X-ray Crystal Structure of the Trimeric N-terminal Domain of Gephyrin

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Gephyrin is a ubiquitously expressed protein that, in the central nervous system, forms a submembrane scaffold for anchoring inhibitory neurotransmitter receptors in the postsynaptic membrane. The N- and C-terminal domains of gephyrin are homologous to the \textit{Escherichia coli} enzymes MogA and MoeA, respectively, both of which are involved in molybdenum cofactor biosynthesis. This enzymatic pathway is highly conserved from bacteria to mammals, as underlined by the ability of gephyrin to rescue molybdenum cofactor deficiencies in different organisms. Here we report the x-ray crystal structure of the N-terminal domain (amino acids 2–188) of rat gephyrin at 1.9-Å resolution. Gephyrin-(2–188) forms trimers in solution, and a sequence motif thought to be involved in molybdoepipin binding is highly conserved between gephyrin and the \textit{E. coli} protein. The atomic structure of gephyrin-(2–188) resembles MogA, albeit with two major differences. The path of the C-terminal ends of gephyrin-(2–188) indicates that the central and C-terminal domains, absent in this structure, should follow a similar 3-fold arrangement as the N-terminal region. In addition, a central \( \beta \)-hairpin loop found in MogA is lacking in gephyrin-(2–188). Despite these differences, both structures show a high degree of surface charge conservation, which is consistent with their common catalytic function.

Gephyrin, a protein of 93 kDa, was identified originally by copurification with the inhibitory glycine receptor from rat spinal cord (1, 2). Biochemical and gene targeting experiments have revealed a crucial role for gephyrin in the clustering of both the glycine receptor and related \( \gamma \)-aminobutyric acid type A receptors at the postsynapse (3–7). Gephyrin has been shown to anchor glycine receptors to the subsynaptic cytoskeleton (8) by interacting with both polymerized tubulin (9) and the cytoplasmic loop region of the glycine receptor \( \beta \)-subunit (10, 11). Similar interactions are also thought to mediate the synaptic localization of \( \gamma \)-aminobutyric acid type A receptors (12). A high concentration of gephyrin at the cytoplasmic face of inhibitory postsynaptic membrane specializations (13, 14) is consistent with gephyrin acting as a synaptic receptor scaffolding protein (reviewed in Ref. 15).

In addition to its synaptic function in the central nervous system, gephyrin has been shown to be involved in the biosynthesis of the molybdenum cofactor (Moco),\(^1\) which is a crucial component of different enzymes catalyzing important redox reactions (16). This was first highlighted by the homology of gephyrin to the \textit{Escherichia coli} enzymes MogA and MoeA, which are known to be implicated in Moco biosynthesis (12). Homology lies within the N- and C-terminal domains of gephyrin, which are separated by an intervening region of \( \sim 170 \) amino acids that harbors multiple protein interaction domains (17). Similar multi-domain proteins comprising both MogA and MoeA homologous domains are found in \textit{Drosophila melanogaster} (cinnamon) (18) and \textit{Arabidopsis thaliana} (Cntx1) (19), in which they have also been shown to be implicated in Moco biosynthesis.

Moco consists of mononuclear molybdenum coordinated by the dithiolene moiety of tricyclic pyranopterins such as molybdopterin. In \textit{E. coli}, Moco is synthesized by multiple enzymes (20, 21) that catalyze the conversion of GTP via precursor \( Z \) to molybdopterin (MPT) followed by the insertion of molybdenum (22–24). Gephyrin has been shown to bind MPT with high affinity and to restore Moco biosynthesis in \textit{E. coli} MogA mutants as well as in Moco-deficient L929 fibroblasts and plants (25). These complementation assays indicate that the N-terminal domain of gephyrin is functionally equivalent to MogA in catalyzing the transfer of molybdenum to MPT (25). The MoeA-like C-terminal domain, in contrast, seems either essential for the conversion of precursor \( Z \) to Moco, because MoeA mutants of \textit{E. coli} accumulate this precursor (26), or may have a function in the transformation of molybdenum into thiomolybdate (27). Thus, in gephyrin, gene fusion has allowed the recruiting of two independent enzymatic activities and a membrane protein scaffolding function into a single polypeptide.

