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Differences in nonenzymatic glycation of histones*

Ágnes Lakatos, Nóra Kiss and Kázmér Jobst

Department of Clinical Chemistry, University Medical School, Pécs, Hungary

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Time course of glucose binding by histone Hl and total histones was followed in isolated histone preparations and in thymus nuclei. In both cases the uptake of glucose by Hl was surprisingly high in contrast to a much lower uptake of glucose by total histones. DNA is not implicated in glycation of histones in nuclei.

Acetylation of histone proteins alters H1-mediated chromatin condensation, the development of nucleosomal higher order solenoid structure and heterochromatin assembly as well as gene expression [1–3]. All these effects of acetylation are due to a change in net positive charge [4]. In contrast to acetylation of histones, there are hardly any data available on their nonenzymatic glycation.

According to the literature glucose reacts with the ε-amino group of histone lysine [5]. Specific glucose binding by lysine residues [6–8] was reported in rat and human serum albumin.

It is, however, not known whether a change in gene control or chromatin condensation has to be ascribed to histone glycation — as has been reported for acetylation. This prompted us to start a detailed investigation on glycohistones. To our surprise we had previously found that the level of glycohistone in the liver cell nuclei of patients who had died of diabetes mellitus was as low as in healthy subjects [9]. Thus to better understand regulation of glycation of the DNA-binding core histones we have examined binding of glucose by the Hl linker histone and by total histones (TH)[1] both in thymus nuclei and in isolated histone preparations. It appeared that the kinetics of glucose binding by histones varied considerably with the different preparations; however, DNA did not play any considerable role in this reaction.

MATERIALS AND METHODS

Total histone and histone H1 were prepared from calf thymus. All steps of isolation were performed at 4°C in the presence of 10000 U/l Aprotinin (Boehringer, Germany), a proteinase inhibitor. The nuclei were isolated at pH 7.2 in isotonic sucrose solution (0.01 M Tris, containing 3 mmol/l calcium chloride) [10]. Two different series of experiments were performed. Glucose (300 mM) dissolved in 66% ethanol containing 3 mM $CaCl_2$ was either incubated with isolated H1 (IH1) or isolated total histones (ITH) (10 mg protein) for 2, 4, 8 and 14 days at 37°C or glucose was incubated with intact nuclei for the same periods of time and NH1 and NTH were isolated subsequently.

For preparation of histones the isolated nuclei were homogenized thoroughly in 0.14 M NaCl to release nonhistone and high molecular group proteins [11], and then were centrifuged at 6000 r.p.m. The pellet was extracted with 150 mM Tris/HCl buffer, pH 7.0 and then, following centrifugation, with 0.35 M NaCl. From part of the pellet obtained in this way ITHs were

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[1] Abbreviations: IH1, isolated H1; ITH, isolated total histones; NBT, nitro blue tetrazolium; NH1, nuclear H1; NTH, nuclear total histones; TH, total histones.

extracted with 0.2 M H_2SO_4 according to Johns [12]. From the remaining pellet IH1 was extracted 2 × 5 min with 5% $HClO_4$ [11, 13]. After centrifugation, the supernatants were dialyzed against distilled water for 48 h and then lyophilized.

In a separate experiment TH was extracted [12] from the 0.14 M NaCl pretreated nuclei with 2.0 M NaCl according to Johns [12] to check up whether there is any difference in glucose binding between the preparation obtained by 2.0 M NaCl extraction and that obtained with 0.2 M sulphuric acid.

The samples withdrawn from the incubation mixtures at different time intervals were dialyzed at 15 ± 4°C, till free of glucose, and lyophilized. Binding of glucose by histones was determined using the fructosamine reaction [14], consisting in reduction of nitro blue tetrazolium (NBT, Serva, Germany) to blue formazan by the Amadori product formed in the aldehyde-amine condensation of protein and glucose [15]. Absorbance at 546 nm was monitored.

Glucose binding by histones was expressed as the difference in absorbance of the NBT reaction product per g protein measured by the biuret reaction. All the data presented are means of three separate experiments.

