IA-2 (insulinoma-associated protein 2) is a protein-tyrosine phosphatase receptor located in secretory granules of neuroendocrine cells. Initially, it attracted attention due to its involvement in the autoimmune response associated with diabetes. Later, it was found that upon exocytosis, the cytoplasmic domain of IA-2 is cleaved and relocated to the nucleus, where it enhances the transcription of the insulin gene. A concerted functioning of the whole receptor is to be expected. However, very little is known about the structure and function of the transmembrane and extracellular domains of IA-2. To address this issue, we solved the x-ray structure of the mature ectodomain of IA-2 (meIA-2) to 1.30 Å resolution. The fold of meIA-2 is related to the SEA (sea urchin sperm protein/enzyme) domains of mucins, suggesting its participation in adhesive contacts to the extracellular matrix and providing clues on how this kind of molecule may associate and form homo- and heterodimers. Moreover, we discovered that meIA-2 is self-proteolyzed in vitro by reactive oxygen species, suggesting the possibility of a new shedding mechanism that might be significant in normal function or pathological processes. Knowledge of meIA-2 structure should facilitate the search of its possible ligands and molecular interactions.

Protein-tyrosine phosphatases (PTP), together with the corresponding kinases, regulate cell division, growth, differentiation, and metabolism (1). There are cytoplasmic PTP as well as transmembrane receptors PTP (RPTP). The latter also participate in cell-cell and cell-matrix contacts, possess an impressive diversity of adhesive and multimerization modules (2), and have been involved in human diseases such as cancer, autoimmunity, and degenerative processes (1).

Two paralog RPTPs, IA-2 (insulinoma-associated protein 2, also termed PTP35 or ICA512) and IA-2 β (PTPR2, also known as phogrin or IAR), were identified as major autoantigens in type-1 diabetes mellitus (3). They have a signal peptide, an ectodomain, a single-pass transmembrane region, and a single intracellular PTP domain.

IA-2 and IA-2 β are prominent in the secretory granules (SG) of brain, pituitary, pancreatic islet, and adrenal endocrine cells (4). Although the physiological ligands for these receptors are unknown and their function is poorly understood, they are involved in hormone and neuropeptide secretion. Indeed, single- and double-knock-out mice lacking IA-2 suffer from glucose intolerance, impaired insulin secretion, and abnormal secretion of pituitary hormones and female infertility (5, 6).

Processing of pro IA-2 by furin-like hormone convertases produces mature IA-2 (7), which lacks the signal peptide and an adjacent fragment (residues 1–448). Mature IA-2 reaches the plasma membrane during exocytosis and comprises extracellular (449–575), transmembrane (576–600), and cytoplasmic domains (601–979). High glucose levels up-regulate IA-2 (8), and insulin exocytosis triggers a Ca2+-dependent and μ-calpain-mediated cleavage of the IA-2 cytoplasmic domain, which, then, is targeted to the nucleus, where it promotes transcription of insulin and other SG genes (7).

An IA-2/β2-syntrophin/utrophin complex may anchor the SG to actin (7). Ca2+, besides activating μ-calpain, promotes the dephosphorylation of β2-syntrophin, which then dissociates from IA-2, allowing targeting the SG to the plasma membrane, cleavage by μ-calpain, and sorting of the cleaved cytoplasmic domain to the nucleus to stimulate insulin synthesis.

Most RPTPs have two cytoplasmic catalytic domains: D1 and D2. D2 is enzymically deficient due to mutations affecting the active site, reversing of which restores catalysis (9). Oxidation of one of the cysteine residues of D2 to a sulfenyl amide by reactive oxygen species (ROS) induces a conformational change coupled to the formation of disulfide linked D2 (10), and
dimerization would lead to an inactivating interaction between cognate D1 (11). Interestingly, the conformational change of D2 is coupled to conformational changes in the ectodomain (12). In this connection, the transmembrane regions have been found to possess dimerization potential per se (13), and conceivably, as part of signaling processes, the whole receptor may undergo coupled conformational changes during association or binding to yet unknown ligands (7).