The crucial role played by gephyrin in Moco biosynthesis was revealed previously by the analysis of gephyrin knockout mice, which in addition to an impaired synaptic localization of inhibitory neurotransmitter receptors, shows only basal levels of Moco-dependent enzyme activities in peripheral organs (5, 7). Notably, hereditary Moco deficiencies in humans cause symptoms that are similar to those observed upon the loss of inhibitory neurotransmission in gephyrin knockout mice (5). Both are characterized by severe neurological abnormalities such as increased muscle tone, mental retardation, microcephaly, myoclonus, and tonic-clonic seizures (28). Moreover, a microdeletion in the gephyrin gene has been identified recently in a

\(^1\) The abbreviations used are: Moco, molybdenum cofactor; MPT, molybdopterin; bicine, \( N,N' \)-bis(2-hydroxyethyl)glycine.
Moco-deficient patient (29). However, the lack of molybdoenzyme activities such as sulfite oxidase in affected patients as well as in gephyrin knockout mice (5) could cause neurological disorders through the accumulation of toxic metabolites (28, 30). Therefore, it is not yet clear whether the role of gephyrin in Moco biosynthesis and receptor clustering at the postsynaptic membrane are two interdependent or completely separate processes.

Here we present the three-dimensional structure of the N-terminal domain of gephyrin from rat and show that it forms a trimer both in solution and in the crystalline state. Overall, the structure of gephyrin displays high homology to that of E. coli MogA. Differences include a MogA insertion of unknown function as well as different paths of the C-terminal ends. The predicted orientations of the C-terminal linker regions of gephyrin suggest 3-fold symmetry for the entire molecule. A highly conserved region is discussed as being the putative active site based on sequence homologies and mutagenesis data.

EXPERIMENTAL PROCEDURES

Gephyrin Cloning, Expression, and Purification—Gephyrin cDNA encoding residues 2–188 was amplified by polymerase chain reaction using synthetic oligonucleotides and cloned into the expression vector pSET (Invitrogen) using the restriction sites NheI and EcoRI. This strategy placed a His tag derived from the vector on the N terminus. The correct sequence of the construct was verified by DNA sequencing. Expression was achieved in E. coli BL21 (DE3) cells (Invitrogen) following standard protocols. Expression of gephyrin (2–188) was induced with 1 mM isopropyl-β-D-thiogalactopyranoside for 3 h. The cells were harvested by centrifugation and lysed by sonication in a buffer containing 50 mM Tris, pH 8.8, 100 mM NaCl, and 5 mM β-mercaptoethanol. The supernatant was cleared by centrifugation and loaded on to a Ni2+-Sepharose (Amersham Pharmacia Biotech) column pre-equilibrated in lysis buffer. The column was then extensively washed with lysis buffer, and a 0–500 mM imidazole gradient in lysis buffer was applied to elute gephyrin (2–188). Fractions containing pure gephyrin-(2–188) were concentrated and loaded onto a Superdex 200 (Amersham Pharmacia Biotech) column in a buffer containing 20 mM bicine, pH 9.3, and 100 mM NaCl. The total yield of gephyrin-(2–188) was 60 mg from 1 liter of the E. coli culture.

Chemical Cross-linking Analysis—Gephyrin-(2–188) was incubated with glutaraldehyde concentrations as indicated (Fig. 1A). In buffer containing 50 mM Hepes, pH 8.0, and 100 mM NaCl at room temperature for 30 min. The reaction was quenched by the addition of 50 mM Tris, pH 8.0, for 30 min at room temperature. Samples were separated by 10% SDS-polyacrylamide gel electrophoresis under reducing conditions, and the bands were visualized by Coomassie Brilliant Blue staining.

