Midkine is a 13-kDa heparin-binding growth factor with 45% sequence identity to pleiotrophin. Pleiotrophin has been demonstrated to bind to protein-tyrosine phosphatase \( \zeta \) (PTP\( \zeta \)) with high affinity. In this study, we examined the binding of midkine to PTP\( \zeta \) by solid-phase binding assay. Midkine and pleiotrophin binding to PTP\( \zeta \) were equally inhibited by soluble pleiotrophin and also by some specific glycosaminoglycans. For both bindings, Scatchard analysis revealed low (3.0 nM) and high (0.58 nm) affinity binding sites. These results suggested that PTP\( \zeta \) is a common receptor for midkine and pleiotrophin. Midkine is structurally divided into the N- and C-terminal halves, and the latter exhibited full activity for PTP\( \zeta \) binding and neuronal migration induction. The C-terminal half contains two heparin-binding sites consisting of clusters of basic amino acids, Clusters I and II. A mutation at Arg78 in Cluster I resulted in loss of the high affinity binding and reduced neuronal migration-inducing activity, while mutations at Lys83 and Lys84 in Cluster II showed almost no effect on either activity. Chondroitinase ABC-treated PTP\( \zeta \) exhibited similar low affinity binding both to the native midkine and midkine mutants at Arg78. These results suggested that Arg78 in midkine plays an essential role in high affinity binding to PTP\( \zeta \) by interacting with the chondroitin sulfate portion of this receptor.

PTP\( \zeta \)/RPTP\( \beta \)\(^{1} \) is a receptor-like protein-tyrosine phosphatase, which is abundantly expressed in the central nervous system as a chondroitin sulfate proteoglycan (1–4). PTP\( \zeta \) is composed of an N-terminal carboxyl anhydrate-like domain, a fibronectin type III domain, a serine, glycine-rich domain that is thought to be chondroitin sulfate attachment region, a transmembrane segment, and two tyrosine phosphatase domains (1, 2). There are three splice variants of this molecule: (a) the full-length PTP\( \zeta \) (PTP\( \zeta \)-A); (b) the short form of PTP\( \zeta \), in which most of the serine, glycine-rich region is deleted (PTP\( \zeta \)-B); and (c) the secreted form (PTP\( \zeta \)-S), which corresponds to the extracellular region of PTP\( \zeta \)-A and is also known as 6B4 proteoglycan/phosphacan (3, 5). All these splice variants are expressed as chondroitin sulfate proteoglycans in the brain (6), suggesting that chondroitin sulfate plays an essential role in receptor function.

Several proteins such as contactin, tenascin, L1, NCAM, and TAG1 have been reported to bind PTP\( \zeta \) (7–9). Contactin is thought to be a neuronal receptor of PTP\( \zeta \) expressed on glial cells (7). Recently, we found that PTP\( \zeta \) binds with pleiotrophin/heparin-binding growth-associated molecule (10), in that a chondroitin sulfate portion of PTP\( \zeta \) constitutes a part of the pleiotrophin binding site and regulates the affinity of PTP\( \zeta \)-pleiotrophin binding (10). We further demonstrated that pleiotrophin-induced neurite outgrowth and neuronal migration were suppressed by chondroitin sulfate, polyclonal antibodies against the extracellular domain of PTP\( \zeta \), and sodium vanadate, a protein-tyrosine phosphatase inhibitor. These findings suggested that PTP\( \zeta \) expressed on neurons is a signal transducing receptor for pleiotrophin (10, 11).

Pleiotrophin has 45% sequence identity to midkine, forming a new family of heparin-binding growth factors. These molecules share many biological activities (12, 13); both proteins promote neurite outgrowth (14–16), enhance plasminogen activator activity in aortic endothelial cells (17), and oncogenically transform NIH3T3 cells (18, 19). These findings suggest that they use a common or highly related receptors.

