Memo Is Homologous to Nonheme Iron Dioxygenases and Binds an ErbB2-derived Phosphopeptide in Its Vestigial Active Site

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HER2/ErbB2 is a member of the epidermal growth factor (EGF/ErbB) family of receptor tyrosine kinases, which in humans includes the EGF receptor (EGF receptor/ErbB1), ErbB3 (HER3), and ErbB4 (HER4) (1, 2). ErbB signaling regulates cell growth, differentiation, and migration, and loss of any of the ErbBs is embryonic lethal in mice with defects found in multiple organ systems (3). ErbB signaling depends on the presence of the Shc adaptor protein, and the association of Memo with ErbB2 was thought to be indirect (10).

To investigate the role of Memo in ErbB2 signaling, we have determined the 2.1 Å crystal structure of human Memo. We show that Memo is structurally homologous to a class of non-heme iron dioxygenases, but we are unable to detect metal binding or enzymatic activity. Memo binds directly to an ErbB2-derived peptide encompassing Tyr-1227 using its vestigial enzymatic active site. Memo thus represents a new class of phosphotyrosine-binding protein.

**EXPERIMENTAL PROCEDURES**

**Protein Expression and Purification**—A cDNA encoding full-length human Memo was subcloned into the pHT vector, which adds a cleavable N-terminal hexahistidine tag (11). Memo expression was induced in *Escherichia coli* with 0.4 mM isopropyl-1-thio-β-D-galactopyranoside overnight at 22 °C, and Memo protein was purified using a nickel-chelating column. The N-terminal His tag was removed by overnight treatment with tobacco etch virus protease at 4 °C followed by a Uno-Q column. The Uno-Q flow-through was concentrated and chromatographed on a Superdex 75 size exclusion column. Fractions containing monomeric Memo were exchanged into distilled water with 1 mM dithiothreitol and concentrated to 5
mg/ml. Selenomethionine-containing Memo was produced by expression in *E. coli* strain B834(DE3) grown in the presence of selenomethionine and purified similarly to native protein.

**Crystallization and Structure Determination**—Crystals were grown by the hanging drop method by mixing protein 1:1 with 22.5% polyethylene glycol 3350/0.1M MES, pH 5.5, and suspending the mixture over the polyethylene glycol solution at 20 °C. Crystals belong to space group P2₁2₁2₁ with cell dimensions *a* = 139.8 Å, *b* = 88.8 Å, and *c* = 98.1 Å and contain four molecules in the asymmetric unit. Diffraction data were collected from a selenomethionine crystal at the selenium absorption edge at beamline X25 of the National Synchrotron Light Source at Brookhaven National Laboratory (see Table 1). Diffraction data from a native crystal soaked for 5 min in pH 8.0 mother liquor containing 5 mM ZnCl₂ were collected at SGX-CAT at the Advanced Photon Source at Argonne National Laboratory. Diffraction data were processed using the HKL package (12), 32 selenium sites were found by the program SnB (13), and the program SOLVE (14) was used to refine the selenium positions. The program RESOLVE (14) was used for density modification, and the program O (15) was used for model building. The resulting atomic model was refined against selenomethionine data extending to 2.1 Å Bragg spacings using CNS (16) and REFMAC (17).

**Purification of Shc**—p52Shc was cloned into a pET21 vector, which adds a C-terminal hexahistidine tag. Shc was expressed in BL21(DE3) (codon plus R/L) *E. coli* by adding 1 mM isopropyl-1-thio-β-D-galactopyranoside for 4 h at 4 °C and purified over a HisTrap HP column (Amersham Biosciences). Shc-containing fractions were exchanged against Tris-HCl, pH 8.4, 150 mM NaCl, 2.5 mM CaCl₂ buffer.

**Peptide Pull-down Assay**—Phosphorylated and nonphosphorylated YD peptides (SPAFDLNYWDQNSSEKKK) and YC peptides (GGAVENPEYLVPREGTKKK) were coupled via captoethanol. The addition of a larger stoichiometric excess of ZnCl₂ resulted in irreversible precipitation of Memo. A 2-ml sample of this Memo preparation was sent to the Chemical Analysis Laboratory at the University of Georgia for metal analysis.