Detection of Molybdenopterin Bound to Gephyrin-(2–188)—Molybdenopterin bound to gephyrin was analyzed according to Johnson and Rajagopalan (26) with minor modifications. 1 mg of gephyrin-(2–188) was oxidized overnight at room temperature by the addition of acidic iodine solution (1% I2/2% KI/1% HCl). Residual iodine was reduced by the addition of ascorbic acid, and 1% Tris base was added to adjust the pH to 8.5. After centrifugation, alkaline phosphatase (2 units, Sigma-Aldrich) and 7 mM MgCl2 were added to the supernatant for dephosphorylation (4 h at 37 °C). The sample was applied to a 0.5-mM QA-Sephadex (acetate form) column and washed (5 ml of water), and form A dephospho was eluted with 5 ml of 100 mM acetic acid. High pressure liquid chromatography was performed using a C18 column (Hypersil ODS, 5 μm, 25 cm) coupled to a fluorescence detector (370/450 nm). 1 ml of the QA-E-Sephadex eluate was injected and eluted with 50 mM ammonium acetate/7% methanol at 1 ml/min. The lower limit for quantitative measurement of the form A dephospho derivative of molybdenopterin was 0.03 pmol/injection corresponding to about 0.3 pmol of MPT/sample.

Crystalization, Data Collection, and Data Processing—Gephyrin-(2–188) was crystallized at a concentration of 18 mg/ml using the hanging drop vapor diffusion method. Crystals grew in drops of a mixture of 1 μl of protein solution and 1 μl of reservoir solution containing 25% (w/v) polyethylene glycol 1500, 0.1 M sodium citrate, pH 5.6, and 10% isopropanol. Cryotreatment was performed by a quick transfer of the crystals into a solution containing 30% (w/v) polyethylene glycol 1500, 0.1 M sodium citrate, pH 5.6, 10% isopropanol, and 20% 2-methyl-2,4-pentanediol followed by flash cooling in a gaseous nitrogen stream. Data to 1.9 Å were collected at the European Synchrotron Radiation Facility (Grenoble) beamline ID14 EH2 at a wavelength of 0.933 Å, with a 2θ oscillation range. X-ray data were processed using the program packages DENZO (31), SCALEPACK (31), and TRUNCATE (32). The crystals belong to the rhombohedral space group R3 and contain 1 molecule/asymmetric unit (2.32 Å2/Da, Matthews coefficient (33)). The unit cell dimensions in the R3 hexagonal setting were a = 65.77 Å and c = 114.40 Å. The data collection statistics are summarized in Table I.

Structure Determination—The structure of gephyrin was solved by molecular replacement using the E. coli MogA structure (34) as a search model, in which nonidentical residues were exchanged to alanine. The program package AMoRe (35) was employed using data between 15 and 4.5 Å. This resulted in a clear peak with a correlation coefficient of 38.7 and an R-factor of 45.0 after rigid body refinement, which corresponded to the correct solution. Automated model building and refinement were performed with the program ARP/wARP (36) and REFMAC (32), and 7% of the reflections were selected at random and set aside for the R-free calculation (37). Model building was completed by tracing missing areas (helix 1, residues 25–33; 43–52, helix 8) manually with the program TURBO (38) in several steps alternated with cycles of automated refinement using data to 1.9 Å. The automated refinement included both positional and restrained B-factor refinement applying bulk solvent correction with CNS (39, 40). Solvent molecules were included in the last stages of interactive model correction and automatic refinement. The final model was refined to an R-factor of 19.9 (Rfree = 21.9) (Table II). 91.6% of all residues lie in the most favored regions of the Ramachandran plot, and no residues are located in disallowed areas, as determined with the program PROCHECK (41).

RESULTS

Oligomerization State of Gephyrin-(2–188)—The N-terminal domain of gephyrin (residues 2–188) expressed in E. coli forms trimers in solution as detected by gel filtration chromatography. The recombinant protein (calculated molecular mass of 21.2 kDa) eluted at a volume of 13.2 ml from a Superdex 200 gel filtration column compared with a volume of 13.6 ml determined for bovine serum albumine (molecular mass of 66 kDa) (data not shown); this is consistent with an oligomeric structure. Chemical cross-linking experiments corroborate this conclusion. Incubation of gephyrin-(2–188) with increasing concentrations of the cross-linking reagent glutaraldehyde caused the appearance of two adducts, the sizes of which corresponded to dimeric and trimeric forms of gephyrin-(2–188) (Fig. 1).

