Intramolecular Interactions between the Juxtamembrane Domain and Phosphatase Domains of Receptor Protein-tyrosine Phosphatase RPTPμ

REGULATION OF CATALYTIC ACTIVITY*

Received for publication, September 24, 1999, and in revised form, February 28, 2000

Elles Feiken, Ingrid van Etten, Martijn F. B. G. Gebbink‡, Wouter H. Moolenaar§, and Gerben C. M. Zondag

From the Division of Cellular Biochemistry, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

RPTPμ is a receptor-like protein-tyrosine phosphatase (RPTP) whose ectodomain mediates homotypic cell-cell interactions. The intracellular part of RPTPμ contains a relatively long juxtamembrane domain (158 amino acids; aa) and two conserved phosphatase domains (C1 and C2). The membrane-proximal C1 domain is responsible for the catalytic activity of RPTPμ, whereas the membrane-distal C2 domain serves an unknown function. The regulation of RPTP activity remains poorly understood, although dimerization has been proposed as a general mechanism of inactivation. Using the yeast two-hybrid system, we find that the C1 domain binds to an N-terminal noncatalytic region in RPTPμ, termed JM (aa 803–955), consisting of a large part of the juxtamembrane domain (120 aa) and a small part of the C1 domain (33 aa). When co-expressed in COS cells, the JM polypeptide binds to both the C1 and the C2 domain. Interestingly, the isolated JM polypeptide fails to interact with either full-length RPTPμ or with truncated versions of RPTPμ that contain the JM region, consistent with the JM-C1 and JM-C2 interactions being intramolecular rather than intermolecular. Furthermore, we find that large part of the juxtamembrane domain (aa 814–922) is essential for C1 to be catalytically active. Our findings suggest a model in which RPTPμ activity is regulated by the juxtamembrane domain undergoing intramolecular interactions with both the C1 and C2 domain.

Protein-tyrosine phosphatases (PTPs)1 play important roles in signal transduction pathways regulated by tyrosine phosphorylation. Members of the superfamily of PTPs use the same catalytic mechanism and are broadly classified into transmembrane or receptor-like PTPs (RPTPs) and intracellular, nonreceptor PTPs (reviewed in Refs. 1 and 2). Members of the RPTP subfamily are type I membrane proteins consisting of a variable ectodomain, a single membrane-spanning region, and in most cases, two conserved intracellular phosphatase domains. The RPTPs are further classified according to the structure of their ectodomains (reviewed in Refs. 3 and 4). The large variety in ectodomain structure suggests the existence of an equal number of putative ligands, yet in most cases the corresponding ligands have not been identified.

RPTPμ is the prototype member of a subfamily of RPTPs that mediate homophilic cell-cell interactions via their ectodomains and, hence, are thought to play a role in cell adhesion-mediated processes (5–8). The ectodomain of RPTPμ shows similarities with that of cell-cell adhesion molecules and consists of an N-terminal “MAM” domain, which is critical for mediating cell-cell adhesion (9), followed by an Ig-like domain and four fibronectin type III repeats (10). Its intracellular part consists of a juxtamembrane domain of 158 amino acids (aa), which is relatively long compared with that in other RPTPs, and two tandem phosphatase domains referred to as C1 and C2. As in most other RPTPs, the membrane-proximal C1 domain of RPTPμ is catalytically active, whereas the membrane-distal C2 domain shows no activity, at least in vitro (11). The C2 domains of most RPTPs have been proposed to play a regulatory role (12), but how it might contribute to RPTP activity is not known.

