MeCP2 Behaves as an Elongated Monomer That Does Not Stably Associate with the Sin3a Chromatin Remodeling Complex*

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MeCP2 is a transcription factor that recognizes and binds symmetrically methylated CpG dinucleotides to repress transcription. MeCP2 can associate with the Sin3a/histone deacetylase corepressor complex and mediate repression in a histone deacetylase-dependent manner. In extracts from rodent tissues, cultured cells, and Xenopus laevis oocytes, we find that only a small amount of mammalian MeCP2 interacts with Sin3a and that this interaction is not stable. Purification of rat brain MeCP2 (53 kDa) indicates no associated proteins despite an apparent molecular mass by size exclusion chromatography of 400–500 kDa. Biophysical analysis demonstrated that the large apparent size was not because of homo-multimerization, as MeCP2 consistently behaves as a monomeric protein that has an elongated shape. Our findings indicate the MeCP2 is not an obligate component of the Sin3a corepressor complex and may therefore engage a more diverse range of cofactors for repressive function.

Cytosine methylation is an important epigenetic mark on vertebrate genomes (1). In mammals, methylation occurs mostly in the context of the CpG dinucleotide and can account for about 70–80% of genomic CpGs (2). CpG methylation acts as an additional means of controlling genome function, beyond DNA base pair sequence. Because DNA methylation is copied faithfully to the newly replicating DNA strands during cell division, these marks can be maintained across development and act as a form of epigenetic memory. There are two general mechanisms by which CpG methylation is believed to function. First, modification of cytosines in the recognition sequence of DNA-binding proteins can inhibit their binding to cognate sequences and thus deny access to regulatory regions. Second, proteins have been identified that specifically bind the methyl-CpG dinucleotide via a methyl-CpG binding domain (MBD)3 or, in the case of Kaiso (4), by a zinc finger domain. These proteins can interact with methylated CpGs and affect nearby genes by repressing transcription and modulating chromatin structure (5).

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‡ The abbreviations used are: MBD, methyl-CpG binding domain; HDAC(s), histone deacetylase(s); DTT, dithiothreitol; Ni-NTA, nickel-nitrilotriacetic acid; BSA, bovine serum albumin; EGS, ethylene glycol-bis succinimidyloxyuccinate; ADH, alcohol dehydrogenase; AE, anion exchange; CE, cation exchange; r, recombinant.

There are five mammalian members of the MBD family: MeCP2 and MBD1–4. With the exception of MBD3, all MBD family members bind methylated CpG dinucleotide specifically. MBD1 (6–8), MBD2 (9–12), MBD3, and MeCP2 (13) are all transcriptional repressors. Elucidation of the relationship between MBD proteins and their partner corepressors is a prerequisite for understanding how DNA methylation represses transcription and modulates chromatin structure. Several methyl-CpG-binding proteins have been shown to associate with histone deacetylases (HDACs) (10, 14, 15) or histone methyltransferases (16, 17). MBD2 and MBD3 are stable components of the NuRD chromatin remodeling complex (10, 12), and Kaiso can be purified in a complex containing NCoR (18). In this study, we have asked whether MeCP2 is also part of a stable repression complex.

MeCP2 dysfunction is the sole identified determinant of Rett syndrome, the most common inherited form of mental retardation in females (19). The majority of Rett syndrome point mutations in the MECP2 gene cluster in the MBD and the transcriptional repression domain (20), suggesting that methyl-CpG binding and transcriptional repression are important functional determinants of MeCP2 in vivo. MeCP2 has been shown to interact with the Sin3a-HDAC chromatin remodeling complex (14). In Xenopus laevis it was reported that MeCP2 partially cofractionated with the Sin3a complex and proposed that xMeCP2 occurs in a stable complex with xSin3a (21, 22). The link between MeCP2 and Sin3a has been strengthened recently with the identification of MeCP2 target genes. Both MeCP2 and Sin3a bind the promoter region of the brain-derived neurotrophic factor (Bdnf) gene (23–25) and modulate its expression. A similar situation occurs at the X. laevis xHairy2a gene, where MeCP2 and Sin3a bind upstream of the promoter region and repress transcription (26).2