Structure Determination—The structure of gephyrin-(2–188) expressed in E. coli was crystallized in the rhombohedral space group R3. The crystals diffracted x-rays to 1.9 Å resolution (Table I) and contain 1 molecule/asymmetric unit. The three-dimensional structure of gephyrin-(2–188) was solved by molecular replacement using the E. coli MogA structure (34) as a search model. The final model has been refined to a crystallographic R-factor of 19.9 (Rfree = 21.9) with good stereochemistry (Table II) and includes residues 13–181 and 90 water molecules. The N-ter-
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TABLE I
X-ray data collection statistics

|                | Values in parentheses are for last shell limits, 1.97–1.90 Å. |
|----------------|-------------------------------------------------------------|
| Wavelength     | 0.933 Å                                                     |
| Resolution     | 30–1.9 Å                                                    |
| Completeness (%)| 99.6 (97.6)                                                 |
| Total reflections| 40,270 (3601)                                               |
| Unique reflections| 14,512 (1427)                                              |
| Redundancy     | 2.8 (2.5)                                                  |
| \(R_{merge}\)  | 5.9 (33.2)                                                  |
| \(\text{Vol}l\)     | 17.2                                                        |

TABLE II
Refinement statistics

|                | rms, root-mean-square. |
|----------------|------------------------|
| Resolution range | 30–1.9 Å                |
| Number of reflections | 14,511                 |
| \(R_{factor}\) | 19.9%                  |
| \(R_{free}\) | 21.9%                  |
| Residues | 169 (out of 186)       |
| Number of protein/solvent atoms | 1280/90                |
| Average B factor | 28.8 Å2                |
| Rms \(\Delta\) bond length | 0.006 Å                |
| Rms \(\Delta\) bond angles (°) | 1.31°               |
| Ramachandran plot | residues in most favoured and additionally allowed regions 100%^b |

a \(R_{factor} = \sum_{hkl} |F_{calc} - k|F_{calc}|/\sum_{hkl} |F_{calc}| .

b As defined in PROCHECK (41).

The structural differences mediated by the sequence insertion in MogA as well as the different directions of the C-terminal ends result in major differences in the exposed surfaces of the trimers (Fig. 4, yellow arrows). The differences in the electrostatic surfaces of gephyrin-(2–188) and MogA noted on the top of the molecule can be attributed to the insertion of the \(\beta\)-hairpin loop as well as the different paths of the C termini. Gephyrin shows a prominent negatively charged surface as well as small positively charged islands on the top of the trimer (Fig. 4a) as compared with a reduced negative surface charge of MogA (Fig. 4b). Interestingly, the bottom of the trimer that forms the interface to the intervening domains of gephyrin is characterized also by a mostly negatively charged surface and closely resembles the electrostatic profile of the corresponding surface of MogA (Fig. 4, c and d).

The high degree of sequence similarity and the conserved function of the N-terminal domain of mammalian gephyrin and E. coli MogA suggest that the homologous domains from Caenorhabditis Q23069, Drosophila cinnamon, and Arabidopsis Cnx1 may have similar overall architectures (Fig. 3). Significant structural differences can therefore only be expected at the respective N- and C-terminal ends that are longer or form linkers to additional domains. The sequence alignment shown in Fig. 3 highlights this conservation and indicates a set of conserved residues that may be implicated in the enzymatic
function of these proteins.