Both forms of IA-2 have only the inactive D2 domain and homo- and heterodimerize (14). Since co-expression of IA-2 and IA-2 β together with other active RPTPs reduces the enzymic activity, heterodimerization has been proposed as a regulatory mechanism (14).

It has also been noted that the absence of known adhesive modules in the ectodomain of IA-2 and IA-2 β would make them odd among RPTPs. In fact, with the exception of the subtypes R7 (PCPT1) and R8 (IA-2 and I-A2 β), all RPTPs have a large variety of extracellular modules apt for the interaction with the extracellular matrix and cell-cell contact (1, 2, 15).

To shed some light on the organization and function of the mature ectodomain of IA-2 (hereafter meIA-2), we report its x-ray structure at 1.30 Å resolution. It was found that meIA-2 possesses a widespread, ferredoxin-like fold. This fact went unnoticed in previous works because meIA-2 lacks sequential homology to known proteins. Knowledge of meIA-2 structure allowed us to perform a more sensitive search of sequential homology and uncover the meIA-2 relationship to the SEA (sea urchin sperm protein, enterokinase, agrin) family of proteins (16), which are specialized domains for oligomerization and interaction with the extracellular matrix. Thus, IA-2 possesses a typical adhesive ectodomain that would allow its interaction with a plethora of different molecules and structures. This finding may be important in the development of a comprehensive model of SG trafficking and regulation of the insulin secretion. It also opens the opportunity of exploring the role of the adhesive ectodomain in normal and pathological processes.

**EXPERIMENTAL PROCEDURES**

**Miscellaneous**—Unless otherwise indicated, reagents were from Sigma. The broad protease inhibitor mixture was Set III (AEBSF, aprotinin, bestatin, E-64, leupeptin, and pepstatin A; Calbiochem). Metals were determined by atomic absorption spectroscopy. Changes in the solvent-accessible surface area (ASA) upon dimerization were calculated as ΔASA_{AB} = ASA_A + ASA_B - ASA_{AB}. Size exclusion chromatography was carried out at 20 °C using an AKTA fast protein liquid chromatography system and a Superose 12 column (GE Healthcare, Uppsala, Sweden) equilibrated with 20 mM sodium phosphate, 150 mM NaCl, pH 7.0.

For the detection of contaminant proteases (17), meIA-2 (∼15 nmol) was incubated 3 weeks at 20 °C or 3 h at 37 °C in 200 μl of 25 mM Tris-HCl, 100 mM sodium chloride, 5 mg/ml α-casein, pH 7.4. At different times, 60-μl aliquots were withdrawn, and the reaction was stopped by adding 60 μl of 1.8 M trichloroacetic acid. After a 30-min incubation at 0 °C, the precipitate was removed, and the absorbance at 280 nm measured to assess the extent of proteolysis. Sequence Analysis—Alignments were performed using Clustal (18) with default parameters. Hidden Markov models (HMM) analyses and searches were carried out with HMMER (19). The 65 SEA seed sequences in Pfam and 24 additional sequences identified by PSI-Blast of meIA-2, MUC1, and MUC16 were compiled, and the latter three sequences were structurally aligned by examination of the corresponding superimposed structures. The remaining 86 sequences were aligned to the structural alignment using ClustalX in the “profile alignment mode.” Previously, the SEA sequence termini were slightly extended to ensure proper register with the structural alignment. The 89 sequences so aligned (supplemental data file 1) were trimmed to the first and last non-gapped columns and used to build a new HHM profile (supplemental data file 2).

**Computational Chemistry**—Molecular dynamic simulations (MDS) were carried out using GROMACS 3.3 (20) and GROMOS 53a6 force field (21). The initial structures were the crystallographic dimers (see Fig. 1, a and b) embedded in a ∼200-nm³, dodecahedral periodic cell and solvated with ∼5900 water molecules (22). Conditioning, cut-off schemes, temperature and pressure coupling, boundary conditions, and long range electrostatics were as described (23). The system was an isobaric-isothermal ensemble at 300 K and 1 bar, with weak temperature tropistics were as described (23). The system was an isobaric-isothermal ensemble at 300 K and 1 bar, with weak temperature and pressure coupling (0.1 and 1.0 ps⁻¹, respectively). All hydrogen atoms were considered explicitly, and protein covalent bonds were constrained using LINCS (24). Cluster analysis (25) was carried out with a cut-off value of 0.2 nm.