In addition to Sin3a, several other factors have been reported to bind mammalian MeCP2, including DNMT1, CoREST, Suv39H1, and c-SKI (14, 27–29), although the contribution of these factors to MeCP2-mediated repression is not known. Native MeCP2 has not been purified previously from mammalian sources, leaving open the possibility that it may exist in a novel multiprotein complex. Here we investigate the association of MeCP2 with the Sin3a complex from both mammalian sources and X. laevis and we purify native MeCP2 from rat brain. We conclude that MeCP2 does not stably associate with the Sin3a complex. Moreover, we find no evidence that MeCP2 forms a stable association either with itself or with other proteins in nuclear extracts. Hydrodynamic analysis of MeCP2 shows that it behaves as an elongated monomeric molecule. These findings raise the possibility that DNA-bound MeCP2 interacts differently with partner proteins compared with its unbound form. In addition, the results suggest that MeCP2 might interact with a range of cofactors in addition to Sin3a.

2 I. Stancheva, personal communication.
**Experimental Procedures**

**Cell Culture**—NG-108 cells were a gift from Rod Brehm (University of Toronto) and were maintained in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% bovine calf serum, nonessential amino acids, sodium pyruvate, and antibiotics (Invitrogen).

**Chromatography**—Chromatography buffers were filtered through a 0.2-μm filter before application to fast protein liquid chromatography or disposable columns. Anion exchange buffer 20 mM Tris-HCl (pH 7.9), 0.2 mM EDTA, 1 mM DTT, 10% glycerol, supplemented with 100 mM NaCl (AE100), or 1000 mM NaCl (AE1000). Cation exchange buffer was 20 mM Hepes (pH 7.6), 0.2 mM EDTA, 1 mM DTT, 10% glycerol, supplemented with 100 mM NaCl (CE100), or 150 mM NaCl (CE150), or 400 mM NaCl (CE400). Ni-NTA affinity buffers (N) were 50 mM NaH2PO4, 300 mM NaCl, 10% glycerol (N0), or 20% imidazole (N100), or 250 mM imidazole (N250). Gel filtration buffers were made in 20 mM Hepes-KOH (pH 7.9), 3 mM MgCl2, 10% glycerol, supplemented with 150 mM KCl (GF150) or 500 mM KCl (GF500).

**Isolation of Rat Brain Nuclei and Nuclear Protein Extraction**—Rat brains (~450 brains obtained from Pel-Freeze Biologicals) were ground to a fine powder in liquid nitrogen with a mortar and pestle. The brain powder was diluted 5 volumes to 1 in ice-cold buffer A containing 10 mM Hepes (pH 7.5), 25 mM KCl, 0.15 mM spermine, 0.5 mM spermidine, 1 mM EDTA, 2 μM succrose, 10% glycerol, and complete protease inhibitors (Roche Applied Science) followed by homogenization in a 60-ml Dounce (Braun) on a Potter S (Braun) motorized homogenizer (five strokes at 1100 rpm). The homogenate was layered onto a 10-mL cushion of buffer A pre-made by swelling 5 column volumes of buffer A at 4 °C and resuspended in 1 volume of buffer B containing 5 mM Hepes (pH 7.9), 1.5 mM MgCl2, 10 mM KCl, 0.5 mM EDTA, complete protease inhibitors (Roche Applied Science) and incubated on ice 10 min. The nuclei were pelleted at 250 × g and resuspended in 1 volume of buffer C containing 5 mM Hepes (pH 7.9), 50% glycerol, 1.5 mM MgCl2, 0.2 mM EDTA, and complete protease inhibitors (Roche Applied Science) supplemented with 100 mM NaCl. The extraction was allowed to proceed for 1 h on ice, and then the nuclei were pelleted at 13,000 rpm for 20 min at 4 °C. The supernatant was taken as the nuclear extract and dialyzed to the indicated salt concentration.