Proposed MPT Binding Site—The highest sequence conservation among all known MogA-related polypeptides (Fig. 3) maps to a loop region connecting β-strand 3 and helix 4 (Fig. 5b). The conserved sequence motif GGTG in this loop region, which connects back to α-helix 4, forms the base of a 15 × 14 × 23-Å wide cavity flanked by residues from the tip of β-strand 1, α-helix 1, and the loop connecting β-strand 2 and α-helix 3 (Fig. 5b). This cavity has been hypothesized as forming the MPT binding site because of its stringent conservation and the occurrence of residual density in close proximity to the GGTG motif in MogA (34). In the present structure, no residual density that could be attributed to MPT binding was found in this area. This is in agreement with no measurable detection of any form A dephospho derivative of molybdopterin by spectroscopic methods in the sample used for crystallization (data not shown; see “Experimental Procedures”). However, mutagenesis experiments strongly implicate this region in MPT binding, and three mutations in the MogA-like domain of Arabidopsis Cnx1 have been reported recently to affect Moco biosynthesis. First, mutation of Asp-515 to Asn (equivalent to gephyrin Asp-61), a residue that is conserved among all MogA-like sequences (Figs. 3 and 5b), has been shown to increase MPT binding and apparently block insertion of molybdenum into MPT (42). In the gephyrin structure, Asp-61 is at the base of the proposed MPT binding site and points toward Gly-87, which is consistent with substitutions at this site affecting MPT binding. In addition, Asp-61 is surrounded by two highly conserved aspartate residues, Asp-24 and Asp-94 (Figs. 3 and 5b). Notably, the MogA mutations D49A and D82A (equivalent to gephyrin Asp-61 and Asp-94) have also been suggested to bind MPT tighter than the wild-type protein. Collectively, these data underscore the important role of aspartate residues in the catalytic mechanism that transfers molybdenum to MPT (34, 42).

A second class of mutations in Cnx1, V557G (equivalent to gephyrin Val-103) and N597L (equivalent to gephyrin Asn-143) (Figs. 3 and 5b), causes a dramatic decrease in MPT binding (42). Val-103 (α-helix 4) points into a hydrophobic pocket surrounded by the residues Leu-70, Ile-71, and Ile-141. Its position is also determined by hydrogen bonding of Arg-36 to the back-
bone oxygen of Val-103. Asn-143 is part of the central parallel β-sheet and locates to β-strand 6 next to the antiparallel β-strand 5. Therefore, substitutions at these positions may have secondary effects on MPT binding by affecting the overall structure of the binding region.

Interestingly, in the trimer the proposed MPT binding site of Fig. 3. Sequence alignment of the Mog-A like domains from gephyrin (Rattus norvegicus), Caenorhabditis elegans protein (data base code Q23069, 35.1% homology to gephyrin), cinnamon (D. melanogaster, 41.6% homology), Cnx1 (A. thaliana, 41.97% homology), and MogA (E. coli, 28.5% homology). Secondary structure elements are shown for rat gephyrin above the sequence and for E. coli MogA below the sequence (including the respective numbering). Residues conserved in all proteins are boxed, and residues involved in trimer interface contacts are highlighted in green. The potential insertions of two alternatively spliced sequence cassettes are indicated by arrows after residues Thr-21 (cassette 1, ALRAMSPLGTPFFV SHKFGC) and Glu-98 (cassette 5, KFPFTPFGCLQRG).

Fig. 4. Electrostatic potential maps of gephyrin-(2–188) (a and c) and MogA (b and d). Regions where electrostatic potential < −30 k_BT are shown in red and those > +30 k_BT are shown in blue (k_BT, Boltzmann constant; T, absolute temperature). The surface changes between the gephyrin-(2–188) and the E. coli MogA structures caused by the insertion of a loop in MogA and the different paths of the C-terminal ends are indicated by yellow arrows. The surfaces viewed from the top (a and b) and bottom (c and d). White arrows indicate slight differences in the trimer interface as seen from the top.
one monomer is also close to α-helix 7 of a neighboring molecule (Fig. 2a, black arrow). There, the tip of the loop region containing the conserved GGTG motif is coordinated by hydrogen bonds between Arg-106 from one monomer and the backbone carbonyls of Phe-90 and Ala-91 of the adjacent monomer. Phe-90 is one of the conserved trimer interface residues (Fig. 3), which packs into a hydrophobic pocket contributed by residues from two molecules. A similar coordination is seen in the MogA structure, in which Arg-94 (gephyrin homologue Arg-106) contacts the backbone carbonyl of Gly-77 from a neighboring monomer. Similarly, Pro-78 (gephyrin homologue Phe-90) packs into a hydrophobic pocket at the same site, and the residues involved in these interactions are conserved among MogA-like sequences (Fig. 3). These shared features may provide a possible explanation for the conserved trimeric structure of these enzymatically active polypeptides.