One major unresolved question is how ligand binding may influence the catalytic activity of RPTPs to affect signal transduction events. A recently proposed model involves dimerization, as inferred from the crystal structure of RPTPα (13). This model suggests that ligand binding induces the formation of a symmetrical dimer in which the catalytic site of one molecule is blocked by specific interactions with a helix-turn-helix segment (termed the “wedge”) in the juxtamembrane domain of the other (13). There is no wedge-like region present directly upstream of the C2 domain, suggesting a fundamental difference between the C1 and C2 domains. Based on these structural studies, dimerization has been proposed to be a universal mechanism of inactivation of RPTPs (reviewed in Ref. 14). Consistent with this, earlier studies had already indicated that the leukocyte-specific RPTP CD45 can form homodimers (15) and that artificial induction of CD45 dimerization may lead to loss of function (16). Using an epidermal growth factor receptor-CD45 chimera, a part of CD45 homologous to the inhibitory helix-turn-helix wedge in RPTPα was recently shown to inhibit CD45 function after ligation by epidermal growth factor (17), in support of the dimerization model. On the other hand, however, the crystal structure of RPTPμ does not reveal such intramolecular interactions between a wedge region and the C1 domain (18). It seems that the catalytic site of RPTPμC1 is unhindered and adopts an open conformation similar to what is observed in
Intramolecular Interactions in RPTPμ

The cytosolic PTP, PTP1B (19). It was suggested that the RPTPμ dimer may be the consequence of crystallization, because dimers were not found in solution. Furthermore, some residues important for the proposed dimerization mechanism are less conserved in RPTPμ (18, 20), suggesting that RPTPμ may not be regulated by dimerization (reviewed in Refs. 14 and 20).

Here we present evidence for a new type of interdomain interaction involved in the regulation of RPTPμ activity. In a search for potential binding partners of the C1 domain using the yeast two-hybrid system, we isolated a cDNA clone encoding part of RPTPμ itself, consisting of a large part of the juxtamembrane domain and a small part of the C1 domain. We show that this “JM” segment can interact with both the C1 and C2 domain and present evidence suggesting that this interaction is intramolecular rather than intermolecular. We further show that the juxtamembrane domain is essential for catalytic activity of the C1 domain. Based on these findings, we propose a model in which the juxtamembrane domain may contribute to the regulation of RPTPμ activity.

EXPERIMENTAL PROCEDURES

Cloning of library—Plasmid pMT2-HA-cl.2 was constructed by subcloning the insert from pVP16-clone 2 into a modified pMT2 vector containing an HA tag, pMT2-HA-RPTPμC1, C1M, and C2 plasmids were constructed by polymerase chain reaction amplification using primers 5′-TATGTCGACAACGAGCTAGATTCTTCTTTC-3′ and 5′-CCGGATTCTCTTTTATCG-3′. RPTPμC1 was fused to the Gal4 DNA binding domain by SalI-EcoRI subcloning into pMD4 (23) containing a trp1 marker. Selection of C-terminal yeast strain Y190, together with a pVP16-based (24) human marker. Yeast transformants expressing the reporter genes were selected on medium lacking histidine and supplemented with 25 mM 3-amino-1,2,4-triazole. Positive colonies were identified by β-galactosidase filter assays.

cDNA Constructs—Plasmid pMT2-HA-cl.2 was constructed by subcloning the insert from pVP16-clone 2 into a modified pMT2 vector containing an HA tag, pMT2-FLAG-RPTPμC1, C1M, and C2 plasmids were constructed by polymerase chain reaction amplification and standard cloning procedures. The wild type and mutant first PTP domain were amplified by sense (5′-TATGTCGACAACGAGCTAGATTCTTCTTTC-3′) and antisense (5′-CCGGATTCTCTTTTATCG-3′) primers using hFL and hFLm constructs as template (5), respectively. The second PTP domain was amplified using sense (5′-ATTACCTGACG-GAGCTCACAACATCTGGTACG) and antisense (5′-TCATTTAGAACACC-ATCAGCAGAATTCA) primers and hFL as template. All polymerase chain reaction products were verified by sequencing. pMT2-FLAG-RPTPμC1 was constructed by inserting an EcoRI fragment containing the second PTP domain into plasmid pMT2-FLAG-RPTPμC1. HA- and Myc-tagged constructs encoding the juxtamembrane region and first catalytic domain (RPTPμC1) were generated using primers 5′-TATGTCGACAACGAGCTAGATTCTTCTTTC-3′ and 5′-TGGATTCTCTTTTATCG-3′. The results of the co-immunoprecipitation analysis are summarized in Fig. 2B.