Midkine and pleiotrophin are structurally composed of two domains (the N- and C-terminal halves), each of which is tightly held through three or two disulfide bridges, respectively (20). The C-terminal half of midkine binds strongly to heparin and exhibits neurite outgrowth-promoting and plasminogen activator-enhancing activities (21, 22). On the other hand, the N-terminal half of midkine, which shows relatively weak heparin binding activity, does not promote neurite outgrowth or enhance plasminogen activator activity (21, 22). NMR spectroscopy revealed two clusters of basic amino acids in the C-terminal half of midkine, Clusters I and II, both of which interact with heparin oligosaccharides (23). Experiments using various midkine mutants indicated that Cluster II plays an essential role in its plasminogen activator-enhancing effect (22).

In this study, we examined the PTP\( \zeta \)-midkine interaction using various midkine mutants. Native PTP\( \zeta \) exhibited high affinity binding to midkine, and the binding properties were essentially the same as those of pleiotrophin. Moreover, PTP\( \zeta \)-midkine binding was inhibited by the presence of pleiotrophin. These observations suggested that midkine and pleiotrophin

---

\(^{1}\) The abbreviations used are: PTP, protein-tyrosine phosphatase; RPTP, receptor-like protein-tyrosine phosphatase; BSA, bovine serum albumin; CHAPS, N-(3-cholamidopropyl)dimethylammonio-1-propanesulfonic acid; ELISA, enzyme-linked immunosorbent assay.

---

Nobuaki Maeda, Keiko Ichihara-Tanaka, Terutoshi Kimura, Kenji Kadomatsu, Takashi Muramatsu, and Masaharu Noda

From the Division of Molecular Neurobiology, National Institute for Basic Biology, and Department of Molecular Biomechanics, Graduate University for Advanced Studies, Okazaki 444-8585, and the Department of Biochemistry, Nagoya University School of Medicine, Tsurumai-cho, Showa-ku, Nagoya 466-8550, and the Peptide Institute Inc., 4-1-2, Ina, Minoh, Osaka 562-0015, Japan

This paper is available on line at http://www.jbc.org
share a common binding site on PTPζ. PTPζ binds to the C-terminal half of midkine, but not to the N-terminal half. A mutation R78Q in Cluster I reduced the binding affinity, while mutations K83Q, K84Q, and K83Q/K84Q in Cluster II did not affect binding. Furthermore, in these midkine mutants, the strength of binding affinities and the neuronal migration-inducing activities were highly correlated. These findings suggested that basic amino acids in Cluster I of midkine and pleiotrophin are crucial for high affinity binding to PTPζ to transduce signals in neurons.

**EXPERIMENTAL PROCEDURES**

**Materials**—Chondroitin sulfate A from whale cartilage, chondroitin sulfate B from pig skin, chondroitin sulfates C and D from shark cartilage, chondroitin sulfate E from squid cartilage, heparan sulfate from bovine kidney, keratan sulfate from bovine cornea, and chondroitinase ABC were purchased from Seikagaku Corp. Heparin was obtained from Sigma. 125I-Bolton-Hunter reagent was purchased from DuPont NEN. Chroma Spin columns were obtained from CLONTECH. Maxisorp immunoplates were purchased from Nunc. Dulbecco’s modified Eagle’s medium, F-12 medium, and B-27 supplement were purchased from Life Technologies, Inc. Transwells were obtained from Corning Coster Corp. Micro BCA kit was from Pierce. PTPζ-S was purified as reported elsewhere (24). The N- and C-terminal half domains of human midkine (1–59 and 60–121, respectively) were synthesized as described previously (25). Mouse midkine mutants, R78Q, K83Q, K84Q, K83Q/K84Q and R78Q/K83Q/K84Q were prepared by site-directed mutagenesis (21, 22). Mutations are indicated by the amino acid residues (in one-letter code) in the wild-type and the mutant, preceding and following the numbers of the altered residues, respectively.