**Induction-coupled Argon Plasma (ICAP) Spectrometry Metal Analysis**—*E. coli* cells containing the Memo plasmid were grown in Terrific Broth medium supplemented with 0.1 mM ZnCl₂. Memo was purified as described above. Superdex 75 column fractions containing Memo (~3 μM) were incubated with 5 μM ZnCl₂ for 1 h, concentrated to 42 mg/ml bovine serum albumin for 1 h at 4 °C. For Fig. 4B, mutants of Memo carrying an N-terminal Myc tag were generated by the mega-primer method and ligated into pcDNA3.1. All sequences were verified prior to transfection into SKBr3 cells using FuGene (Roche Applied Science). 3 days later, cells were collected and lysed in Nonidet P-40 lysis buffer. Coupled beads were incubated with 0.5 ml of SKBr3 cell lysates for 1 h at 4 °C. Proteins bound to the beads were subjected to SDS-PAGE and Western blotting with specific antibodies: Memo, Shc antibody (10) and Myc antibody (Santa Cruz Biotechnology Inc.).

**Isothermal Titration Calorimetry (ITC)**—ITC was performed using a VP-ITC apparatus (MicroCal, Northampton, MA). Memo protein was dialyzed into 20 mM MES, 150 mM NaCl, pH 5.5, and 0.1 mM dithiothreitol. A 16-mer YD or pYD peptide (SPAFDNLYWDQNSSEKKK) was dissolved in the dialysate at a concentration of 0.35 mM and titrated into the 0.04 mM Memo solution in 25 injections at 15 °C. To examine whether Memo-pYD interactions are coupled to zinc binding, ITC experiments at pH 7.4 were performed in the absence or presence of zinc. Memo protein was dialyzed into 20 mM Hepes, 150 mM NaCl, pH 7.4, and 0.1 mM dithiothreitol. The pYD peptide was dissolved in the dialysate. 0.4 mM peptide was titrated into 0.035 mM Memo solution. In a parallel experiment, 0.035 mM ZnCl₂ was included in both the protein and the peptide buffer. Data analysis was performed using the Origin software supplied by MicroCal.

**RESULTS**

**Memo Structure**—The crystal structure of full-length human Memo was determined by single wavelength anomalous diffraction, and the resulting atomic model was refined to 2.1 Å resolution (Table 1). All but the 5 N-terminal amino acids were modeled. Memo adopts a single domain structure with a mixed four-helical/three-stranded β-sheets arrangement (Fig. 1A). Secondary structure elements have been designated with numbers (strands) or letters (helices) in the order of their appearance in the primary sequence (Figs. 1A and 2). A search for structural homologs using the program DALI revealed Memo to be homologous to LigB, the catalytic β-subunit of the protocatechuate 4,5-dioxynase LigAB from *Sphingomonas paucimobilis* (18, 19) (Fig. 1, B and D). Superposition of Memo and LigB structures results in an r.m.s.d. of 2.86 Å for 190 matched α-carbon positions with 21 of the 190 matched residues (11%) identical. LigAB is a class III nonheme iron(II)-dependent extradiol-

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**TABLE 1**

| Crystallographic data | Crystal |
|-----------------------|--------|
| **Data collection**    |        |
| Wavelength (Å)         | 0.97997 |
| Space group            | P2₁2₁2₁ |
| Unit cell (Å)          | a = 139.8, b = 88.8, c = 98.1 |
| Resolution (Å)         | 30.0-2.10 (2.18-2.10) |
| Number of molecules    | 4 |
| per asymmetric unit    | 4 |
| Completeness (%)       | 99.0 (98.2) |
| Redundancy             | 66.0 |
| Unique reflections     | 71,140 |
| R_f (%)                | 13.6 (60.6) |
| R_ref (%)              | 7.9 (2.8) |
| I/σ                   | 227.7 (62.6) |
| 1/α                   | 8.5 (2.4) |

| **Refinement**         |        |
| Mean r.m.s.d. bond angle (°) | 1.5 |
| Mean B-value            | 14.0 |
| Protein data bank code  | 3BCZ |

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**Memo Structure and Function**

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type catechol dioxygenase that catalyzes oxidative cleavage of substituted catechols in bacterial aromatic degradation pathways (20). Despite low (<15%) sequence identities, sequence homology between Memo and class III nonheme iron(II)-dependent dioxygenases is detected by PSI-BLAST after multiple iterations (21).

