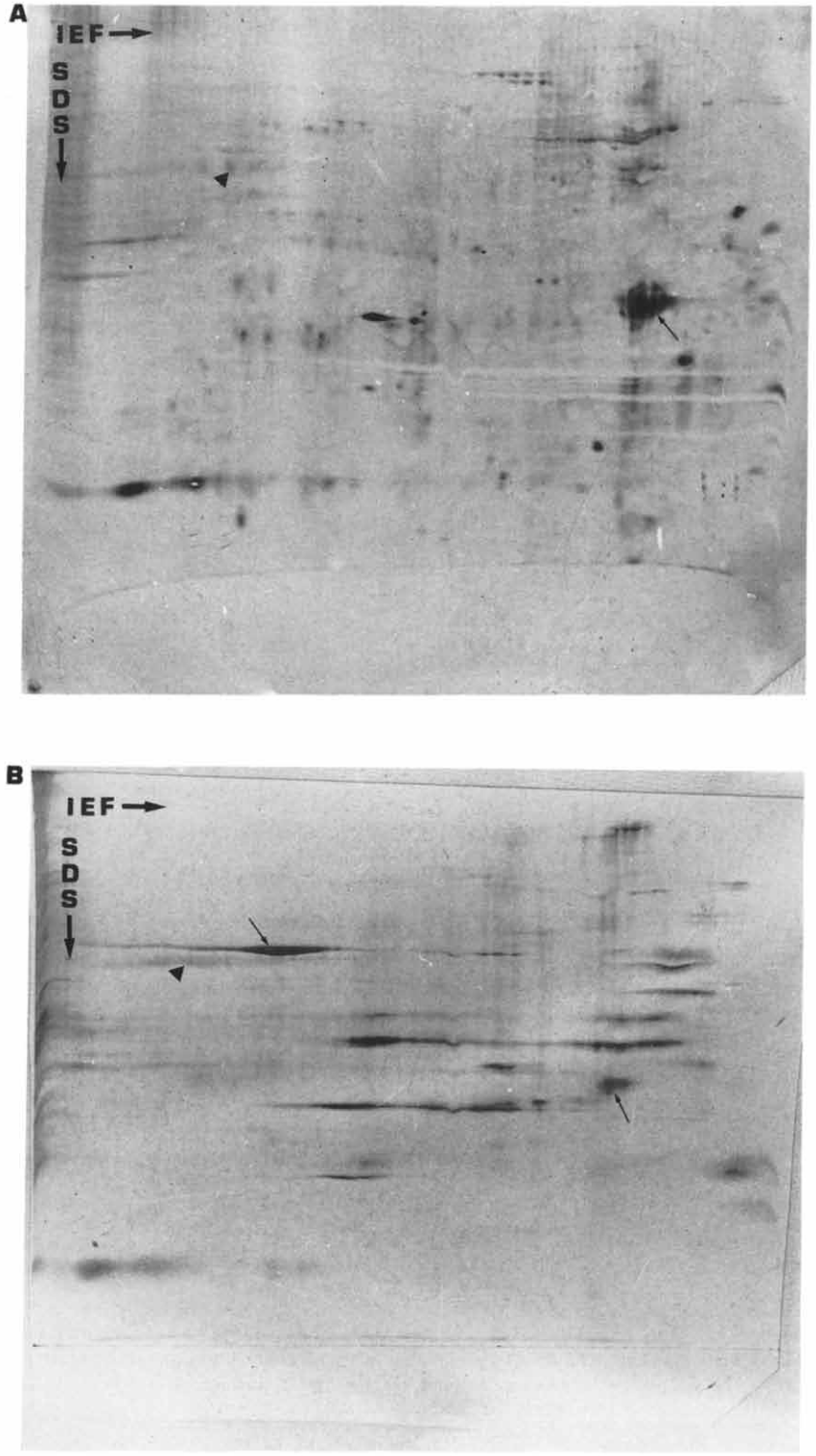

Fig.3. Bidimensional electrophoresis of nuclear proteins from chicken liver, stained with concanavalin A-peroxidase. The arrows indicate the internal standard proteins, human transferrin (upper left arrows, absent in A) and ovoalbumin (lower right arrow). The arrowhead indicates lamin A. (A) Glycoproteins from pore complex-lamina fraction. (B) Glycoproteins from chromatin, fraction II.

glycosylated lamins B and C are probably present but their identification remains doubtful.

No protein was stained if concanavalin A was added together with 0.2 M methylglycoside, which blocks the lectin active site.