125I-Labeling of PTPζ-S—PTPζ-S was purified from rat brain and labeled as described previously (10, 24). Briefly, dried 125I-Bolton-Hunter reagent (100 μCi) was solubilized with samples (10 μg of protein in 100 μl of 100 mM sodium phosphate buffer, pH 8.0), followed by incubation for 3 h on ice and then mixed with 30 μl of 1 M glycine, pH 7.5. After a 2-h incubation at 4 °C, free 125I-Bolton-Hunter reagent was removed by passing through a Chroma Spin 30 column equilibrated with 0.05% Triton X-100, 0.5 mg/ml BSA, 0.15 mM NaCl, 10 mM sodium phosphate, pH 7.2. The specific radioactivity of the sample thus prepared was 3.3 × 106 cpm/μg.

Binding Assay—Wells of Nunc Maxisorp Immunoplates were coated with 35 μl of 1–5 μg/ml midkine or pleiotrophin in 5 mM Tris-HCl, pH 8.0, at 4 °C overnight. The wells were washed three times with phosphate-buffered saline and then blocked with 1% BSA/phosphate-buffered saline for 1 h at room temperature. 125I-PTPζ-S diluted in 0.5% BSA, 2 mM CaCl2, 2 mM MgCl2, 0.1% CHAPS, 0.15 mM NaCl, 10 mM sodium phosphate, pH 7.2, was added to the coated wells. When inhibition experiments were performed, inhibitors (pleiotrophin or glycosaminoglycans) were premixed with 125I-PTPζ-S to midkine. On the other hand, the N-terminal half of midkine also exhibited high (Kd = 0.55 nM) and low (Kd = 2.4 nm) affinity binding sites. The N-terminal half of midkine showed no binding.

**RESULTS**

**Binding of PTPζ-S to Midkine**—Fig. 1 shows the binding profile of 125I-labeled PTPζ-S to human midkine-coated ELISA plates. Scatchard analyses of the binding of PTPζ-S to midkine showed low (Kd = 3.0 nm) and high (Kd = 0.58 nm) affinity binding sites (Fig. 1B), which are similar to those of pleiotrophin-PTPζ binding (10). As shown in Fig. 1, the C-terminal half of midkine exhibited exactly the same binding properties to PTPζ-S as native midkine. On the other hand, the N-terminal half of midkine showed no binding activity to PTPζ-S (Fig. 1). Soluble pleiotrophin premixed with PTPζ-S inhibited the binding of PTPζ-S to pleiotrophin-coated ELISA plates (Fig. 2). In a similar dose-dependent manner, soluble pleiotrophin also inhibited the binding of PTPζ-S to midkine on the plates (Fig. 2), suggesting that pleiotrophin and midkine bind to the same binding site on PTPζ-S with a similar affinity. However, fairly high concentrations of pleiotrophin were required for inhibition (10–30 μg/ml) compared with the Kd values of midkine- or pleiotrophin-PTPζ-S binding obtained by solid-phase binding assay. These observations suggested that substrate-bound forms of midkine and pleiotrophin exhibit orders of stronger affinity to PTPζ-S than the soluble forms.

Midkine has two clusters of basic amino acids (Clusters I and II) located at the surface on one side of the C-terminal half domain, which are considered to be heparin binding sites (23). Cluster I contains Lys76, Arg78, and Lys83, and Cluster II contains Lys86, Lys88, and Arg86; amino acids were numbered according to mouse midkine. Among these, Lys76, Arg78, Lys83, and Lys86 are conserved in midkine and pleiotrophin of all species examined to date. On the other hand, Lys84 is conserved only in midkine of various species but is changed to Arg in pleiotrophin, and Arg86 is changed to Leu in pleiotrophin of various species and midkine of some species (23).

Five mouse midkine mutants were prepared, in which some of the basic amino acids in the Cluster I and/or II were changed.