**Immunoprecipitation**—MeCP2 or Gal4 antibodies were incubated with 500 μg of rat brain nuclear extract at 4 °C for 4 h. Protein A-Sepharose beads (Amersham Biosciences) were added to the reaction and incubated for 1 h at 4 °C. Beads were washed four times with 20 mM Hepes (pH 7.9), 0.1 mM NaCl, 10% glycerol, 0.2 mM EDTA, 0.01% Triton X-100. Bound proteins were eluted in Laemmli buffer and run on an 8% denaturing polyacrylamide gel. The gel was blotted to nitrocellulose and incubated with the primary antibody (MeCP2) or the secondary antibody, followed by horseradish peroxidase (Amersham Biosciences). The membranes were visualized by enhanced chemiluminescence (Amersham Biosciences). The MeCP2-containing fractions were identified by Coomassie Blue staining. Western blotting.

**Large Scale Purification of Native MeCP2**—All chromatography and dialyses were performed at 4 °C, and MeCP2 (14, 145, 146, 147, 148, 149, 150, 151) was dialyzed into CE200. The protein from the Ni-NTA elutions was washed with 20 column volumes of N20 and batch-eluted with N250. MeCP2-containing fractions were identified by Coomassie Blue staining and dialyzed into CE200. The protein from the Ni-NTA elutions was loaded onto an Sp-Sepharose column and eluted with a linear gradient of CE200 to CE1000, and the MeCP2-containing fractions were combined and directly loaded onto a Sephacryl S-300 26/60 (Amersham Biosciences) column and eluted with GF500. MeCP2-containing fractions were combined and dialyzed against CE200 and then loaded onto an Sp-Sepharose column. Proteins were eluted by a linear gradient of CE200 to CE1000, and MeCP2-containing fractions were dialyzed and dialyzed into CE200 and stored at −20 °C.

**Mass Spectrometry**—To identify purified proteins, excised bands were in-gel trypsinized, and peptides were eluted from the gel slice. Mass spectrometry analysis was carried out on an Applied Biosystems Voyager DE-STR matrix-assisted laser desorption ionization time-of-flight instrument using a-cyano-4-hydroxycinnamic acid matrix. Spectra were analyzed in MS-Fit and then submitted to Protein Prospector (prospector.ucsf.edu/) for peptide matching.

**Xenopus Oocyte Extract and MeCP2 Chromatography**—Oocyte extract was prepared from one female as described previously (21). The extract (90 mg) was then loaded onto a 10-mL Bio-Rex 70 column exactly corresponding to the initiating ATG and 3 EcoRI site downstream of the endogenous MeCP2 stop codon. The PCR fragment was inserted into the NdeI/EcoRI sites of the bacterial expression plasmid pET30b (Novagen) to create an untagged bacterial MeCP2 expression vector pET30bhMeCP2.

**Expression and Purification of Full-length Undigested MeCP2 in Bacteria**—pET30bhMeCP2 was transformed into BL21 codon plus bacteria (a gift from Robin Allshire). Bacterial cultures (usually 0.5 or 1 liter) were grown in LB at 37 °C until the culture reached an A600 of 0.5 absorbance units. Cultures were induced with 1 μl isopropyl 1-thio-β- D-galactopyranoside for 3 h at 30 °C. Cells were pelleted and lysed in 20 mM Tris-HCl (pH 8.0), 500 mM NaCl, 0.1% Nonidet P-40, complete protease inhibitors (Roche Applied Science) and on ice for 5 min. The lysate was centrifuged at 250 × g at 20 °C for 20 min, and Ni-NTA beads (Qiagen) pre-equilibrated with lysis buffer were added to the supernatant. Recombinant MeCP2 was allowed to bind the beads mixing for 1 h at 4 °C and then applied to a 10-mL disposable column. The column was washed with 20 column volumes of N20 and batch-eluted with N250. MeCP2-containing fractions were identified by Coomassie Blue staining and dialyzed into CE200. The protein from the Ni-NTA elutions was loaded onto an Sp-Sepharose column and eluted with a linear gradient of CE200 to CE1000, and the MeCP2-containing fractions were combined and directly loaded onto a Sephacryl S-300 26/60 (Amersham Biosciences) column and eluted with GF500. MeCP2-containing fractions were combined and dialyzed against CE200 and then loaded onto an Sp-Sepharose column. Proteins were eluted by a linear gradient of CE200 to CE1000, and MeCP2-containing fractions were dialyzed and dialyzed into CE200 and stored at −20 °C.