**Differential Splicing Produces Three Variants of the N-terminal Domain**—Several splice variants of gephyrin that result from insertion or deletion of different exonic sequence “cassettes” of the highly mosaic gephyrin gene have been identified (17, 43, 44). The structure of gephyrin-(2–188) is derived from a splice variant containing cassette 2 (exon 3) within the N-terminal domain and cassette 6 (exon 19) within the C-terminal MoeA-like domain; most of the data available on the postsynaptic function of gephyrin have been obtained with this specific variant. Two other splicing products with possible insertions in the N-terminal domain analyzed here have been described: cassette 1 (exon 2) is spliced in between residues Thr-21 and Val-22 and cassette 5 (exon 13) is spliced in between residues Glu-98 and Ala-99, respectively (Figs. 2 and 3). Thr-21 locates to the end of β-strand 1, which is sandwiched between β-strands 2 and 3 at a central position of the structure (Fig. 2, a and b). The structure of gephyrin-(2–188) shows that the insertion of cassette 1 could be accommodated by extending the loop region connecting β-strand 1 and α-helix 1 without disturbing the present topology, including trimer formation. However, it is conceivable that local changes at the end of β-strand 1 could affect the adjacent sequence GGTG that forms part of the proposed binding site for MPT. The second splicing site, Glu-98, is solvent-exposed in α-helix 4, and an insertion at this position (cassette 5; Fig. 2, a and b) may be achieved without global structural changes simply by extending the linker region between α-helix 4 and β-strand 4. Again, local changes induced by this insertion may also alter the structure of the MPT binding site (Figs. 2 and 5b). Insertion of cassette 5 into α-helix 4 will lead to an exposed extension at the lower surface of the triangular oligomer, which in turn could influence the orientation of the spacer region connecting to the intervening domain as well as the interface between the N-terminal and intervening domains.
A soluble form of the N-terminal domain of gephyrin comprising residues 2–188 was produced in E. coli and crystallized. The data presented here show that gephyrin forms trimers in solution with a highly conserved trimer interface that closely resembles the one observed in E. coli MogA (34). This strongly indicates that trimer formation may be important for the enzymatic function of both proteins. Interestingly, the proposed MPT binding site is formed by a conserved loop region containing the residues GGTG, which are part of a cavity of one monomer that is close to the α-helix 7 of a neighboring molecule. Conserved residues are found to employ similar interactions to maintain the structural coordination of this loop by two neighboring monomers in both gephyrin-(2–188) and E. coli MogA structures. It is conceivable therefore that the conserved function of MogA-like domains in Moco biosynthesis, namely the binding of MPT and the transfer of molybdenum, involves a catalytic center formed at the sites of intersubunit contact within the homotrimeric protein. Presently, mutants of the trimer interface, which would generate monomeric gephyrin or E. coli MogA, are not available to test the role of oligomerization for enzymatic function. However, oligomerization of native gephyrin has been observed previously in sedimentation studies (45) and suggested to play a role in the clustering of inhibitory receptors at developing postsynaptic membrane specializations (15).