**Oxygen-mediated Cleavage**—MeIA-2 (30 μM) in 50 μl of 0.1 M Tris-HCl, pH 7.2, was supplemented, singly or in combination, with the following additives: (a) sodium ascorbate (20 mM), (b) H₂O₂ (3 or 30 mM), and (c) metal ions (Fe³⁺, Cu²⁺; 1 mM). After 2 h at 30 °C, the reaction was stopped by adding SDS-PAGE sample buffer (26).

**Protein Expression and Purification**—MeIA-2 corresponds to residues 449–576 of human IA-2 (Uniport entry: PTPRN_HUMAN) numbered 1–128 for simplicity. Soluble Escherichia coli proteins were subjected to ionic exchange chromatography on a 1.5 × 5.0-cm column Mono Q (GE Healthcare) equilibrated at 20 °C with 20 mM Tris-HCl, pH 8.0, and eluted with a salt gradient (20 ml, 0–0.2 M NaCl in equilibration buffer). Further purification to >95% was achieved by size-exclusion chromatography on Superose 12 (GE Healthcare). The purified protein was concentrated using Centricron (Millipore, Billerica, MA). The product identity and integrity were verified by mass analysis, which yielded the value expected from the sequence within 1 Da.

**Crystallization**—Crystals of meIA-2, in the form of flattened spindles of about 150 × 50 × 50-μm³, were obtained after 2 months at 19 °C with the hanging drop method. The reservoir solution (300 μl) was 25% (w/v) polyethylene glycol 4000, 0.1 M Tris-HCl, 0.2 M CaCl₂, pH 8.5. The drop (2 μl) was a 1:1 blend of reservoir and protein solution (∼10 mg/ml in 10 mM Tris-HCl, 50 mM NaCl, pH 7.4).

**Data Collection and Processing**—X-ray diffraction data were collected at the National Synchrotron Light Source on beamline X6A, at 100 K, using an ADSC Q210 detector (Area Detector Systems Corp., Poway, CA). Before data collection, crystals

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*Note:* The text is a partial representation of the full document, focusing on the experimental procedures and structural analysis of the mature ectodomain of IA-2. The full document would provide detailed methods, results, and discussion relevant to the research.
were soaked in mother liquor supplemented with 10% (w/v) polyethylene glycol 400 and flash-cooled in liquid nitrogen. Two data sets were collected on a single crystal: one at low energy (7.8 keV, 1.589 Å) for phasing purposes and one at high energy (12.6 keV, 0.979 Å) for refinement. A single crystal was used for the collection of all diffraction data. Indexing, integration, scaling, and reduction were performed with the HKL2000 suite of programs (27). Five percent of the measured reflections in the high energy data set was flagged for cross-validation. Relevant data collection parameters are given in Table 1.

Structure Solution, Model Building, and Refinement—The structure was solved by the single-wavelength anomalous dispersion method. Phases were calculated relying on the anomalous signal in the data recorded at low energy. SHELX (28) was used to determine the position of anomalous scatterers, ultimately attributed to calcium ions, and to calculate initial phases. Statistics from SHELXD at 2.00 Å were: CC (all/weak) = 45/28 and PATFOM = 38. SHELXE processing yielded PSEUDO CC = 66, Connectivity = 89, and FOM = 0.63 at 1.65 Å. Further improvement was achieved by density modification with DM (29) (FOM = 0.81; 82/82 residues per monomer; and 76 assigned water molecules). Automated model building was performed with ARP/wARP (30) and allowed 90% of the model to be traced. The initial model was manually completed and refined with the high energy data using COOT (31) and REFMAC5 (32), respectively. This procedure also allowed the identification of three calcium ions and 176 water molecules in the asymmetric unit.

A few residues presented weak or missing side-chain electron density and were modeled as alanine. The final $2F_o - F_c$ Fourier map is of excellent quality and in concordance with the high resolution of the structure, and 94.9 and 5.1% of the residues were found in the most favored and additionally allowed regions, respectively, of the Ramachandran plot. The coordinates and structure factors of meIA-2 have been deposited in the Protein Data Bank (entry 2qt7). Molecular graphics were displayed with MOLMOL (33) and PyMOL.