**Bandshift Analysis**—Increasing concentrations of MeCP2 (50, 100, 250, 500, and 750 ng) were bound to the CG11 probe (30) either methylated or unmethylated. The CG11 probe containing 27 CpGs was generated by digesting out an EcoRI/HindIII (New England Biolabs) fragment from the plasmid pCG11 (30). The 135-bp CG11 fragment was purified by gel electrophoresis and agarose gel extraction. The CpGs in the resulting fragments were methylated with SsIl methyltransferase (New England Biolabs), and the methylated or unmethylated CG11 probe was end-labeled with [32P]dCTP using Klenow (Roche Applied Science). The binding reactions were assembled in buffer containing 6 mM MgCl2, 5% glycerol, 1 mM EDTA, 150 mM KCl, 100 μg/ml poly(dA-dT) (10 μg/ml) at room temperature for 25 min, and then the probe was added and incubated for a further 25 min. The reactions were run on a 1.5% agarose gel and dried onto DE-81 anion exchange paper (Whatman). The bandshifts were exposed on a phosphor screen and analyzed on a Storm 840 PhosphorImager (Amersham Biosciences). Competition with cold methylated and unmethylated probe at 4 °C and 7 °C was performed, and excess was used to demonstrate the specificity of the bandshift.

**EGS Cross-linking**—An EGS (Pierce) stock solution was made fresh 25 mM in MeSO4. 5 μg of MeCP2 was incubated in CE200 (without DTT or EDTA) with increasing concentrations of EGS (0.25, 0.5, 1.0, 2.5, 5.0 mM) in a 50-μl reaction volume. BSA (5 μg, monomer) and ADH (5 μg, tetramer) were included as internal controls for cross-linking efficiency. Cross-linking reactions were carried out at room temperature.
Purification of Native MeCP2

FIG. 1. a, Sin3a is immunoprecipitated from rat brain nuclear extracts by anti-MeCP2 antibodies but not an irrelevant antibody (anti-Gal4). b, Superose 6 size exclusion chromatography of nuclear extracts (N.E.) from rat brain or NG108 cells. Fractions were analyzed by Western blotting with antibodies raised against Sin3a, MeCP2, and HDAC 1/2 as indicated. The elution profile of molecular weight standards and the void volume ($V_v$) is indicated above each Western blot. $f_0$ indicates the reaction for 15 min at room temperature. SDS-PAGE loading buffer (4×) was added, and the proteins were separated on an 8% SDS-polyacrylamide gel followed by Coomassie Blue staining to visualize the proteins. Gel Filtration and Sucrose Gradient Analysis of rMeCP2—A Superose 12 HR 10/30 gel filtration column was pre-equilibrated with gel filtration standards thyroglobin (669 kDa, $R_g = 8.5$), apoferritin (443 kDa, $R_g = 6.1$), B-amylase (200 kDa, $R_g = 5.4$), ADH (150 kDa), BSA (66 kDa, $R_g = 3.5$), and carbonic anhydrase (29 kDa, $R_g = 2$). Recombinant MeCP2 (125 $\mu$g) was loaded onto the column pre-equilibrated with buffer GF500. Fractions (0.5 ml) were collected, and 50 $\mu$g of each fraction was separated on an 8% SDS-polyacrylamide gel and Coomassie Blue-stained to visualize the proteins from the Superose 12 column, and the radius was calculated by using an equation derived from the plotted standards. For sucrose gradient sedimentation ~15 $\mu$g of rMeCP2, 50 $\mu$g of apoferritin (17.7 S), 30 $\mu$g of B-amylase (8.9 S), 50 $\mu$g of ADH (7.4 S), and 50 $\mu$g of BSA (4.3 S) were loaded onto a 13-ml linear 5–20% sucrose gradient made in 0.3M KCl, 20 mM Hepes (pH 7.9), 2 mM EDTA, 10% glycerol, 10 mM β-mercaptoethanol. The gradient was centrifuged for 19 h at 40,000 rpm in a Beckman SW40 rotor at 4 °C. Fractions (0.5 ml) were taken from the top of the gradient, trichloroacetic acid-precipitated, run on a 10% SDS-polyacrylamide gel, Western-blotted using an anti-MeCP2 antibody or on a 10% SDS-polyacrylamide gel, and Coomassie Blue-stained for the indicated standards. Densitometric analysis utilized Gene Tools Analysis Software package (SynGene), and values were adjusted for background. Intensity was plotted by fraction to determine the relative sedimentation coefficient of rMeCP2.