RESULTS

C1 Interdomain Interaction in the Yeast Two-hybrid System—In an attempt to identify proteins that interact with the C1 domain of RPTPμ, we used this domain as bait (RPTPμC1, aa 923–1190) in a yeast two-hybrid screen of a human testis cDNA library. Two positive colonies were identified that contained identical testis-derived cDNA clones, termed clone 1 and clone 2. Strikingly, both clones encode a membrane-proximal region of RPTPμ (aa 803–955) consisting of a large part of the juxtamembrane domain (120 aa) and a small part of the C1 domain (33 aa) (Fig. 1A), which we refer to as either cl.2 or the JM region. As shown in Fig. 1B, co-expression of RPTPμC1 and cl.2/JM in yeast results in the activation of the lacZ reporter gene. These results strongly indicate that the C1 domain undergoes either intermolecular or intramolecular interaction with the JM region.

The Membrane-proximal JM Region Interacts with Both Catalytic Domains of RPTPμ in COS Cells: Evidence for Intramolecular Interactions—To confirm the observed C1-JM interdomain interaction in mammalian cells, HA-tagged clone 2 (HA-cl.2) encoding the JM polypeptide was transiently expressed together with epitope-tagged C1 (FLAG-RPTPμC1) in COS cells (Fig. 2A). When both proteins were co-expressed, C1 was co-precipitated with the anti-HA monoclonal antibody (not shown), whereas HA-cl.2 was co-precipitated with anti-FLAG monoclonal antibody. Thus, the C1-JM interdomain interaction occurs in both yeast and mammalian cells. Given the sequence similarities between the C1 and C2 domains, we tested whether the C2 domain might also interact with JM. As shown in Fig. 2A, this is indeed the case; when HA-cl.2 was co-expressed with the isolated C2 domain (FLAG-RPTPμC2; aa 1191–1452) or with both C1 and C2 domains in tandem (FLAG-RPTPμC1C2; aa 923–1452), HA-cl.2 was co-precipitated with anti-FLAG monoclonal antibody. It thus appears that the JM polypeptide does not discriminate between the C1 and C2 domain for binding in COS cells. It is of note that the RPTPμC1C2 construct, containing both phosphorylation domains, yields a stronger binding signal than either C1 or C2 (Fig. 2A, last lane), as one would expect if each catalytic domain binds one HA-cl.2 molecule (JM polypeptide).

We next examined whether the nature of the JM-C1 and JM-C2 interactions is intramolecular or intermolecular. To this end, COS cells were transfected with various epitope-tagged RPTPμ constructs and then subjected to immunoprecipitation and blotting assays. Strikingly, whereas HA-cl.2 (JM polypeptide) co-precipitates with the individual phosphorylation domains as well as the tandem C1C2 domain (Fig. 2A), HA-cl.2 fails to interact with longer versions of RPTPμ; either a Myc-tagged polypeptide consisting of a large part of the juxtamembrane domain and the C1 domain (JC1, aa 814–1190) (not shown) or full-length RPTPμ (Fig. 2C). Furthermore, we find that HA-tagged JC1 does not co-precipitate with Myc-tagged JC1 (Fig. 2B) nor with full-length RPTPμ (Fig. 2C). The results of the co-immunoprecipitation analysis are summarized in Fig. 2D. From these findings we conclude that the observed JM-C1/C2 interactions do not occur between different RPTPμ molecules.
Thus, our results can only be explained by the JM-C1/C2 interactions being intramolecular rather than intermolecular.

**Mutational Analysis: Effects of Point Mutations on the JM-C1/C2 Interaction**—To determine which residues are involved in the interaction between the JM and C1 domains, we transfected RPTP\(\mu\) constructs in which the following critical residues were mutated: cysteine 1095 to a serine (C1095S) and glutamate 896 to an arginine (E896R). The conserved cysteine 1095 is essential for catalytic activity of the C1 domain of RPTP\(\mu\). Mutation of cysteine 1095 to a serine (C1095S) was shown to completely abolish phosphatase activity (11). Glutamate 896 is analogous to aspartate 228 of RPTP\(\alpha\). In the RPTP\(\alpha\) dimer, this residue is located in the N-terminal wedge that inserts into the catalytic pocket of the C1 domain of the juxta-posed RPTP\(\alpha\) molecule and thereby may block its activity (13). Glutamate 896 of RPTP\(\mu\) is also analogous to glutamate 624 in CD45; mutation of this residue was shown to abolish the inhibitory effect on T-cell receptor signaling caused by CD45 dimerization (17). We find, however, that the mutation C1095S in FLAG-RPTP\(\mu\)C1 did not affect association with HA-cl.2 (Fig. 3A). We also find that the mutation E896R in HA-cl.2 does not affect the association with the C1 domain (Fig. 3B). Taken together, catalytic activity and glutamate 896 are not essential for the association between the juxtamembrane and the C1 domain of RPTP\(\mu\).