4. DISCUSSION

The mono- and bidimensional electrophoretic patterns of lamina and chromatin preparations indicate that in all tissues examined glycosylated forms of

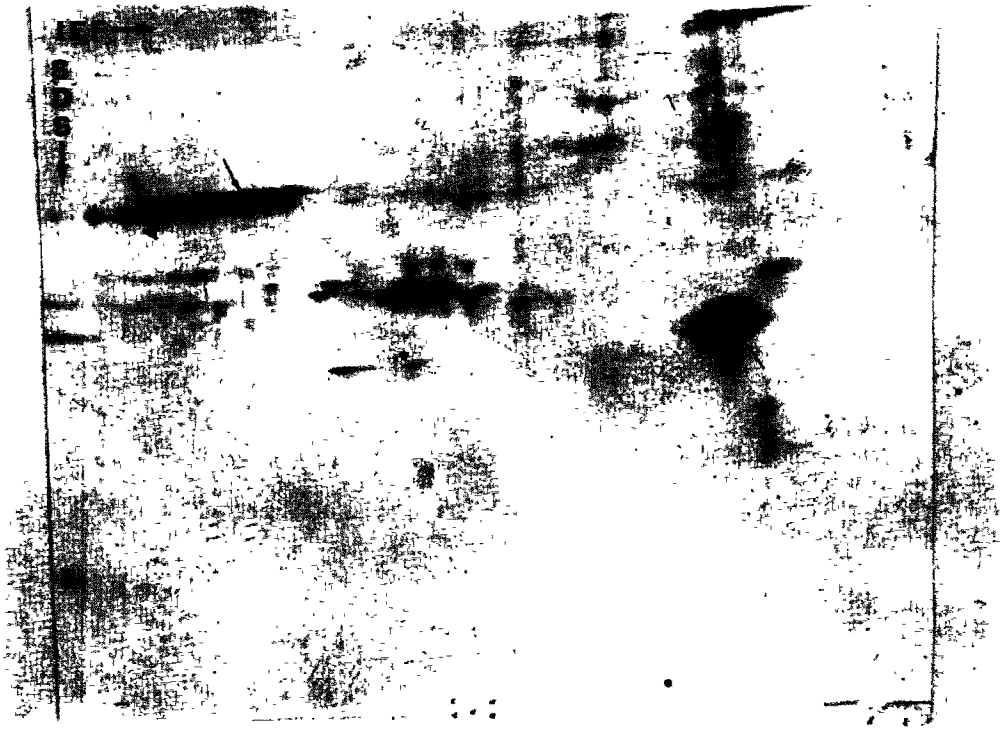

Fig.4. Bidimensional electrophoresis of the glycoproteins from chromatin fraction II, prepared from chicken heart. Staining with concanavalin A-peroxidase. The arrows indicate the internal standard proteins, human transferrin (upper left arrow) and ovoalbumin (lower right arrow). The arrowhead indicates lamin A.

lamins, and particularly of lamin A, are present. Lamin A is identified on the basis of its relative molecular mass, its isoelectric point(s), its multiple forms derived by different levels of phosphorylation and by its presence both in preparations of nuclear lamina and among the chromatin proteins which are only extracted by urea and guanidine, as are those from our fraction II. When lamina preparations are compared to chromatin proteins from fraction II (fig.1B, fig.2), the lamina preparations appear to be enriched, as expected, in the 70–75 kDa glycoprotein, identified with lamin A. On the other hand, the presence of lamins not only in lamina preparations but also in chromatin is documented in the literature, and well described by the detailed electrophoretic analysis by Peters and Comings [16].

Although no quantitative considerations can be made on the basis of these data, it appears that the protein band stained with concanavalin A-peroxidase and which corresponds to lamin A is a weak one, and particularly so in pig liver lamina. This suggests that only a fraction of lamin is glycosylated, and that this fraction can depend not only on the kind of tissue, but also on the animal species, as shown by the comparison of figs 2 and 3. This may explain why the lamin glycosylation has not been reported before; in fact, lamins from rat liver, which have been the most extensively studied, could be not glycosylated at all, or to such a low extent as to escape detection.

Lamin A recognized by concanavalin A should be *N*-glycosylated. We checked, therefore, the published amino acid sequences of lamin A for the presence of the known peptide capable to undergo glycosylation, which is Asn-X-Ser(or Thr) [17]. In human lamin A, according to Fisher et al. [18], 3 such peptides are present. These are in positions 456–458 (Asn-Lys-Ser), 532–534 (Asn-Ser-Thr), and 660–662 (Asn-Cys-Ser). The sequence Asn-Ser-Ser in position 650–652 is not counted because of the presence of a proline in position 653 [19]. The sequence of lamins is highly conserved, and the first two peptides have been found also in lamin C in mouse [20]. The presence of these sites, which is a necessary, although not sufficient, condition for glycosylation, is in accordance with our results.

As regards the role of glycosylated lamins, while nothing can be said at present, it should be noticed that receptors for carbohydrates have been identified in nuclei [21,22], and that many transcription factors have been shown to be glycoproteins, in which the carbohydrate moiety seems to be necessary for the transcription activation [23]. This suggests the existence in the nucleus of specific interactions involving carbohydrates. Glycosylated lamins could be capable of particular interactions with other lamina or chromatin proteins, possibly producing some effect on the anchorage of chromatin to the nuclear periphery.

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