Molecular Weight and Frictional Coefficient Calculations—Calculations to determine molecular weight and frictional coefficient ($f_0$) were applied as described (31, 32) using Equations 1 and 2,

$$M_r = 6\pi \eta_{pol} s_{20,W} R_g N(1 - \rho_{20,W})$$

(Eq. 1)

$$f_{pol} = 6\pi \rho_{20,W} R_g s_{20,W} V(3 M/4 \pi N)^{1/3}$$

(Eq. 2)

where $R_g$ is the Stoke's radius (cm), $s_{20,W}$ is the sedimentation velocity ($S \times 10^{-13}$), $\eta_{pol}$ is the viscosity of water at 20 °C (0.01002 g s$^{-1}$ cm$^{-1}$), $N = \text{Avogadro's number (6.022 × 10^{23} mol}^{-1}$), $\rho_{20,W}$ is the density of water at 20 °C (0.9981 g cm$^{-3}$), $V$ is the partial specific volume (used 0.725 cm$^3$ g$^{-1}$).

RESULTS

Biochemical Analysis of MeCP2 in Nuclear Extracts—Previous studies have demonstrated that Xenopus (21) and mammalian MeCP2 can associate with the Sin3a complex (14), but the properties of the mammalian Sin3a/MeCP2 interaction remain to be investigated. We confirmed that in rat brain nuclear extracts Sin3a is immunoprecipitated by antibodies against MeCP2 (Fig. 1a), but the amounts are small relative to input. In order to evaluate the relative amount of MeCP2 residing in a stable Sin3a complex, nuclear extracts from either rat brain or NG108 (mouse-rat neural-glial fusion) tissue culture cells were separated by size exclusion chromatography, and the resulting fractions were analyzed by Western blot with antibodies against MeCP2, Sin3a, and HDACs 1 and 2, which are components of the Sin3a complex. To our surprise most of Sin3a-containing fractions were devoid of detectable MeCP2. The majority of Sin3a eluted over the apparent molecular mass range of 500 kDa to 2 Mda, showing significant overlap with HDAC 1/2-containing fractions (Fig. 1b). In contrast, MeCP2 eluted with an apparent molecular mass of 400–500 kDa (Fig. 1b).