LigB catalytic activity is mediated by an iron ion, which is coordinated by His-12, His-61, Glu-242, and a water molecule (19) (Figs. 1B and 3). A third histidine, His-195, is believed to function as a general base during catalysis. Each of these histidines is conserved in Memo (His-49, His-81, and His-192, respectively), but Glu-242 is replaced by a cysteine (Cys-244) (Fig. 3). A glutamate to cysteine substitution at this site does not necessarily imply loss of metal binding; a cysteine side chain is capable of coordinating many metal ions including iron, and the side chain of Asp-189 in Memo is positioned so that it could coordinate a metal ion bound at this site and balance the positive charge (22) (Fig. 3). Furthermore, cysteine, aspartic acid, and three histidines at the noted positions are all conserved in a subset of Memo/LigB homologs and appear to define a subfamily of proteins with the Memo/LigB fold (Fig. 2).

A 2.3 Å crystal structure of the uncharacterized E. coli protein ygiD (JW3007) (Protein Data Bank accession code 2pw6) has recently been released, and this protein proves to be another distant structural homolog of Memo. The ygiD protein shares 11% sequence identity to Memo, clearly shares the same structural topology with Memo, and results in a DALI Z-score of 13.0 (values greater than 6.0 are taken to indicate highly significant structural homology) (18) (Fig. 1C). The ygiD protein binds three zinc ions, one of which is positioned in a site homologous to the putative metal-binding site in Memo. This zinc ion is ligated by a water molecule and three histidine residues (His-22, His-57, and His-234), which correspond to residues His-49, His-81, and Cys-244 in Memo, respectively.

No Evidence for Strong Metal Binding by Memo—No electron density consistent with a bound metal ion is apparent in Memo. Memo crystals were grown at pH 5.5, however, and at this pH
the histidines are likely to be protonated and less able to coordinate a metal ion. X-ray crystallographic, UV absorption, and fluorescence experiments were thus carried out at neutral pH to look for evidence of metal ion binding by Memo. Since iron(II) readily oxidizes and iron(III) is almost insoluble in aqueous solution, cobalt(II), which has a similar ionic radius and overlapping coordination properties as iron(II) (23, 24), is frequently used as a surrogate for iron or zinc binding.

Cobalt-protein complexes exhibit characteristic peaks at 500–700 nm in UV-visible absorption spectra and another peak at 350 nm if a cysteine is involved in cobalt coordination.

FIGURE 2. Sequence alignment of Memo homologs and LigB. The alignment among Memo family members was performed by ClustalW and includes Xenopus (Xe, CAJ83509), Drosophila melanogaster (Dm, AAL29902), Caenorhabditis elegans (Ce, NP_741570), Arabidopsis thaliana (At, AAM64443), Schizosaccharomyces pombe (Sp, NP_594340), Saccharomyces cerevisiae (Sc, YPR0088W), Magnetospirillum magnetotacticum (Mm, YP_423069), Desulfuromonas acetoxidans (Da, ZP_01311659), and Pelobacter carbinolicus (Pc, YP_357065). The alignment between Memo and LigB is based on structural superposition of the two proteins. Identical amino acids are highlighted in green, and similar residues in yellow. The number in parentheses indicates the number of amino acids inserted relative to the alignment. The letter p above the Memo sequence indicates residues involved in ErbB2 peptide binding based on mutagenesis results. The red circles below the LigB sequence indicate residues coordinating the ferric ion. The letter b indicates the residue acting as potential catalytic base in LigB.