**The Juxtamembrane Domain Is Essential for Catalytic Activity of the C1 Domain**—To examine how the distinct domains of RPTP\(\mu\) contribute to catalytic activity, we determined tyrosine phosphatase activity in immune complexes using a non-radioactive tyrosine phosphatase assay (see “Experimental Procedures”). We measured the activity of both full-length RPTP\(\mu\) and different epitope-tagged constructs of RPTP\(\mu\) (Fig. 4, A and B) expressed in COS cells. We found that the isolated C1 and C2 domains as well as C1C2 are inactive (Fig. 4B). In marked contrast, however, N-terminal extension of the C1 domain leads to phosphatase activity as inferred from the JC1 polypeptide being active in the assay. In other words, the juxtamembrane domain is required for activity of the C1 domain. This is consistent with reports on LAR and RPTP\(\alpha\), which show that the isolated C1 domains require at least part
of the juxtamembrane domain for activity in vitro (12, 25). The present data also indicate that the C2 domain is not required for activity of the C1 domain, although we cannot exclude the possibility that the C2 domain may somehow contribute to the activity of C1. Finally, we found that the E896R mutation in the juxtamembrane domain of HA-RPTPμJC1 does not affect
In the present study, we have shown that the juxtamembrane domain of RPTPα can bind to both the first and the second phosphatase domain (C1 and C2) and that this interaction is likely to be intramolecular rather than intermolecular. Furthermore, we have presented evidence that the juxtamembrane domain is required for the C1 domain to become fully active.

**DISCUSSION**

In the present study, we have shown that the juxtamembrane domain of RPTPα can bind to both the first and the second phosphatase domain (C1 and C2) and that this interaction is likely to be intramolecular rather than intermolecular. Furthermore, we have presented evidence that the juxtamembrane domain is required for the C1 domain to become fully active.

Through yeast two-hybrid analysis, we found that the RPTPα C1 domain binds to an RPTPα molecule, termed JM, consisting of a large part of the juxtamembrane domain and a small part of the C1 domain. Our COS cell experiments revealed that the JM segment interacts not only with C1 but also with the C2 domain of RPTPα. These results would be consistent with RPTPα forming dimers, in which the JM region of one molecule interacts with the juxtaposed C1 and/or C2 domains in the partner RPTPα molecule, analogous to what has been proposed for the C1 domain of RPTPα (13). Inconsistent with a homodimerization model, however, is our finding that the JM segment fails to interact with extended, C1-containing versions of RPTPα C1. JM also fails to interact with full-length RPTPα. We also did not detect any interdomain interactions between versions of RPTPα that comprise both JM and C1. These results are most readily explained by a model in which JM-C1/C2 binding represents an intramolecular interaction within one single RPTPα molecule. Mutational analysis indicates that the interaction is independent of RPTPα catalytic activity and of glutamate 896 in the helix-turn-helix segment, which is analogous to that in the corresponding motif of CD45, where it has been implicated in dimerization-dependent inhibition of CD45 activity caused by dimerization (17).

In a recent crystallographic study on the RPTPα C1 domain (residues 874–1168), it was concluded that the protein behaves as a monomer in solution and that C1-C1 dimerization is most likely a consequence of crystallization (18). The C1 crystal structure revealed that the catalytic site is unhindered and adopts an open conformation. Caution is needed, however, to extrapolate findings obtained with RPTPα C1 to the full-length molecule, particularly because the juxtamembrane domain was excluded from crystallographic analysis and, hence, any JM-C1 interaction would go undetected. The N terminus of the C1 domain used for crystallization starts at the helix-turn-helix segment (at the membrane-distal end) very close to the boundary of the C1 domain. This would imply that a more membrane-proximal part of the juxtamembrane domain (immediately N-terminal to the helix-turn-helix structure) is involved in the observed JM-C1/C2 interaction. Further crystallization studies using N-terminally extended versions of the C1 domain are required to clarify this point.