RESULTS

Crystal Structure—MeIA-2 crystallized in the orthorhombic space group P2$_1$2$_1$2$_1$, and the structure was refined to 1.30 Å (see Table 1 and "Experimental Procedures" for the details). The asymmetric unit contains a protein dimer (Fig. 1) whose monomers superimpose with a C$_{e}$ root-mean-square (r.m.s.) deviation of 0.57 Å. The monomer main axes are 18.5, 14.4, and 11.6 Å in length. The corresponding values for the dimer are 30.5, 21.5, and 18.3 Å. There was no electron density for residues 1–19 and 111–128, which cannot be the result of local disorder for the presence of the missing residues would lead to unrealistic crystal density. It will be shown below that the absence is due to proteolysis during crystallization.

The monomer possesses a ferredoxin-like or (βαβ)×2 fold (34) (Fig. 1c). The antiparallel, four-stranded β sheet is characteristically twisted. The hydrogen bond ladder and strand pairing is regular, except for the presence of a β bulge at the beginning of β2 and a distortion involving the last and first residues of β1 and β4, respectively.

In the interface of the asymmetric unit dimer (Fig. 1a), despite involving equivalent protein surfaces, some of the side chains adopt non-equivalent rotamers to maximize the contacts. The total buried surface is ~1000 Å$^2$ (more than 10% of the dimer surface), corresponding to β4-β4 and αα-αα pairing, with numerous van der Waals interactions, six backbone, and six side-chain intramolecular hydrogen bonds.

A second dimerization mode can be observed in the crystal due to the antiparallel zippering β2-β2 (Fig. 1b). As in the asymmetric unit, an extended, eight-stranded antiparallel β sheet is so formed, which allows continuous growing of extended chains with antiparallel, two-fold screw axes along the c cell dimension. In this case, the interface buries 1100 Å$^2$ and involves, in addition, antiparallel pairing α1-α1, 10 hydrogen bonds, and numerous van der Waals contacts.

Layers of the above chains are further stabilized by two additional contacts per monomer mediated by calcium ions, which bury ~350 Å$^2$ each (supplemental Fig. 1). Metal binding causes the non-equivalence of the monomers: the asymmetric metal binding site 1 involves atoms from two A monomers, the asymmetric site 2 bridges one monomer A to one monomer B (of a different asymmetric unit), and site 3 is built with atoms from a single A monomer.

Most carboxylates are grouped on the exposed faces of the α helices, whereas positive charges concentrate on a small region close to the C terminus of β3 (not shown). There is a patch of hydrophobic residues in the exposed face of the β sheet. This non-polar surface is close to the C terminus and might be covered by the 17 residues that precede the transmembrane segment and become proteolyzed during crystallization (see below). On the other hand, the internal faces of the helices and β sheet are tightly filled with aliphatic and aromatic side chains, forming a classical hydrophobic core.

A search for structural similarity to meIA-2 using DALI (35) retrieved over 200 matches. A large variety of proteins, with diverse origin, shape, and functions, contains modules with this

| Table 1: Data collection, phasing, and refinement statistics |
| --- |
| **Data collection**                                         |     |
| Wavelength, Å                                             | 0.9793 | 1.5895 |
| Space group                                               | P212121 | P212121 |
| Unit cell Å                                               | a = 31.8 | a = 31.8 |
|                                                          | b = 66.8 | b = 66.8 |
|                                                          | c = 73.1 | c = 73.1 |
| Resolution, Å                                             | 30.00-1.30 (1.32-1.30) | 30.00-1.65 (1.68-1.65) |
| Rmerge, %                                                 | 0.03 (0.21) | 0.03 (0.16) |
| I/σ(I)                                                   | 43.1 (5.3) | 54.6 (6.8) |
| Completeness, %                                           | 97.7 (81.4) | 94.7 (50.7) |
| Redundancy                                                | 7.6 (4.3) | 9.5 (3.9) |
| **Reefinement**                                           |     |
| Resolution, Å                                             | 30.00-1.30 |
| No. of reflections                                        | 36265 |
| Rmerge/Rfree                                              | 0.220/0.242 |
| Protein atoms                                             | 1383 |
| Calcium atoms                                             | 3 |
| Water molecules                                           | 176 |
| **Bond r.m.s.**                                           |     |
| Lengths and angles, Å$^2$                                 | 0.008, 1.161 |