Biochemical Purification of MeCP2 from Rat Brain—The predicted molecular mass of MeCP2 based on its amino acid sequence is between 52.4 and 53 kDa, depending on species. This is much smaller than the apparent molecular weight observed by size exclusion chromatography, indicating that MeCP2 may exist in a multiprotein complex. To test this possibility, a large scale biochemical purification of rat brain MeCP2 was devised (Fig. 2a). During the purification, MeCP2, Sin3a, and HDAC 1/2 were tracked by Western blotting. Superose and MonoQ columns efficiently separated MeCP2 from the majority of the Sin3a and HDAC 1/2-containing fractions, confirming that the majority of MeCP2 from nuclear extract is absent from the Sin3a complex. After four purification steps, three polypeptides were detected by SDS-PAGE, of which the middle band was identified as MeCP2 by Western blot. The final size exclusion chromatography step was applied to fractions eluted from the Ni-NTA column. SDS-PAGE of the resulting fractions (Fig. 2d) demonstrated that the apparent molecular weight of MeCP2 (400–500 kDa) remained constant over the purification. The SDS-PAGE analysis also showed a 90-kDa band whose elution profile overlapped with MeCP2. This protein was identified by mass spectrometry (data not shown) as topoisomerase I. We were able to rule out
the possibility that MeCP2 associated with topoisomerase I as MeCP2 antibodies were unable to immunoprecipitate topoisomerase I from rat brain nuclear extract (Fig. 2e). Furthermore, an independent purification starting with 10 mg of rat brain extract yielded MeCP2 with the same Superose 12 size exclusion profile but in the absence of detectable topoisomerase I (data not shown). We conclude that MeCP2 in rat brain extracts, despite its large apparent molecular weight, does not exist in a complex with other proteins.

Biochemical Analysis of MeCP2 from X. laevis Oocytes—A previous report (21) suggested that X. laevis MeCP2 cofractionated with X. laevis Sin3a (xSin3a). To revisit this observation, we made extracts from X. laevis oocytes and separated it by Bio-Rex 70 ion exchange chromatography (Fig. 3, a and b), using the procedures described previously (21). Western blots using antibodies directed against xMeCP2 and xSin3a were used to monitor the elution of these proteins (Fig. 3b). Proteins from the Bio-Rex 70 step were separated by size exclusion chromatography, and fractions were analyzed by Western blot (Fig. 3e). The xSin3a protein elutes in a complex with an apparent mass of 500 kDa to 2 mDa, whereas xMeCP2, like mammalian MeCP2, elutes with an apparent molecular mass of 400–500 kDa. In agreement with our observations in mammalian extracts (Fig. 1 and Fig. 2) and an independent study of Xenopus oocyte extracts (33), X. laevis MeCP2 does not coelute with fractions containing the majority of the xSin3a corepressor complex.

Purification of Recombinant Untagged Human MeCP2—To examine further the discrepancy between the observed molecular weight (400–500 kDa) and the monomeric molecular weight (53 kDa) of MeCP2, we developed a four-step scheme to obtain pure recombinant MeCP2, free of protein tags, from bacterial lysates (rMeCP2) (Fig. 4, a and b). To ensure that this protein was active and suitable for further biophysical analy-
Fig. 3. a, X. laevis oocyte extract was separated by Bio-Rex 70 ion exchange chromatography followed by size exclusion chromatography on a Superose 6 column. b, Western blot analysis of input oocyte extract and Bio-Rex 70 exchange 500 mM NaCl (BE500) elution using xSin3a and xMeCP2 antibodies. The lower band in the MeCP2 Western blot is likely a degradation product. c, Superose 6 size exclusion chromatography of the BE500 elution Western blotted for xSin3a and xMeCP2. Elution profile of molecular weight standards (kDa) and the void volume (V0) is indicated above.

Fig. 4. a, a four-step chromatographic purification procedure (Ni-NTA, Sp-Sepharose, Sephacryl-S300, and Mono S) was used to purify untagged recombinant MeCP2 (rMeCP2) from bacterial extracts. b, Coomassie Blue stained gel showing a typical purification of rMeCP2 with protein molecular weight marker (left) and rMeCP2 (right). c, rMeCP2 has the capacity to specifically recognize methylated (Meth) DNA. Increasing amounts of rMeCP2 (50, 100, 250, 500, and 750 ng) were incubated with methylated (left) or unmethylated (right) CG11 probe. Cold competitor DNA, either methylated (Comp M+) or unmethylated (Comp M-) were added to show specificity of bandshift.

sis, we confirmed that untagged rMeCP2 specifically interacted with a methylated probe (Fig. 4c). This bandshift was successfully competed by cold methylated competitor but not by unmethylated competitor (Fig. 4c). Most importantly, rMeCP2 maintained an apparent molecular mass of 400–500 kDa by size exclusion chromatography (Fig. 5a), identical to that seen for native MeCP2.

