BINDING OF MYELIN BASIC PROTEINS TO CHROMATIN FRACTIONS OF MOUSE BRAIN

Jim W. GAUBATZ

Department of Biochemistry, College of Medicine, University of South Alabama, MSB 2170, Mobile, AL 36688, USA

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1. Introduction

Whereas most mammals have a single myelin basic protein (MBP), the myelin component of rodent brain has two well-characterized basic proteins [1,2]. The subcellular distribution of MBP has been thoroughly studied, and it is known that variable amounts of MBP are found with nuclei following subcellular fractionation [3]. It also appears that these proteins can lead to problems in quantitating individual histones because MBP migrate with mobilities very similar to histones in acetic acid–urea or sodium dodecylsulfate (SDS) polyacrylamide gel systems [4].

Employing standard methods for purifying nuclei and fractionating chromatin with micrococcal nuclease into transcriptionally active and inactive subsets of DNA sequences [5,6], it was observed that these proteins not only co-isolated with nuclei but, indeed, were bound to all fractions of chromatin starting with the basic subunit or nucleosome. Furthermore, MBP resemble histones and other basic chromosomal proteins in their binding properties and solubility behavior. This observation has important implications for in vitro studies with brain nuclei where the binding of these proteins could influence the structure and function of chromatin.

2. Experimental

Nuclei were isolated from brain tissue of C57BL male mice. Immediately after removal, brain tissue was disrupted in a hand-driven, glass–Teflon homogenizer containing 10 vol. ice-cold 0.32 M sucrose, 10 mM Tris–HCl (pH 7.4) and 3 mM $MgCl_2$. The homogenate was passed through 8 layers of cheese cloth pre-soaked in buffer and was subjected to 10 strokes of a tight pestle in a Dounce homogenizer. Nuclei were collected by sedimentation of 1000 × *g* for 5 min and were washed 5 times by suspending the nuclear pellet with several passes of a Teflon pestle and a glass receptacle in the above medium containing 1% Triton. Microscopic examination of the final fraction revealed intact nuclei free of cytoplasmic contamination. Subsequent procedures were performed in the presence of 1 mM phenylmethylsulfonyl fluoride as a proteinase inhibitor. Purified glial and neuronal nuclei were separated on a step gradient composed of 1.8 M and 2.4 M sucrose containing 1 mM $MgCl_2$. This molarity of sucrose prevents sedimentation of cell debris and most myelin while centrifugation at 100 000 × *g* for 45 min leaves the neuronal nuclei at the 2.4 M sucrose surface and pellets the glial nuclei [7].

Myelin was purified from mouse brain tissue as in [9]. Basic proteins were acid-extracted from myelin or chromatin fractions by the addition of 1/10 vol. 4 N H_2SO_4. The extracts were sonicated and left at 5°C for 1 h or longer. Insoluble material was removed by centrifugation at 10 000 × *g* for 10 min. The acid-soluble proteins were concentrated by dialysis against an excess of acidified ethanol.

Chromatin was fractionated following limited digestion with micrococcal nuclease according to [5,8]. Nucleosomes were separated on 5–20% linear sucrose gradients as in [10]. DNA fragments were purified from gradient fractions as in [10] and proteins were extracted from parallel gradients as above. Total proteins were precipitated with 2 vol. ethanol and were dissolved in 1% SDS, 5% 2-mercaptoethanol, 10 mM Tris (pH 6.8), 1 mM EDTA, and 10% glycerol. Total proteins were electrophoresed on a discontinuous SDS–polyacrylamide gel system, prepared as in [14]. Electrophoresis of acid-extracted proteins was on 15% polyacrylamide gels containing 0.37% Triton, 6 M urea and 0.9 N acetic acid [11]. The size of DNA fragments were determined on 5% polyacrylamide gels under non-denaturing conditions [12].

3. Results

By shearing chromatin with the enzyme micrococcal nuclease, it is possible to fractionate fragments into either transcriptionally-enriched or -depleted DNA sequences [5,8]. The method in [5,8] separates chromatin into 4 fractions following nuclease digestion. A supernatant fraction (S1) of proteins and acid-soluble oligodeoxynucleotides released by enzyme digestion is obtained by sedimenting the nuclei. Lysing the nuclei with 1 mM EDTA releases 2 classes of nucleosomes that can be distinguished by their solubility behavior in moderate salt concentrations (100 mM NaCl). The mononucleosomes in the salt-soluble and -insoluble fractions may be isolated by sucrose gradient sedimentation as shown in fig.1. The average DNA chain length of the salt-soluble mononucleosomes (MN1) is 145 basepairs, whereas the salt-insoluble fraction contains mononucleosomes (MN2) with residual internucleosomal or spacer DNA for a combined length of 170 basepairs (not shown). In addition, the salt-insoluble fraction contains oligonucleosomes. The higher molecular mass chromatin (P2 fraction) that is not released by 1 mM EDTA treatment presumably contains the bulk of inactive DNA. In a typical fractionation experiment (5% DNA digested), 25% of the mouse brain chromatin is solubilized by 1 mM EDTA treatment with about equal amounts of salt-soluble and salt-insoluble nucleosomes. The remainder (70%) comprises the P2 fraction.