* Data were collected on a single crystal.
* Values in parentheses are for the highest-resolution shell.
versatile fold. However, the angles between strands and helices vary among them, as well as the length and complexity of the connecting loops. Like some of the proteins with this fold, melA-2 has a characteristic kink in $\alpha_2$ (Fig. 1).

Although the examples of similar architecture are many, one particularly interesting one is that of SEA: eukaryotic extracellular domains that are located in heavily glycosylated environments, contain linked carbohydrates, and might regulate or assist binding to extracellular moieties (16). The least-square superimposition of melA-2 and two SEA members with known three-dimensional structure (36, 37) (Fig. 2) results in r.m.s. deviation of 2.5 Å (MUC1; 65% of melA-2 backbone atoms) and 2.3 Å (MUC16; 56% of melA-2 backbone atoms). Although the structural similarity between melA-2 and the above SEA domains is high, a distinct segment between $\beta 1$ and $\alpha 1$ is absent in melA-2. This segment is called the YT turn because it contains threonine and tyrosine residues well conserved among mucins (37).

Homology to SEA—No sequence relationship between melA-2 and other protein domains was previously reported (38), and neither melA-2 sequence corresponds to a protein family in Pfam (39). Nevertheless, the HMM profile (19) for SEA in Pfam (entry PF01390) does not take into account explicit conformational information. Since the x-ray structure of melA-2 adds to that of two bona fide SEA members recently solved (36, 37), it was natural to ask whether a new HMM profile incorporating structural information would be able to identify both SEA and melA-2 domains. To that end, the 65 SEA seed sequences in Pfam and 24 additional sequences identified by PSI-Blast of melA-2, MUC1, and MUC16 were compiled and aligned, combining sequential and structural information. The aligned sequences were used to build a new HHM profile (supplemental data file 2). The HMM search using the new HMM profile retrieves all known SEA members and also the extracellular juxtamembrane domains of IA-2 and IA-2 from different species; it does so without including false positives and with sensitivity as good as that the currently used HMM for detection of SEA domains (not shown). Incidentally, an HMM profile built from a conventional Clustal alignment of the above 89 sequences is only slightly less sensitive (not shown), indicating that structural considerations are not crucial for relating the sequences of melA-2 and SEA domains.

When unaligned sequences of IA-2, IA-2, and crystallized SEA domains are individually aligned to the new HMM profile, highly significant matches are found (Fig. 3), the alignment is consistent with the secondary structure of the characterized domains, and a set of common conserved residues is revealed. In the alignment shown, the similarity between SEA and melA-2 is particularly evident for the C-terminal helix and strands. These findings, along with the structural and topological correspondence, lend support to the hypothesis that SEA and melA-2 may have diverged from a common ancestor.

Significance of melA-2 Dimers—Analytical size exclusion chromatography indicated the existence of a monomer-dimer equilibrium at 0.1–10 $\mu$M concentrations. $R_s$ values were 21.0 and 24.0 Å for monomer and dimer, respectively, which is compatible with the size and shape of melA-2. By fitting the dependence of $R_s$ with protein concentration (not shown), a $K_d$
above 20 μM, higher order association takes place. Cross-linking experiments using disuccinimidyl suberate or glutaraldehyde also evidenced concentration dependent dimerization (not shown).

Further, it was found by MDS that after 20 ns, the asymmetric unit dimer undergoes a local conformational change, but it does not dissociate (supplemental Fig. 2a). Although the overall fold of the monomers remains the same, in one of them, the kink at Val-89 in α2 is straightened and α1 changes its orientation, making the dimer significantly asymmetric. The conformational change obliterated a helix-flanked and water-filled groove under the sheets (see the center of the structure in Fig. 1a). In the simulated dimer, as water is drained away from the groove, the helices move and fill the void, concealing the hydrophobic side chains of Leu-88 and Val-89 from the solvent. This has a cost, however, for the previously buried Leu-41 becomes solvent-exposed.