Comparison of the crystal structures of the N-terminal domain of gephyrin and E. coli MogA reveals a very high conservation of the basic protein fold from bacteria to mammals, a finding that is consistent with the conservation of Moco biosynthesis. Both structures reveal a similar molecular surface involved in MPT binding and implicate the same key residues in their enzymatic function as deduced from mutagenesis data (34, 42). Functional conservation has been also demonstrated via complementation of MogA function in Moco-deficient E. coli by gephyrin (25) or Cnx1 (42). Likewise, the expression of gephyrin rescues the Cnx1 mutant of Nicotiana plumbaginifolia (25). Moco synthesis is a multi-step pathway (20, 21) that ultimately catalyzes the conversion of GTP via a precursor Z to MPT (22, 23). Both the MogA and the MoeA domains from E. coli as well as their counterparts from mammals (gephyrin) and plants (Cnx1) have been shown to bind MPT, and there is evidence that both domains must act interdependently to catalyze the efficient transfer of molybdenum to MPT (25). This may explain the genetic fusion of both activities in eukaryotic proteins such as Drosophila cinnamon, Arabidopsis Cnx1, and mammalian gephyrin.

The structures of gephyrin-(2–188) and E. coli MogA show three structural differences, namely an insertion of a β-hairpin loop in MogA with unknown function, changes in the loop region connecting α-helix 2 and β-strand 2, and a different orientation of their C-terminal ends. The C-terminal end of gephyrin-(2–188) indicates that the N-terminal domain is connected to the intervening region via a short helix in an arrangement suited to maintain the 3-fold symmetry for the entire molecule. Interestingly, the bottom surface of gephyrin-(2–188) shows an overall negatively charged electrostatic surface that closely resembles that of MogA. The conservation of this surface is remarkable, because it seems solvent-exposed in MogA but part of the interface in gephyrin. The disorder of the last seven residues in the gephyrin structure (2–188) as well as the presence of an adjacent proline-rich sequence also indicate that the intervening domain may not be tightly packed against the N-terminal domain. This may be important to accommodate the functions of gephyrin at the postsynaptic membrane (15).

Gephyrin has dual functions as an enzymatic protein in Moco biosynthesis, explaining its wide tissue distribution (17), and as a scaffolding protein at postsynaptic membranes in the central nervous system. There it regulates inhibitory receptor clustering by forming a subynaptic protein scaffold, which anchors inhibitory receptors to the cytoskeleton. In addition, gephyrin may be involved in signal transduction processes that control translation at synaptic sites (15). Sequencing of different gephyrin cDNAs and analysis of the genomic gene structure have revealed extensive alternative splicing that may account for the functional diversity of gephyrin at the level of protein subdomain organization (17, 43, 44). The gephyrin isoform that contains cassettes 2 and 6 has been shown to bind to the β-subunit of the glycine receptor (7, 11). Insertion of cassette 5 into the N-terminal domain abolishes this interaction (44), implicating the N-terminal MogA-like domain indirectly in glycine receptor subunit clustering. However, we could not detect any binding of a 51-amino acid peptide corresponding to most of the M3 loop of the glycine inhibitory receptor (11) to the N-terminal domain of gephyrin-(2–188) in vitro (data not shown). This suggests that the N-terminal domain of gephyrin may not be involved directly in glycine receptor clustering. Therefore, the splicing of casette 5 into α-helix 4 most likely results in an exposed extension at the bottom part of the gephyrin-(2–188) oligomer and may thus influence the linker region connecting the N-terminal domain to the intervening domain; this in turn may alter the conformation required for glycine receptor binding. Two other isoforms, both of which lack the first 114 residues (43), do not bind the glycine receptor β-subunit loop sequence (44). Again, the resulting different fold of the residual N-terminal domain may influence the conformation of the spacer or linker sequences or even the entire intervening region. No data on the possible insertion of casette 1 into β-strand 1 are yet available, and its effects on the receptor-binding properties and/or Moco biosynthesis activity of gephyrin have yet to be determined.

The structure of the trimeric N-terminal domain of gephyrin reported here serves as a starting point to understand this multifunctional protein. Further structural analysis will be required to fully understand the function of gephyrin in Moco biosynthesis and at the synapse, including a precise mapping of molecular interactions of molecules such as tubulin, profilin, PIN, RAPT1, and collybistin (reviewed in Ref. 15) with the different subdomains of gephyrin. Such experiments should also unravel whether the N-terminal domain of gephyrin analyzed here may have functions other than a role in Moco biosynthesis.

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