Chromosomal proteins from the various fractions were compared electrophoretically with MBP, the only acid-soluble proteins in myelin [2,3]. SDS–polyacrylamide gel analysis of total proteins associated with mouse brain chromatin gives a very complex electrophoretic pattern, and moreover, the MBP are not completely resolved from the histones owing to their similarity in size (not shown). However, analysis of acid-extracted, basic chromosomal proteins greatly simplifies the electrophoretic pattern. The low-pH electrophoresis system in [13] again does not completely resolve the MBP from histones as shown in fig.2. Another alternative is the Triton–urea low-pH system, commonly used to study histone variants [11]. We have found that 0.37% Triton–6 M urea will effectively separate MBP from histones on 15% polyacrylamide gels (fig.3).

As expected, the majority of the basic proteins bound to chromatin fractions are the histones. Unexpectedly however, there were substantial amounts of MBP (fig.3d) bound to MN1 (fig.3a) and MN2 (fig. 3b) mononucleosomes, as well as the residual nuclease-treated chromatin (P2 fraction, fig.3c). Sedimenting nuclei through high molarities of sucrose previously shown to strip away myelin [7] did not appear to diminish the occurrence of these basic proteins as seen in fig.3f (neuronal mononucleosomes), fig.3g (neuronal P2 fraction) and fig.3h (glial cell chromatin).

To assess the relative strength of MBP binding, purified brain nuclei were extracted twice with

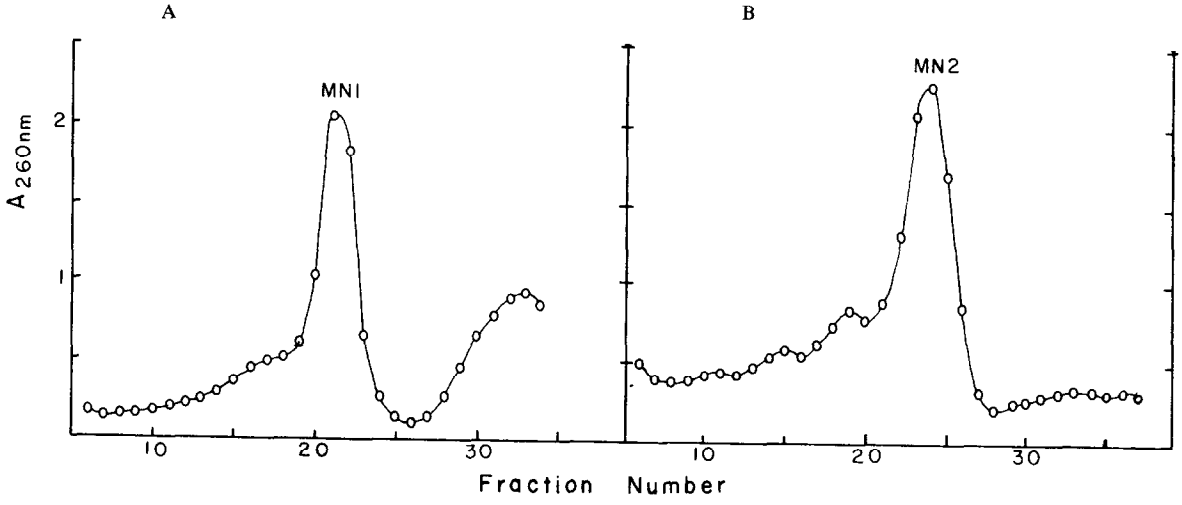


Fig.1. Sucrose gradient analyses of chromatin particles released from mouse brain nuclei digested with micrococcal nuclease and treated with 1 mM EDTA: (A) salt-soluble fraction; (B) salt-insoluble fraction.

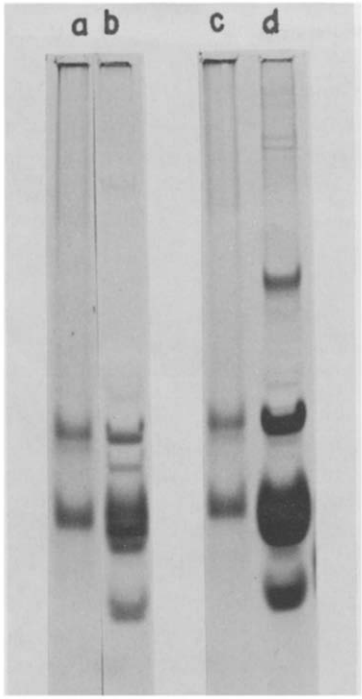
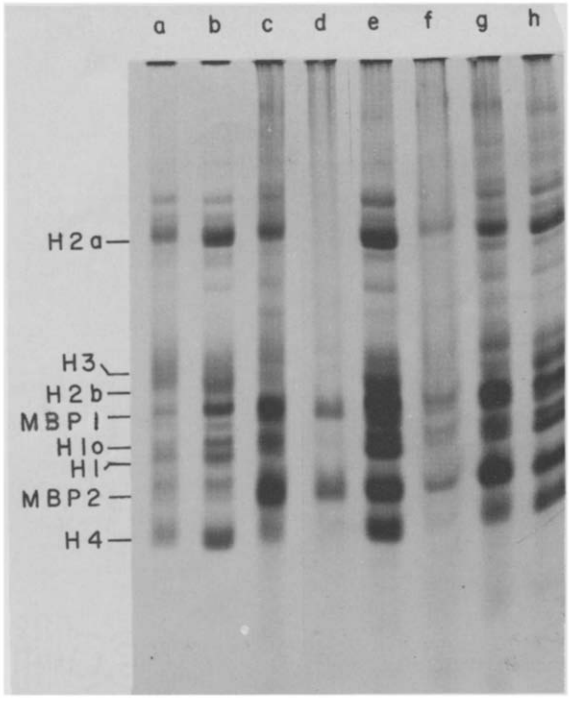