**FIG. 1.** Midkine binds to PTPζ through the C-terminal half. A, wells of ELISA plates were coated with wild-type human midkine (●), the C-terminal half (○), or the N-terminal half of midkine (●), and the binding of 125I-PTPζ-S was measured by solid-phase binding assay. B, 125I-PTPζ-S binding to midkine (●), C-terminal half (○), or N-terminal half of midkine (●) was analyzed using Scatchard plots. Midkine exhibited high (Kd = 0.55 nm) and low (Kd = 2.4 nm) affinity binding sites, but the N-terminal half of midkine showed no binding.
to glutamine: R78Q, K83Q, K84Q, K83Q/K84Q, and R78Q/K83Q/K84Q (21, 22). As shown in Fig. 3, K83Q, K84Q, and K83Q/K84Q exhibited essentially the same binding activities to PTPz-S as the native midkine, suggesting that Cluster II is not essential for midkine-PTPz binding. In contrast, R78Q and R78Q/K83Q/K84Q exhibited only low affinity binding to PTPz-S, suggesting that Cluster I plays an important role in the high affinity binding between PTPz and midkine (Fig. 3 and Table I).

Effects of Chondroitinase ABC Digestion of PTPz-S on the PTPz-midkine Binding—Chondroitin sulfate chains of PTPz play an essential role in its high affinity binding to pleiotrophin (10). Chondroitinase ABC digestion of PTPz-S reduced its affinity also to midkine (Fig. 4). In contrast to the intact PTPz-S showing high ($K_d = 0.5 \text{ nM}$) and low ($K_d = 3 \text{ nM}$) affinity binding sites, chondroitinase ABC-digested PTPz-S exhibited only a low affinity binding site ($K_d = 8.8 \text{ nM}$) (Fig. 4, A and B). In addition, R78Q (Fig. 4, C and D) and R78Q/K83Q/K84Q (Table I), which have a mutation at Arg 78, showed a single binding site to intact PTPz-S with a $K_d$ value of 2.8 nm, in a similar affinity range to the chondroitinase ABC-digested PTPz-S (–8 nm). This suggested that Arg 78 is involved in binding to chondroitin sulfate to make up the high affinity binding site.

Influence of Glysosaminoglycans on PTPz-midkine Binding—Previously, we reported that pleiotrophin-PTPz-S binding is inhibited strongly by heparin, moderately by heparan sulfate, and chondroitin sulfate C, and very weakly by chondroitin sulfate A (10). Glysosaminoglycans inhibited midkine-PTPz-S interactions similarly (Fig. 5). Heparin strongly inhibited binding of PTPz-S to midkine ($IC_{50} = 10 \text{ ng/ml}$), heparan sulfate showed moderate inhibition ($IC_{50} = 100 \text{ ng/ml}$), and keratan sulfate exerted almost no effect. On the other hand, various types of chondroitin sulfate exerted diverse influences on midkine-PTPz-S binding. Chondroitin sulfate D and chondroitin sulfate E strongly inhibited binding ($IC_{50} = 70 \text{ ng/ml}$ for both types of chondroitin sulfate). Chondroitin sulfate B and chondroitin sulfate C showed moderate inhibitory effects ($IC_{50} = 500 \text{ ng/ml}$ and 1000 ng/ml, respectively), but chondroitin sulfate A exerted almost no effect ($IC_{50} > 100 \text{ \mu g/ml}$). Similar sensitivities to the various chondroitin sulfates were observed for pleiotrophin-PTPz binding (data not shown; data partly shown in Ref. 10).

Cell Migration-inducing Activity of Midkine—We reported previously that pleiotrophin induced cell migration of cortical neurons (11). Midkine also induced neuronal migration in Boyden chamber cell migration assay with essentially the same dose dependence profile as that of pleiotrophin (data not shown; see Fig. 3A of Ref. 11). Boyden chamber cell migration assay indicated that the C-terminal half of midkine exhibited full cell migration-inducing activity but the N-terminal half was devoid of activity (Fig. 6A). Midkine mutants, K83Q, K84Q, and K83Q/K84Q, which have amino acid replacements in Cluster II, showed normal levels of activity. In contrast, R78Q and R78Q/K83Q/K84Q exhibited low cell migration-inducing activity (Fig. 6B). These results suggested that Cluster I is sufficient for the neuronal migration-inducing activity of midkine.