It seems likely that the interdomain interactions found in RPTPα also occur in other members of the RPTP family. It is of note that the juxtamembrane domain of RPTPα, in common with the other MAM domain-containing RPTPs, is about 70 residues longer than that in all other RPTPs (10); the significance of this extension remains unknown. It will be interesting to see whether JM-C1/C2 interactions are a specific feature of the MAM domain-containing subfamily of RPTPs. Our results support the view that dimerization is not involved in the regulation of RPTPα activity, in contrast to what has been proposed for the regulation of RPTPs and CD45 (14, 20). In fact, there is no direct evidence that RPTPα dimers are catalytically inactive. Parts of RPTPα containing the inhibitory wedge and the C1 domain, are catalytically active and probably act as active monomers in solution (25, 26). The RPTP dimerization concept has become even more complex since the C1 domain of RPTPα was reported to interact with the C2 domain of RPTPβ but not the RPTPα-C2 with RPTPαC1 (27). This apparent C1-C2 heterodimerization requires the wedge region of RPTPα, which was thought to bind the "pseudo-active" site in the juxtaposed RPTPαC2 domain (27). Although the precise cellular role of the C2 domain remains unknown, the latter result does suggest that the C2 domain is involved in a variety of protein-protein interactions. Very recently, structural studies on the tandem phosphatase domains of RPTP LAR revealed a monomeric con-
Intramolecular Interactions in RPTPμ

FIG. 4. Analysis of tyrosine phosphatase activity. A, phosphatase activity of full-length RPTPμ using a nonradioactive tyrosine phosphatase assay kit (Roche Molecular Biochemicals). Anti-RPTPμ (3D7) immunoprecipitates of COS-7 cells transfected with empty expression vector (control) or with pMT2-hFL were used in the nonradioactive phosphatase assay. The absorbance at 405 nm is a reciprocal measure for phosphatase activity. Absorbance at 490 nm is the reference wavelength. B, analysis of phosphatase activity of different parts of RPTPμ, using anti-FLAG immunoprecipitates from lysates of COS-7 cells transfected with empty expression vector (control 1), pMT2-FLAG-RPTPμC1, pMT2-FLAG-RPTPμC2, or pMT2-FLAG-RPTPμC1/C2, and anti-HA immunoprecipitates from lysates of COS-7 cells transfected with empty vector (control 2) and pMT2-HA-RPTPμC1 or pMT2-HA-RPTPμC1/E896R.

FIG. 5. Proposed model of intramolecular interactions regulating RPTPμ activity. Two possible conformations of RPTPμ are schematically drawn. One conformation represents the inactive state of the molecule, in which the second catalytic domain (C2) of RPTPμ interacts with the juxtamembrane domain thereby inhibiting the first catalytic domain (C1). In the other conformation, C1 interacts with the juxtamembrane domain rendering an active RPTPμ molecule as C1 is active in this conformation. For details see “Discussion.”