Fig.2. Polyacrylamide gel electrophoresis of basic proteins extracted with 0.4 N H_2SO_4 from mouse myelin (a,c)., mouse brain nuclei (b), and calf thymus chromatin (d). Electrophoresis was on 18% polyacrylamide, 2 M urea gels in the acid system of [13].



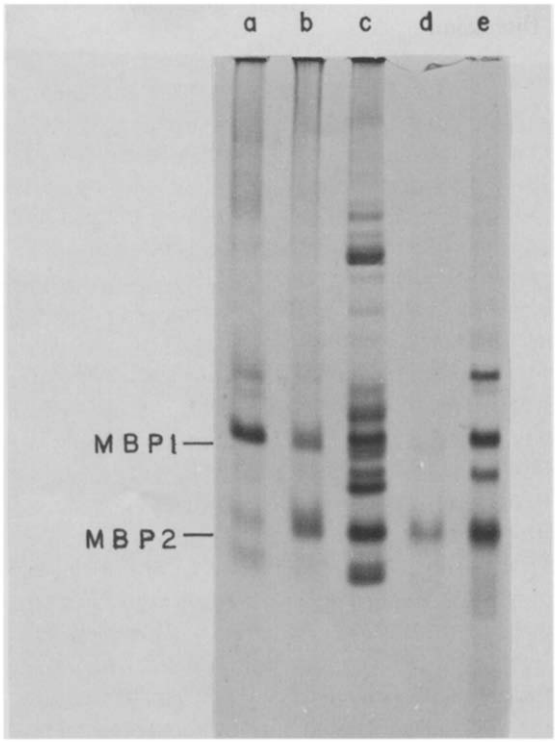


Fig.4. Comparison of basic proteins from: myelin (b); 0.35 M NaCl brain nuclear extract (a); 0.35 M NaCl unextracted nuclear proteins (c); and 0.5 M NaCl extracted, 2% trichloroacetic acid insoluble (d) and soluble (e) nuclear fractions.

350 mM NaCl by gentle homogenization. While some MBP was dissociated by 0.35 M NaCl, most of the MBP remained bound to chromatin as shown in fig.4. Increasing the ionic strength to 0.5 M NaCl removed the MBP and histone H1. Furthermore, the MBP were soluble in 2% trichloroacetic acid but insoluble in 10% trichloroacetic acid (fig.4). In this regard they resemble HMG 1 and 2 proteins [15]. On the other hand, MBP behave like non-H1 histones in that only histone H1 and $H1_o$ were extracted from chromatin with 5% perchloric acid.

Fig.3. Electrophoretic analysis of acid-extracted proteins isolated from: mouse brain chromatin fractions MN1 (a), MN2 (b), and P2 (c); myelin (d); total brain chromatin (e); MN1 and MN2 (f) and P2 (g) fractions of purified neuronal nuclei; and glial cell chromatin (h).

4. Discussion

From the results presented in fig.3,4, it is apparent that MBP bind chromatin and chromatin subunits, and they bind rather tightly. This is not surprising in view of the fact that MBP are histone-like proteins with respect to their size and charge [2,16]. Indeed, the structure of MBP1 is very similar to histone H1, and MBP1 is extensively phosphorylated as is histone H1 [2]. Furthermore, MBP are only partially removed from brain chromatin by 0.35 M NaCl treatment, in agreement with the salt-dissociation pattern of histone H1 [10]. Alternatively, there are differences between H1 and MBP1 such as their distribution and relative content of basic amino acids [2,16]. This probably reflected in the finding that the MBP are not soluble in 5% perchloric acid, whereas H1 is soluble.

It seems reasonable to assume that during tissue and cell disruption, MBP are released from the membrane and migrate to nuclear sites. Nevertheless, the possibility exists that a fraction of these proteins may be located in the nucleus in vivo. Since the nuclear isolation procedures used here are very commonly employed in chromatin studies, it seems important to point out the presence of MBP binding to brain chromatin. This is an observation that could easily be overlooked because of the difficulty in separating these proteins from histones. It also appears that the presence of significant amounts of MBP in chromatin preparations could influence certain types of in vitro experiments. For example, the binding of MBP to chromatin could affect RNA polymerase binding sites and loading capacities. Therefore, this possibility should be taken into account when such studies are performed with brain nuclei.

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