Influence of Glysosaminoglycans on Midkine-induced Neuronal Migration—Midkine-induced neuronal migration was inhibited strongly by heparin, moderately by heparan sulfate, but
not by keratan sulfate (Fig. 7). As in the case of midkine-PTPζ-S binding, various types of chondroitin sulfate exerted diverse effects on midkine-induced neuronal migration. Chondroitin sulfate A exhibited almost no effect (Fig. 7). On the other hand, midkine-induced neuronal migration was inhibited strongly by chondroitin sulfate E and moderately by chondroitin sulfates B, C, and D (Fig. 7). Similar inhibitory effects by chondroitin sulfates were observed for pleiotrophin-induced neuronal migration (data not shown; data partly shown in Ref. 11).

**DISCUSSION**

In this study, we demonstrated that midkine binds to PTPζ. The characteristics of binding of midkine to PTPζ were indistinguishable from those of pleiotrophin (10), suggesting that PTPζ is a common receptor of midkine and pleiotrophin. Here, the C-terminal half domain of midkine was revealed to be sufficient for the binding. The C-terminal half domain of midkine exhibits various activities: strong heparin-binding activity, neurite promoting activity, and tissue plasminogen activator enhancing activity (21, 22). On the other hand, specific functions have not been found for the N-terminal half of midkine, although it weakly binds to heparin (21, 22, 26).

NMR spectroscopy indicated that there are two heparin-binding sites in the C-terminal half domain: Cluster I, which is composed of Lys276, Arg278, and Lys299 and Cluster II, which is composed of Lys83, Lys84, and Arg86 (23). On the other hand, in the N-terminal half domain, the basic amino acids do not form...
clusters which are expected to interact with the sulfate groups on heparin (23, 26). Our data showed that PTPζ-midkine binding was significantly affected by the mutation of Arg78, but not by mutations of Lys83, Lys84, or Lys85 + Lys86. Here, mutation of Arg78 resulted in loss of high affinity binding between midkine and PTPζ (Fig. 3), and the chondroitin sulfate portion of PTPζ plays an essential role in formation of the high affinity binding site (Fig. 4). Therefore, it seems that Arg78 of midkine is involved in binding to chondroitin sulfate on PTPζ. In support of this idea, various chondroitin sulfate preparations differentially affected midkine-PTPζ binding (Fig. 5). Among various chondroitin sulfate species, there was a significant difference in the inhibiting activity. This finding suggested that there must be a specific structural motif of chondroitin sulfate that strongly inhibits midkine-PTPζ binding. However, the nature of this structure is not known at present because commercially available chondroitin sulfate samples contain considerable heterogeneity. Nevertheless, it is possible to speculate that Arg78 of midkine recognizes a specific structure of chondroitin sulfate on the PTPζ molecule, which is also present in chondroitin sulfates C, D, and E, but not in chondroitin sulfate A. An oversulfated structure is one of the candidates; however, the fine structure of chondroitin sulfate chains of PTPζ must be determined to further clarify this point. A similar finding was reported for DSD-1-PG, a chondroitin sulfate proteoglycan expressed in the rodent central nervous system, that is recognized by a monoclonal antibody 473HD (27). DSD-1-PG exhibited neurite outgrowth-promoting activity, which was blocked by 473HD or by chondroitinasin ABC digestion of this proteoglycan (27). The binding of 473HD to DSD-1-PG was inhibited by chondroitin sulfates C and D, but not by chondroitin sulfates A or B (27, 28), suggesting that a specific structural motif of chondroitin sulfate plays an important physiological function in the brain.