FIGURE 3. Stereo image of a superposition of the LigB active site residues with the homologous residues in Memo. Memo residues are colored blue, and LigB residues are colored yellow.
The UV-visible spectrum of a 0.03 mM solution of Memo in the presence of 0.5 mM CoCl₂ failed to show either of these characteristic peaks. Fortuitously, Trp-16 is located in the potential metal-binding site of Memo, which enables fluorescence quenching experiments to test for substrate binding near this site, but FeSO₄, Fe(NH₄)₂(SO₄)₂, and CoCl₂ titrated into Memo solutions failed to show any quenching of tryptophan fluorescence (data not shown). Memo crystals were soaked for 5 min in mother liquor adjusted to pH 8.0 and supplemented with 5 mM cobalt chloride or 5 mM zinc chloride, but difference Fourier maps calculated with diffraction data from these and native crystals showed no evidence for cobalt or zinc binding (Supplemental Fig. 1). The potential metal-binding site is accessible to solvent in Memo crystals.

A Memo sample was submitted to the Chemical Analysis Laboratory at the University of Georgia for analysis by ICAP spectrometry. Purified Memo was incubated with ZnCl₂ and then dialyzed against a solution without zinc. ICAP measurements showed a zinc concentration of 0.30 ppm for the Memo sample and 0.02 ppm for the dialysate. The calculated zinc concentration for 42 μM Memo would be 2.75 ppm with a protein:zinc stoichiometry of 1:1, which is ~10-fold greater than the experimental value.

Memo binds pYD—Memo was identified in a screen of cell lysates for proteins that bind to an immobilized 16-residue phosphopeptide encompassing Tyr-1227 from rat ErbB2 (pYD) (10). The adaptor protein Shc also bound pYD, and depletion of Shc from the cell lysates diminished the ability of Memo to associate with pYD (10). For this reason, it was thought that interactions between Memo and pYD were likely to be indirect and possibly mediated by Shc. To test this proposal, purified Memo protein was assayed for its ability to bind pYD in the absence of other factors. Contrary to expectations, immobilized pYD is able to pull down purified Memo, and this ability to bind directly to Memo depends on phosphorylation at the Tyr-1227 site (Fig. 4A). In contrast pYC, a phosphorylated ErbB2 peptide encompassing Tyr-1201, is unable to pull down Memo. Consistent with earlier reports, purified Shc binds to both pYC and pYD (25) (Fig. 4A, lower panel).

A large cleft is present in the Memo structure at the site homologous to the dioxygenase active site (the "vestigial" active site) (Fig. 4C). To test whether this region is important for mediating interactions between pYD and Memo, 10 variant Memo proteins with amino acid substitutions in this cleft were assayed for their ability to interact with pYD in pull-down assays (Fig. 4B). Substitutions H49A, H81A, H192A, C244A, and W16A eliminated detectable pYD binding, and the Y54A

![FIGURE 4. Peptide binding activity of Memo. A, binding of purified Memo and Shc to YC, pYC, YD, and pYD peptides was analyzed by a pull-down assay followed by Western blot using anti-Memo or anti-Shc antibodies. B, binding of Myc-tagged wild-type (WT) Memo and Memo mutants in cell lysates of transiently transfected SKBr3 cells to YD or pYD analyzed by pull-down assays followed by Western blot using anti-Myc or anti-Shc antibodies. The lower panels show endogenous Shc binding to YD or pYD as a control. C, orthogonal views of the Memo surface showing the peptide-binding cleft and the sites of residues mutated for binding studies. Residues Trp-16, His-49, His-81, His-192, and Cys-244, mutation of which abolished peptide binding, are colored red. Residue Tyr-54, mutation of which reduced binding, is colored yellow. Residues Asp-189, Arg-196, Arg-198, and Arg-246, mutation of which had no effect on the binding, are colored green. D, Memo pYD-peptide binding as a function of pH. The buffers used were 50 mM MES for pH 5.5 and 6.5; 50 mM HEPES for pH 7.4.](https://jbc.asm.org/content/283/5/2738/F4.large.jpg)
substitution diminished pYD binding. Expression levels of the H49A and H81A Memo variants were slightly and markedly lower than wild-type Memo, respectively, and failure to observe pYD binding for at least the H81A variant may be due to a decreased amount of this protein. CD spectra of the H81A and H192A variant proteins indicate that they are folded (Supplemental Fig. 2). Cumulatively, these results indicate that the vestigial Memo active site and several histidine residues and Cys-244 in this region are important for pYD binding.