Hydrodynamic Analysis of rMeCP2—Since MeCP2 does not associate stably with other proteins, we entertained the following three possible explanations for the discrepancy in observed versus predicted molecular weight: 1) MeCP2 self-associates to create a homomultimeric complex; 2) MeCP2 interacts with a DNA or RNA component that alters its molecular size; and 3) MeCP2 is a monomeric molecule with an abnormal shape (radius). To address whether MeCP2 might self-associate, chemical cross-linking experiments using glutaraldehyde (data not shown) or EGS were carried out. Although the control protein ADH was cross-linked as expected to give multimers, MeCP2 showed no evidence of multimerization (Fig. 5a). A slight reduction in the size of treated MeCP2 was observed, presumably due to compaction of the protein as a result of intramolecular cross-links. To determine whether MeCP2 is associated with a DNA or RNA component, purified rat MeCP2 was treated with Benzonase, which contains both DNase and RNase activity. Nuclease treatment had no effect on the apparent molecular weight as determined by size exclusion chromatography (data not shown).

To explore more thoroughly the biophysical properties and molecular shape of MeCP2, we carried out hydrodynamic analysis. Seigel and Monty (31) demonstrated that the mass of a protein (or protein complex) can be determined accurately by combining size exclusion chromatography and sucrose gradient sedimentation. rMeCP2 was fractionated by size exclusion chromatography, and the peak elution of protein was determined by the absorbance at 280 nm. This value was used to calculate the apparent radius of MeCP2 to ~6.15 nm (Fig. 5b). To determine the sedimentation coefficient, rMeCP2 was separated on a 5–20% sucrose gradient and an approximate sedimentation coefficient of ~2.28 S (Fig. 5c) was observed. By combining the values from our hydrodynamic analysis, we calculated the derived mass of MeCP2 in solution (Table I). The derived mass (57.8 kDa) falls within ~10% of the predicted monomeric molecular weight of MeCP2 (52.4 kDa). Furthermore, the calculated frictional ratio of ~2.41 indicates that rMeCP2 is a highly elongated molecule (34).

DISCUSSION

Mammalian MeCP2 can interact with Sin3a as a recombinant protein in vitro or by coimmunoprecipitation from nuclear extracts (14). Also, it is reported that Xenopus MeCP2 partially cofractionates with xSin3a, suggesting that the proteins may exist in a stable complex (21). In this study, we have investigated the mammalian MeCP2/Sin3a interaction biochemically, and we found that MeCP2 does not exist in a stable Sin3a complex. Reinvestigation of the biochemical properties of Xenopus oocyte MeCP2 led us to conclude that xMeCP2 does not coelute with the majority of xSin3a either. Therefore, neither amphibian nor mammalian MeCP2 appears to form a stable complex with Sin3a in cellular extracts.

Biochemical purification of MeCP2 from rat brain demon-
strated that MeCP2 does not stably associate with other proteins. Nevertheless, purified rat brain MeCP2 had an apparent molecular mass by size exclusion chromatography of 400–500 kDa, which is nearly 10 times its true molecular mass (52 kDa). By generating recombinant untagged MeCP2, we were able to study in more detail the biophysical properties of MeCP2. rMeCP2 maintained the same large apparent molecular weight as the native rat and *X. laevis* proteins. This large apparent molecular weight was not because of an RNA or DNA component or self-association. Based on the experimentally determined radius and sedimentation coefficient, we were able to deduce the native molecular weight of rMeCP2 and determine the frictional ratio, which indicates molecular shape (31, 32). The derived molecular weight fits closely to the theoretical molecular weight of MeCP2, indicating that, despite its aberrant gel filtration properties, MeCP2 exists as a monomeric protein in solution. The frictional ratio of ∼2.41 is consistent with an elongated molecule. Globular proteins invariably show frictional ratios of <1.5, whereas elongated proteins have frictional ratios in excess of 2.0 (34). As size exclusion chromatography separates proteins largely based on radius (i.e., shape), this finding can account for the large apparent molecular weight of native MeCP2. MeCP2 has 11.5% proline by amino acid composition, and the inherent rigidity of this amino acid (35) may in part give rise to the nonglobular nature of MeCP2. The biological significance of the elongated MeCP2 molecular shape is currently unclear.