Stability of the histone preparations at 37°C was checked by one dimensional thin-layer electrophoresis (PAGE) in 15% polyacrylamide gel containing 3 M acidic urea and 0.15% Triton X-100. The gels were stained with Coomassie blue.

RESULTS AND DISCUSSION

Histones, the basic DNA-binding proteins arranged in the nucleosomal particles, are subcomponents of chromatin [16]. Any change in this highly organized structure may affect not only gene regulation but also the reactivity of the core and linker histones [17].

The kinetics of glucose uptake by ITH extracted either with 0.2 M sulphuric acid or with 2.0 M NaCl, were practically identical. The secondary sulphuric acid treatment of the salt-extracted histones did not change the glycation reaction (Fig. 1).

After incubating the nuclei in 66% ethanol glucose solution in the presence of 10000 U/l Aprotinin no considerable amounts of histone protein were dissociated from the nuclei. The dry material content of the dialysed and lyophilized supernatant of the incubation medium of the nuclei was hardly measurable.

We did not observe any difference in PAGE pattern (Fig. 2) between the controls and the NTH incubated in 66% ethanol solution at 37°C for two weeks.

We did not determine the DNA content or the glucose uptake by the DNA of nuclei. However, after 3 days of incubation of thymus nuclei at 37°C we observed a 5% insignificant DNA decrease in their alcohol-fixed smears using the Schiff-Feulgen method, the result of which was evaluated cytophotometrically [18].

In the preliminary experiment it has been proved that extraction of the histone prepara-

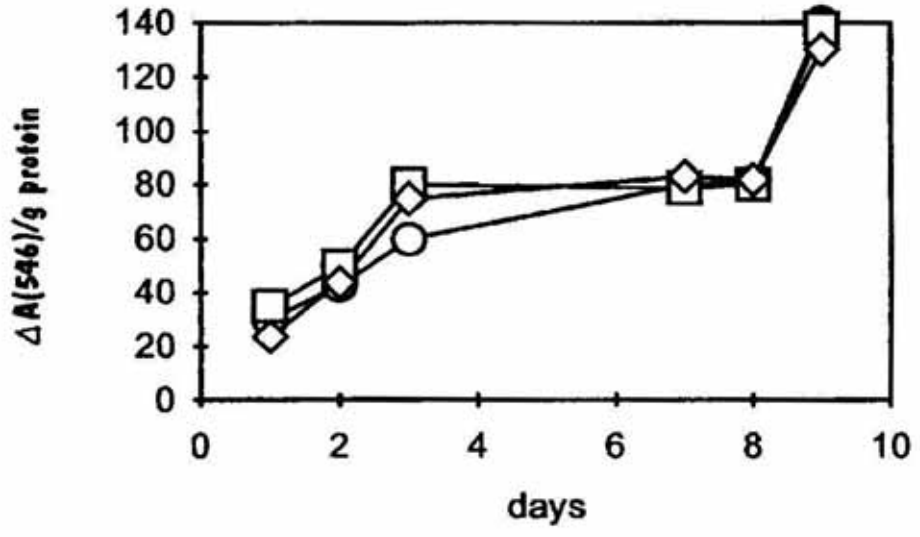

Fig. 1. Kinetics of glucose uptake by total histone, extracted either with 0.2 M sulphuric acid (□), or with 2.0 M NaCl (○), and after secondary extraction of the latter with 0.2 M sulphuric acid (◊).

Fig. 2. Separation of total histone by PAGE stained with Coomassie blue: a) extracted with 0.2 M sulphuric acid, b) extracted with 2.0 M NaCl, c) extracted with 2.0 M NaCl and subsequently treated with 0.2 M sulphuric acid, d) extracted with 0.2 M sulphuric acid following incubation in 60% aqueous ethanol at 37°C for two weeks.

tions with sulphuric or with perchloric acid showed great similarity, but also a few points of divergence. The uptake of glucose both by the NH1 in the nucleus and the H1 glycated after its isolation (Fig. 3) was rapid, showing in both cases closely similar kinetics (and reached saturation values after 14 days). In contrast, the glucose uptake by TH either directly glycated or glycated in the nuclei before extraction showed lower capacity to bind glucose than H1 histone. It was also evident that glycation of TH was about 1.5 times more intensive when incorporated in the nuclei than after their extraction from the nuclei.