Chondroitinasin ABC-treated PTPζ showed markedly reduced binding affinity to midkine. Mutations of midkine at Arg78, Lys83, and Lys84 did not influence binding to the chondroitinasin ABC-treated PTPζ, suggesting that these amino acids do not play an essential role in binding to the core glycoprotein portion of PTPζ. In summary, there seems to be a hierarchy with three steps in the binding between PTPζ and midkine: 1) low affinity binding between midkine and core glycoprotein portion of PTPζ (Kd = ~3 nM); 2) medium affinity binding between midkine and PTPζ bearing general structure of chondroitin sulfate (Kd = ~0.6 nM); and 3) high affinity binding between midkine and PTPζ bearing a specific structural motif of chondroitin sulfate (Kd = ~0.2 nM), which involves a specific contribution of Arg78 of midkine.

Boyden chamber cell migration assay indicated that the mutation of Arg78 of midkine significantly reduced the neuronal migration-inducing activity of this factor (Fig. 6). In contrast, mutations of Lys83 and Lys84 did not influence this activity. These observations suggested that the high affinity binding of midkine and PTPζ is important for the neuronal migration-inducing activity. Here, heparin strongly inhibited midkine- and pleiotrophin-induced neuronal migration, and only the substrate-bound forms of these factors exhibit this activity (11, 17), which is consistent with the finding that PTPζ exhibits very low affinity to soluble pleiotrophin (Fig. 2). In contrast, plasminogen activator-enhancing activity of midkine was markedly reduced by double mutation of Lys83 and Lys84, but not by the single mutation of Arg78, Lys83, or Lys84 (22). The soluble forms of midkine and pleiotrophin enhance plasminogen activator activity. However, it has been suggested that enzymatic dimerization of midkine and pleiotrophin induced by heparin-like oligosaccharides (presumably endogenous heparan sulfate) is required for plasminogen activator-enhancing activity (17). Here, exogenously added heparin could substitute for endogenous heparan sulfate (17). Taken together, these two activities of midkine and pleiotrophin are thought to be mediated by distinct receptors. Neurite-promoting activity of midkine was also markedly reduced by mutation of Arg78, while mutations of Lys83 and Lys84 were less effective (22). These observations suggested that the neurite-promoting and the neuronal migration-inducing activities of midkine are mediated at least partly by the same or similar receptor(s).

Midkine binds to a syndecan family heparan sulfate proteoglycan, rydoucan, with high affinity (29). Pleiotrophin/heparin-binding growth-associated molecule binds to N-syndecan, which is thought to be another pleiotrophin receptor involved in pleiotrophin-induced neurite extension (30). It would be helpful to examine the binding of syndecan family proteoglycans with midkine mutants to determine the physiological significance of these interactions.

Acknowledgment—We thank Akiko Kodama for secretarial assistance.