The importance of histidine residues in Memo for pYD binding suggested that interactions between Memo and pYD might be pH-dependent. Pull-down assays were thus performed at different pHs and show that the strength of Memo binding to pYD indeed varies with pH, with Memo-pYD interactions stronger at lower pH and weaker at higher pH (Fig. 4D). This pH-dependent binding profile is consistent with a requirement for protonated histidines for optimal pYD binding, which suggests that these histidines may balance the charge of the phosphorylated tyrosine.

To assess the strength of pYD binding to Memo, the dissociation constant for this interaction was measured at pH 5.5 by ITC and found to be $32 \mu M$ with a 1:1 stoichiometry (Fig. 5B). Consistent with results of the pull-down assays, no interaction was observed between Memo and unphosphorylated peptide (YD) by ITC (Fig. 5A). To examine whether Memo-pYD interactions are coupled to zinc binding, ITC experiments at pH 7.4 were performed in the absence or presence of stoichiometric amounts of zinc and showed that the presence of zinc had no effect (Fig. 5, C and D). Attempts to produce crystals of a Memo-pYD peptide complex have been unsuccessful. Several hundred conditions have been tried for each of an 8-mer, 12-mer, and 16-mer pYD-derived peptide using both factorial and standard grid screens. In addition, both colorimetric and mass spectrometric assays failed to detect any Memo-specific phosphatase activity.

**DISCUSSION**

Memo was identified based on its ability to co-precipitate with a specific phosphopeptide within the cytoplasmic tail of HER2/ErbB2 and disrupt HER2/ErbB2-mediated cell migration when depleted by RNA interference (10). We report here the crystal structure of human Memo and show that it is homologous to a class of nonheme iron-dependent dioxygenases. Many of the key active site residues are conserved between these dioxygenases and Memo, but an iron-coordinating glutamate found in dioxygenases is substituted with a cysteine in Memo (Cys-244). Although a cysteine side is capable of coordinating iron and other metals, we find no evidence for metal binding in vitro, which would be required for a dioxygenase-like activity. The recent
deposition of atomic coordinates for the structurally homologous ygiD protein indicates that Memo/LigB structural scaffold can also accommodate a zinc ion. In this case, the zinc ion is coordinated by three histidines. Physiological protein-zinc dissociation constants are typically in the 10^{-10}–10^{-12} M range (24), and both X-ray and ICAP experiments failed to detect strong zinc binding by Memo. Our experiments do not rule out the possibility that metal binding or enzymatic activity is a component of Memo activity, but in the absence of additional evidence, such properties seem unlikely.

We do show, however, that Memo represents a new class of phosphotyrosine-binding protein. The dissociation constant of Memo for its cognate phosphopeptide (pYD) is 32 μM, which is lower than values of 50–500 nM typically observed for optimal phosphotyrosine interactions but comparable with values reported for at least some physiologically relevant interactions (26, 27). The dissociation constant of the Shc SH2 domain binding to an EGF receptor-derived phosphopeptide is 69 μM, for example (26). Memo-pYD interactions are weaker at normal cellular pH, however, which casts some doubt on whether pYD binding by Memo represents a physiological interaction. On the other hand, Memo associates with the phosphorylated ErbB2 cytoplasmic tail in cells and is required for ErbB2-driven cell motility (10), which strongly suggests that the direct interaction between Memo and pYD is unlikely to be entirely coincidental. Future experiments will be needed to resolve this issue, but one possibility is that Memo-pYD interactions are enhanced in vivo by the presence of additional factors. The diminished association observed between Memo and pYD in cell lysates depleted of Shc hints that Shc may be one such factor, possibly via its interactions with nearby tyrosine 1201 (pYC) (10).

Much remains to be learned about Memo function in addition to resolving its role in binding to phospho-ErbB2. The presence of cysteine rather than glutamate at a metal-coordinating position in the dioxygenase active site appears to define or enzymatic activity is a component of Memo activity, but in the absence of additional evidence, such properties seem unlikely.

Many intriguing questions. A Memo homolog with the cysteine substitution is present in yeast, for example, which lack receptor tyrosine kinases. This observation suggests that the Memo subfamily evolved functions independent of LigB-like dioxygenase activity before acquiring a role in ErbB signaling. Determining what these functions are and what role they play in different organisms promises to provide a rich ground for future exploration.