Two *bona fide* target genes whose transcriptional activity responds to MeCP2 have been identified: *xHairy2a* (26) and *Bdnf* (23, 24). Sin3a is also localized to each of these promoter regions by chromatin immunoprecipitation analysis (23, 24, 26). These findings provide support for the idea that MeCP2 and Sin3a interact on DNA templates. It is also noteworthy that loss of MeCP2 from the *Bdnf* promoter results in a loss of local histone methylation (23, 24), in agreement with previous evidence that MeCP2 can interact with the histone methyltransferase activity (17). To reconcile these results with the present findings, we propose that MeCP2 enters into a stable association with cofactors that modulate gene expression only when bound to DNA. Given recent evidence for a gene-specific role of MeCP2, it is possible that sustained interactions between MeCP2 and its protein partners rely on the specific DNA sequence architecture of its cognate target promoters. Thus monomeric MeCP2 may act as a multifunctional repressor that recruits Sin3a and perhaps other corepressors in a template-dependent manner. The biochemical properties of MeCP2 support this view. Efficient extraction of MeCP2 from nuclei requires more stringent conditions than does extraction of its methyl-CpG-binding counterparts MBD2 and Kaiso, which are components of large molecular weight chromatin remodeling complexes (30, 36). This biochemical distinction is compatible with the view that methyl-CpG-binding proteins belonging to free multiprotein complexes interact with DNA in a transient manner, whereas MeCP2 remains tightly associated with chromatin and recruits corepressors in its DNA-bound context.

Our findings may have relevance to Rett syndrome, which is

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TABLE I  
Hydrodynamic Properties of rMeCP2

| Theoretical mass | Sedimentation coefficient | Stokes radius | Frictional ratio | Derived mass |
|------------------|--------------------------|---------------|----------------|--------------|
| Da               | $s_{20, w}$              | $nm$          | $f_0$          | Da           |
| 52,437           | −2.28                    | −6.15         | 2.41           | 57,880       |

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Fig. 5. a, BSA (monomer), rMeCP2, and ADH (tetramer) were cross-linked with increasing concentrations of EGS (0.25, 0.5, 1.0, 2.5, 5.0 mM). rMeCP2 did not cross-link, whereas the ADH monomer when cross-linked formed several multimeric species (arrowheads right). b, rMeCP2 was separated by Superose 12 size exclusion chromatography, and fractions were separated by SDS-PAGE and stained with Coomassie Blue. Elution profile of molecular weight standards (kDa) and of the void volume ($V_v$) is indicated above, and calculated radius in nm below. c, rMeCP2 was separated by 5–20% sucrose gradient sedimentation, and the fractions were separated by SDS-PAGE and Western-blotted by using anti-MeCP2 antibodies. The sedimentation profile of protein standards are indicated above, and the calculated sedimentation value in Svedberg units ($S$) is shown below.
caused by mutations in the MECP2 gene (19). The absence of an exclusive MeCP2-Sin3a repression complex leaves open the possibility that other protein partners, including those reported previously (14, 17, 27–29) and others as yet unidentified, play an important role in mediating the effects of MeCP2 on gene expression. Given the broad distribution of Rett mutations within the MECP2 coding sequence, it seems likely that the surface of MeCP2 is capable of multiple intermolecular interactions that are of relevance to neuronal function. Understanding these interactions at clinically relevant MeCP2 target genes is a challenge for the future.

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Note Added in Proof—Since publication of this paper, we have become aware of an earlier study (von Kries et al., 1994) reporting that the chicken protein ARBP has an elongated shape based on its Stokes radius. ARBP was subsequently found to be chicken MeCP2 (Weitzel et al., 1997). The conclusion of von Kries and colleagues is confirmed by this aspect of our study and should have been cited (von Kries, J. P., et al. 2000).

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