REFERENCES

1. Krueger, N. X., and Saito, H. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 7417–7421
2. Levy, J. B., Canoll, P. D., Silvennoinen, O., Barnea, G., Morse, B., Honegger, A. M., Huang, J.-T., Cannizzaro, L. A., Park, S.-H., Druck, T., Huebner, K., Sap, J., Ehrlich, M., Musachio, J. M., and Schlessinger, J. (1993) J. Biol. Chem. 268, 10573–10581
3. Maeda, N., Hamaoka, H., Shintani, T., Nishiwaki, T., and Noda, M. (1994) FEBS Lett. 354, 67–70
4. Barnea, G., Grumet, M., Miller, P., Silvennoinen, O., Levy, J. B., Sap, J., and Schlesinger, J. (1994) J. Biol. Chem. 269, 14349–14352
5. Maurel, P., Rauch, U., Flad, M., Margolis, R. K., and Margolis, R. U. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 5352–5356
6. Nishiwaki, T., Maeda, N., and Noda, M. (1998) J. Biochem. (Tokyo) 123, 458–467
7. Peles, E., Nativ, M., Campbell, P. L., Sakurai, T., Martin, R., Lev, S., Clary, D. O., Schilling, J., Barnea, G., Flowman, G. D., Grumet, M., and Schlessinger, J. (1995) Cell 82, 251–260
8. Grumet, M., Miller, P., Sakurai, T., Karthikeyan, M., Bourdon, M., Margolis, R. K., and Margolis, R. U. (1994) J. Biol. Chem. 269, 12142–12146
9. Miley, P., Maurel, P., Haring, M., Margolis, R. K., and Margolis, R. U. (1996) J. Biol. Chem. 271, 15716–15723
10. Maeda, N., Nishiwaki, T., Shintani, T., Hamaoka, H., and Noda, M. (1996) J. Biol. Chem. 271, 21446–21452
11. Maeda, N., and Noda, M. (1998) J. Cell Biol. 142, 203–216
12. Kadomatsu, K., Tomomura, M., and Muramatsu, T. (1988) Biochem. Biophys. Res. Commun. 151, 1312–1318
13. Muramatsu, T. (1994) Dev. Growth Differ. 36, 1–8
14. Raya, H. (1989) EMBO J. 8, 2933–2941
15. Li, Y.-S., Milner, P. G., Chauhan, A. K., Watson, M. A., Hoffman, R. M., Kodner, C. M., Milbrandt, J., and Deuel, T. F. (1990) Science 250, 1690–1694
16. Muramatsu, H., and Muramatsu, T. (1991) Biochem. Biophys. Res. Commun.
17. Kojima, S., Muramatsu, H., Amanuma, H., and Muramatsu, T. (1995) J. Biol. Chem. 270, 9590–9596
18. Chauhan, A. K., Li, Y.-S., and Deuel, T. F. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 679–682
19. Kadomatsu, K., Hagihara, M., Akhter, S., Fan, Q.-W., Muramatsu, H., and Muramatsu, T. (1997) Br. J. Cancer 75, 354–359
20. Fabri, L., Maruta, H., Muramatsu, H., Muramatsu, T., Simpson, R. J., Burgese, A. W., and Nice, E. C. (1993) J. Chromatogr. 646, 213–226
21. Asai, T., Watanabe, K., Ichihara-Tanaka, K., Kaneda, N., Kojima, S., Iguchi, A., Inagaki, F., and Muramatsu, T. (1997) Biochem. Biophys. Res. Commun. 236, 66–70
22. Akhter, S., Ichihara-Tanaka, K., Kojima, S., Muramatsu, H., Inui, T., Kimura, T., Kaneda, N., Talukder, A. H., Kadomatsu, K., Inagaki, F., and Muramatsu, T. (1998) J. Biochem. (Tokyo) 123, 1127–1136
23. Iwasaki, W., Nagata, K., Hatanaka, H., Inui, T., Kimura, T., Muramatsu, T., Yoshida, K., Tasumi, M., and Inagaki, F. (1997) EMBO J. 16, 6936–6946
24. Maeda, N., Hamanaka, H., Oshira, A., and Noda, M. (1995) Neuroscience 67, 23–35
25. Inui, T., Bodi, J., Kuo, S., Nishio, H., Kimura, T., Kojima, S., Maruta, H., Muramatsu, T., and Sakakibara, S. (1996) J. Pept. Sci. 2, 28–39
26. Muramatsu, H., Inui, T., Kimura, T., Sakakibara, S., Song, X.-J., Maruta, H., and Muramatsu, T. (1994) Biochem. Biophys. Res. Commun. 203, 1131–1139
27. Faisser, A., Clement, A., Lochtner, A., Streit, A., Mandl, C., and Schachner M. (1994) J. Cell Biol. 126, 783–799
28. Nadanaka, S., Clement, A., Masayama, K., Faisser, A., and Sugahara, K. (1998) J. Biol. Chem. 273, 3296–3307
29. Kojima, T., Katsuzi, A., Yamazaki, T., Muramatsu, T., Nagasaka, T., Ohsumi, K., and Saito, H. (1996) J. Biol. Chem. 271, 5914–5920
30. Raulo, E., Chernousov, M. A., Carey, D. J., Nolo, R., and Rauvala, H. (1994) J. Biol. Chem. 269, 12999–13004