REFERENCES
1. Fauman, E. B., and Saper, M. A. (1996) Trends Biochem. Sci. 21, 413–417
2. Neel, B. G., and Tonks, N. K. (1997) Curr. Opin. Cell Biol. 9, 193–204
3. Fischer, E. H., Charbonneau, H., and Tonks, N. K. (1991) Science 253, 401–406
4. Schaapveld, R., Wieringa, B., and Hendriks, W. (1997) Mol. Biol. Rep. 24, 247–262
5. Gebbink, M. F. B. G., Zondag, G. C. M., Wubbolts, R. W., Beijersbergen, R. L., van Etten, I., and Moolenaar, W. H. (1991) J. Biol. Chem. 266, 16101–16104
6. Brady-Kalnay, S. M., Flint, A. J., and Tonks, N. K. (1993) J. Cell Biol. 122, 961–972
7. Sap, J., Jiang, Y.-P., Friedlander, D., Grumet, M., and Schlessinger, J. (1994) Mol. Cell. Biol. 14, 1–9
8. Cheng, J., Wu, K., Armanini, M., O’Houroue, N., Dowbenko, D., and Lasky, L. A. (1997) J. Biol. Chem. 272, 7264–7277
9. Zondag, G. C. M., Koningstein, G. M., Jiang, Y.-P., Sap, J., Moolenaar, W. H., and Gebbink, M. F. B. G. (1995) J. Biol. Chem. 270, 14247–14250
10. Gebbink, M. F. B. G., van Etten, I., Hakeboer, G., Suijkerbuijk, R., Beijersbergen, R. L., Geurts van Kessel, A., and Moolenaar, W. H. (1991) FEBS Lett. 290, 123–130
11. Gebbink, M. F. B. G., Verheijen, M. H. G., Zondag, G. C. M., van Etten, I., and Moolenaar, W. H. (1993) Biochemistry 32, 13516–13522
12. Streuli, M., Krueger, N. X., Thaï, T., Tang, M., and Saito, H. (1990) EMBO J. 9, 2399–2407
Intramolecular Interactions in RPTP-μ

13. Bilwes, A. M., den Hertog, J., Hunter, T., and Noel, J. P. (1996) Nature 382, 555–559
14. Weiss, A., and Schlessinger, J. (1998) Cell 94, 277–280
15. Takeda, A., Wu, J. J., and Maizel, A. L. (1992) J. Biol. Chem. 267, 16651–16659
16. Desai, D. M., Sap, J., Schlessinger, J., and Weiss, A. (1993) Cell 73, 541–554
17. Majeti, R., Bilwes, A. M., Noel, J. P., Hunter, T., and Weiss, A. (1998) Science 279, 88–91
18. Hoffmann, K. M. V., Tonks, N. K., and Barford, D. (1997) J. Biol. Chem. 272, 27506–27508
19. Barford, D., Flint, A. J., and Tonks, N. K. (1994) Science 263, 1397–1404
20. Barford, D., Das, A. K., and Egloff, M.-P. (1998) Annu. Rev. Biophys. Biomol. Struct. 27, 133–164
21. Zondag, G. C. M., Moolenaar, W. H., and Gebbink, M. F. B. G. (1996) J. Cell Biol. 134, 1513–1517
22. Gebbink, M. F. B. G., Zondag, G. C. M., Koningstein, G. M., Feiken, E., Wubbolts, R. W., and Moolenaar, W. H. (1995) J. Cell Biol. 131, 251–260
23. Smit, L., van der Horst, G., and Borst, J. (1996) J. Biol. Chem. 271, 28564–28569
24. Vojtek, A. B., Hollenberg, S. M., and Cooper, J. A. (1993) Cell 74, 205–214
25. Wang, Y., and Pallen, C. J. (1991) EMBO J. 10, 3231–3237
26. Wu, L., Buist, A., den Hertog, J., and Zhang, Z.-Y. (1997) J. Biol. Chem. 272, 6994–7002
27. Wallace, M. J., Fladd, C., Batt, J., and Rotin, D. (1998) Mol. Cell. Biol. 18, 2608–2616
28. Nam, H.-J., Poy, F., Krueger, N. X., Saito, H., and Frederick, C. A. (1999) Cell 97, 449–457
Intramolecular Interactions between the Juxtamembrane Domain and Phosphatase Domains of Receptor Protein-tyrosine Phosphatase RPTP \( \mu \): REGULATION OF CATALYTIC ACTIVITY

Elles Feiken, Ingrid van Etten, Martijn F. B. G. Gebbink, Wouter H. Moolenaar and Gerben C. M. Zondag

J. Biol. Chem. 2000, 275:15350-15356.
doi: 10.1074/jbc.275.20.15350

Access the most updated version of this article at http://www.jbc.org/content/275/20/15350

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 28 references, 15 of which can be accessed free at http://www.jbc.org/content/275/20/15350.full.html#ref-list-1