According to the presented results an extraordinarily rapid uptake of glucose by the lysine-rich histone H1, extracted with chaotropic perchloric acid and only partly associated with DNA, indicates high reactivity of a large number of free amino groups. H1 glycated *in situ* in chromatin took up glucose somewhat slower than after its isolation, which may imply the involvement of "high molecular group" nonhistone proteins.

The glycohistone content values for the two differently treated TH preparations were especially surprising from two points of view. On the one hand, the glucose uptake by NH1 glycated in the nuclei was about 50% higher than that of the corresponding total histone. In the case of isolated preparations 2.5 times as much glucose was taken up by histone H1 as by total histone. This may be partly explained by the fact that in contrast to the 15% content of lysine in TH, histone H1 contains 27% lysine. This data is in good agreement with the 40% higher value for glucose uptake by NHl than by NTH on day 10 [19]. The value of surplus lysine may reflect the free amino groups, which is lent support to by the steep kinetics of H1. However, the difference becomes increasingly smaller with time. On the other hand, the uptake of glucose by chromatin was more rapid than by isolated histones. The glucose uptake by the preparations glycated in the nucleus was 50%

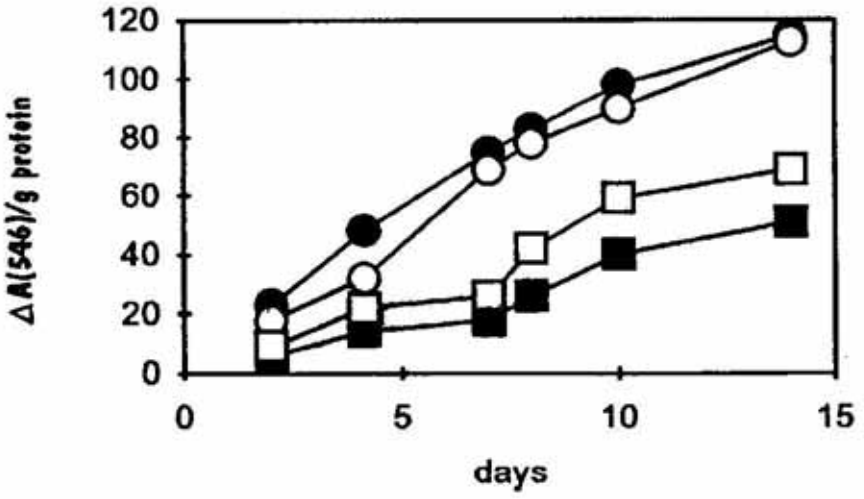

Fig. 3. Time course of the uptake of glucose by total (■) and H1 (●) histones isolated from calf thymus nuclei or histones (□, ○) from the nuclei previously incubated with glucose. The glucose uptake was followed by the NBT- fructosamine test and expressed in terms of ΔA_{546} /g protein.

higher than that of the ITH preparations extracted with sulphuric acid prior to glycation. Actually, we had expected just the opposite. According to the present measurements the uptake of glucose by the ordered nucleosomal structure in the nuclei was greater than that by DNA-free ITH. The reason for this may be that, in the course of the isolation with sulphuric acid, the histones undergo structural, conformational changes which prevent condensation of glucose and primary amine i.e., the Schiff base formation. Because of the similar kinetics of the NH1 and IH1 preparations we believe that this will affect core histones first of all. At the same time, in chromatin, DNA does not influence the uptake of glucose by either H1 or TH.

The low glycohistone values obtained for the liver cell nuclei of diabetics [9] cannot be explained on the basis of the present results. There is, however, no doubt that *in vitro*, histones in chromatin do take up glucose [20]. Therefore when interpreting the course of events *in vivo*, the 159-day-long lifetime of liver histones should be taken into account [5, 21], as well as the inhibited intracellular uptake of glucose in insulin deficiency.

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