vitermes trinervoides[27] and α-D galactosidase, β-D galactosidase and β-D glucosidase from the alimentary tract of *Locusta migratoria*[23].

The effect of substrate concentration on the activity of α-D and β-D galactosidase and α-D glucosidase was determined under optimal pH conditions. Care was taken to ensure that substrate availability did not become rate-limiting during the course of the reactions. The apparent Michaelis constant (K_m) and V_{max} were calculated from Lineweaver-Burk plots of the data for each enzyme (table 2). The affinities of the 3 enzymes for their respective substrates compare reasonably well with values reported elsewhere in various other insect species[21,23,25,28]. In the present study the activity of α-D glucosidase did not show simple Michaelis-Menton kinetics (table 2); the Lineweaver-Burk plot being bi-phasic. Similar bi-phasic plots have been reported for β-D glucosidase and β-D galactosidase from the gut of *Locusta* with similar substrates to those used here, although in the locust the plot for α-D glucosidase was linear[23]. A number of alternative explanations may be advanced. There may be 2 different enzymes or isoenzymes present, each with different affinities for the substrate. Some support for this comes from the fact that 3 β-glucosidase components have been reported in the crop of *Locusta*[29] and biphasic Lineweaver-Burk plots have also been reported for β-glucosidase activity in this species[23]. A second possibility is that a single enzyme is involved but that there is some change in the kinetic parameters of the system at different substrate concentrations. Clearly further studies are necessary before the significance of such bi-phasic plots can be explained in relation to the physiology of digestion in *Callosobruchus maculatus*.

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Nonhistone protein with high affinity for histone H1 and HMG 14 protein

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Summary. Specific interaction of a nonhistone protein from mouse spleen chromatin with histones HI, H2A and HMG 14 protein is shown. Some implications of these findings are briefly discussed.

Interactions of nonhistone proteins with particular components of chromatin have drawn the attention of many investigators, as these proteins are the most probable candidates for the role of elements controlling the genome's expression. So, any nonhistone component specifically interacting with some chromatin elements and not interacting with others is important. The more so if such a protein belongs to the transcriptionally active chromatin fraction.

In the present paper I report the results of the investigation of the interaction of one particular nonhistone protein, PS_1, described by us earlier[1,2]. This protein is selectively released from the nuclei of mouse spleen under conditions of mild hydrolysis with micrococcal nuclease or DNAse I, i.e. when preferential hydrolysis of actively transcribed genes[3-5] takes place. This fact makes this protein a very interesting subject of investigation. Moreover, this protein, according to its properties[1] is quite different from other nonhistone proteins, namely HMG proteins, which are also selectively released from nuclei under similar conditions[6]. Earlier, we showed that PS_1 protein does not interact with DNA[7]. In the work described here, I found that this component interacts very specifically with histones H1, H2A and HMG protein 14.

Materials and methods. PS_1 protein was purified by preparative electrophoresis in the presence of SDS and electro-eluted from the gel as described earlier[2]. Purified protein was extensively dialyzed against 1000 vols of 0.5 M sodium phosphate buffer pH 7.5 containing 1% of Triton × 100 (Merck), followed by dialysis against another 1000 vs of the same buffer without Triton × 100. After the dialysis PS_1 protein was iodinated with NaI^{125} (USSR) using the chloramine T procedure, as described[8]. The specific radioactivity of the iodinated protein was $2.5-3.5 \times 10^6$ cpm/μg.

To study the interaction of I^{125}-PS_1 with histones we applied the method of electrophoretic transfer of histones onto nitrocellulose paper after the electrophoresis in the presence of SDS[9]. Total histone was prepared from mouse spleen nuclei by the conventional technique[10]. Histones were loaded on the 0.6 mm polyacrylamide slab gel, and electrophoresed in the presence of SDS using 4–15% discontinuous Laemmli system[11]. After the electrophoresis the gel strip containing electrophoretically separated histone fractions was cut out with a microtome blade and immersed for 3 h in a solution of 50 mM NaCl, 10 mM Tris-HCl, pH 7.2, 1 mM EDTA (Serva) and 1% Triton × 100 to remove SDS and renature the proteins[9]. Afterwards the gel strip was soaked in 5.4% CH_3COOH for 40 min. The gel strip was placed on 2 sheets of Whatmann 3MM paper presoaked in 5.4% acetic acid covered with a nitrocellulose sheet (BAO85, Schleicher and Schuell) followed by another 2 Whatman 3MM sheets and put into a special device for electrophoretic transfer. Histones were transferred onto nitrocellulose paper for 4 h at 2 A, 60 V in 5.4% acetic acid.