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REFERENCES

1. Holbro, T., and Hynes, N. E. (2004) Ann. Rev. Pharmacol. Toxicol. 44, 195–217
2. Yarden, Y., and Sliwkowski, M. X. (2001) Nat. Rev. Mol. Cell. Biol. 2, 127–137
3. Olayioye, M. A., Neve, R. M., Lane, H. A., and Hynes, N. E. (2000) EMBO J. 19, 3159–3167
4. Hynes, N. E., and Lane, H. A. (2005) Nat. Rev. Cancer 5, 341–354
5. Burgess, A. W., Cho, H. S., Eigenbrot, C., Ferguson, K. M., Garrett, T. P., Leahy, D. J., Lemmon, M. A., Sliwkowski, M. X., Ward, C. W., and Yokoyama, S. (2003) Mol. Cell. 12, 541–552
6. Schlessinger, J. (2000) Cell 103, 211–225
7. Dankort, D. L., Wang, Z., Blackmore, V., Moran, M. F., and Muller, W. J. (1997) Mol. Cell. Biol. 17, 5410–5425
8. Segatto, O., Pelicci, G., Giuli, S., Digiesi, G., Di Fiore, P. P., McGlade, J., Pawson, T., and Pelicci, P. G. (1993) Oncogene 8, 2105–2112
9. Hellyer, N. J., Kim, M. S., and Koland, J. G. (2001) J. Biol. Chem. 276, 42153–42161
10. Marone, R., Hess, D., Dankort, D., Muller, W. J., Hynes, N. E., and Badache, A. (2004) Nat. Cell. Biol. 6, 515–522
11. Geisbrecht, B. V., Boytain, S., and Pop, M. (2006) Protein Expression Purif. 46, 23–32
12. Orwinowski, Z., and Minor, W. (1997) Methods Enzymol. 276, 307–326
13. Weeks, C. M., and Miller, R. (1999) J. Appl. Crystallogr. 32, 120–124
14. Terwilliger, T. C. (2003) Methods Enzymol. 374, 22–37
15. Jones, T., Zou, J.-Y., Cowan, S., and Kjeldgaard, M. (1991) Acta Crystallogr. Sect. A 47, 110–119
16. Brunger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gross, P., Grosse-Kunstleve, R. W., Jiang, J. S., Kuszewski, J., Nîgles, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonon, T., and Warren, G. L. (1998) Acta Crystallogr. Sect. D Biol. Crystallogr. 54, 905–921
17. Murshudov, G. N., Vagin, A. A., and Dodson, E. J. (1997) Acta Crystallogr. Sect. D Biol. Crystallogr. 53, 240–2510
18. Holm, L., and Sander, C. (1996) Science 273, 595–603
19. Sugimoto, K., Senda, T., Aoshima, H., Masai, E., Fukuda, M., and Mitsui, Y. (1999) Structure (Camb.) 7, 953–965
20. Noda, Y., Nishikawa, S., Shiozuka, K., Kadokura, H., Nakajima, H., Yoshida, K., Katayama, Y., Morohoshi, N., Haraguchi, T., and Yamasaki, M. (1990) J. Bacteriol. 172, 2704–2709
21. Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. (1997) Nucleic Acids Res. 25, 3389–3402
22. Harding, M. M. (1999) Acta Crystallogr. Sect. D Biol. Crystallogr. 55, 1432–1443
23. Bertini, I., and Luchinat, C. (1984) Adv. Inorg. Biochem. 6, 71–111
24. Lippard, S., and Berg, J. (1994) Principles of Bioinorganic Chemistry, University Science Books, Mill Valley, CA
25. Ricci, A., Lanfrancone, L., Chiar, R. Belardo, G., Perica, C., Natali, P. G., and Segatto, O. (1995) Oncogene 11, 1519–1529
26. Zhou, M. M., Harlan, J. E., Wade, W. S., Crosby, S., Ravichandran, K. S., Burakoff, S. J., and Fesik, S. W. (1995) J. Biol. Chem. 270, 31119–31123
27. Pawson, T. (2004) Cell 116, 191–203