After the transfer the nitrocellulose strip was soaked for 1.5 h in 3 changes of 0.1 M NaCl, 10 mM Tris-HCl, pH 7.9, 3% of bovine serum albumin (Serva) with shaking, followed by the addition to this solution of 2×10^6 cpm of I^{125}-PS_1. After incubation for another hour the filter strip was extensively washed with several changes of the same solution fo 3 h to remove all unbound radioactivity, and air-dried. Autoradiography was performed with the help of RM-1 film (USSR).

Results and discussion. In figure A one can see the nitrocellulose filter strip onto which histones were electrophoretically transferred from the gel. The strip was stained with amido-black as described[9]. It should be noted here that only histones and no other bands were visible on the stained nitrocellulose strip. The same was also true for the original gel.

The results of autoradiography of such a strip following the incubation with I^{125}-PS_1 protein are presented in figure B. It is evident that the radioactivity was bound to both histone H1 subfractions and to the other 3 components. However, the intensity of these components was much lower than that of histone H1.

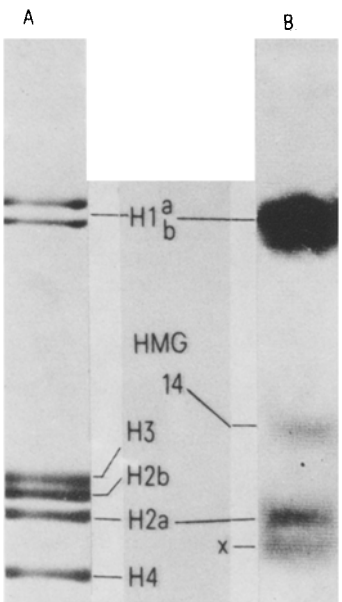
The other 3 proteins which interacted with I^{125}-PS_1 protein were identified by their migrational distance and by superimposing the X-ray film over the filter strip with the transferred histones. It was found that the position of the 1st one corresponds to that of histone H2A, and of the 2nd one to that of HMG 14. The 3rd protein (designated by X in figure B) was located between the bands of histones H2B and H4. It is a yet unidentified protein, possibly representing a degradation product of histone H1.

Another point of interest revealed in these experiments was that proteins HMG 14 and X were not visible neither on the original gel conventionally stained with Coomassie Brilliant Blue R-250 nor on the amido black-stained nitrocellulose filter strip after electrophoretic transfer (fig. A). Therefore, the levels of these proteins in the total histone preparation were far below the sensitivities of both staining methods. Thus, the affinity of PS_1 for these components has to be rather high to reveal them.

The data presented here clearly demonstrate a high affinity of PS_1 protein to H1 histone and in addition a highly selective interaction with histone H2A and HMG 14 protein. The binding of PS_1 to histone H1 is not surprising as, according to the amino acid composition of the former[2] it must be slightly negatively charged, and H1 histone carries a strong positive charge. At the same time, selective interaction of PS_1 protein with histone H2A and HMG 14 protein suggest its functional significance, as the positive charges of these components do not differ essentialy from those of other histones; H3, H2B and H4. It is interesting to note here that recently reported data[12] suggest the location of H2A histone close to the ends of the nucleosome, just on the border with the internucleosomal linker region, whereas HMG 14 protein is located at both ends of nucleosome core[13,14].

The results presented in this paper suggest the specific localization of PS_1 protein in the internucleosomal linker region where it may interact with all components for which it has high affinity, namely, histones H1 and H2A and HMG 14 protein.

Another important conclusion which may be drawn from the data of these experiments as well as from the results reported by us earlier[7] (inability of PS_1 protein to form complexes with DNA) is that PS_1 is included in the chromatin structure due to its interactions with proteins and not with DNA.



A Electrophoretically transferred histones stained with amido black[9]. *B* Autoradiography of the nitrocellulose filter strip containing transferred histones with I^{125}-PS_1 bound protein.

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