Neither did the second dimer observed in the crystal (Fig. 1b) dissociate during a 20-ns MDS. In this case, only minor fluctuations around the crystallographic coordinates were observed (supplemental Fig. 2b).

Proteolysis of meIA-2—Crystals of meIA-2 were subjected to SDS-PAGE, mass analysis, and Edman degradation. The analyses showed that chain hydrolysis takes place during crystallization and at several positions close to the termini (Fig. 1d).

Further experiments evidenced that proteolysis takes weeks at room temperature, is enhanced by a broad specificity protease inhibitor mixture, and has a narrow optimal range of pH close to 7.0 (Fig. 4). The enhancement of proteolysis by the protease inhibitor mixture remains unexplained, but it has precedents (36). On the other hand, the proteolysis rate is independent of meIA-2 concentration (in the 7–370 μM range, not shown), and assays with the highly susceptible substrate casein (17), for up to 3 weeks, gave no evidence of contaminant proteases in the preparations of meIA-2. The rate of cleavage was, however, significantly reduced by metal chelators such as EDTA (not shown) or DTPA (Fig. 4) and by protein denaturants (2 M urea, not shown). These results, along with the fact that a contaminant protease with the observed specificity (attacking both the C termini and the N termini, on a variety of side chains) would be unlikely, indicate that the cleavage does not depend on protein diffusion, is mediated by bound metals, and is conformation dependent.

The above features, in addition to the characteristic pH dependence (40), are indicative of a ROS-mediated cleavage reaction (26). It was also found that the cleavage reaction is inhibited by free radical scavengers (mannitol, dimethyl sulfoxide, and β-mercaptoethanol; not shown), and most impor-
slightly, cleavage does not take place under a nitrogen atmosphere (Fig. 5a). Thus, all the above evidences lead to the conclusion that the observed proteolysis is mediated by oxygen radicals.

Atomic absorption spectroscopy showed that the metal content (calcium, iron, copper, zinc, manganese, nickel, magnesium, and cobalt) of meIA-2 preparations was so low that less than 1% of the pure protein preparations would carry bound metals. On one hand, this confirmed that Ca^{2+} observed by x-ray diffraction comes from the crystallization buffer, and on the other hand, this confirms that traces of bound metal suffice to promote proteolysis.

Slowness of the cleavage reaction, then, could be due to the scarcity of active metals and redox species in the meIA-2 solutions. To test this possibility, millimolar concentrations of ascorbate, redox-active metals, and H_{2}O_{2} were added. Under these rather mild conditions for the generation of ROS, meIA-2 is so rapidly and severely damaged that it becomes undetectable by SDS-PAGE (Fig. 5, b and c) after 2 h. More diluted redox additives produce weak SDS-PAGE bands. However, the products failed to yield sequenceable ends (other than the initial) and originated very complex mass spectra, which precluded assignment. These results uncovered the extreme sensitivity of meIA-2 to ROS. Indeed, several protein controls included in the assays were resistant to the above oxidative conditions (not shown), as most proteins are (40).

**DISCUSSION**

IA-2 is currently under close scrutiny because of its involvement in two main aspects of diabetes: deficient insulin secretion and autoimmunity. Due to the complexity of the secretory machinery and to the multidomain and transmembrane character of IA-2, the physiological role of this protein is not properly understood. Even less is known on the events that make IA-2 one of the primary autoantigens upon the inflammatory destruction of pancreatic islet cells.

In a recent work, we demonstrated that the isolated, mature ectodomain of IA-2 attains a highly stable three-dimensional structure (38), and herein, we describe that structure at 1.30 Å resolution. It turns out that meIA-2 possesses a widespread ferredoxin-like fold, although previous sequence similarity searches failed to suggest that fact.

The structure, topology, and glycosylation potential of meIA-2 directed our attention to a subgroup of the ferredoxin-like family: that of the glycosylated extracellular domains of transmembrane proteins called SEA. Making use of HMM profiles for the detection of vanishingly low sequential similarity, the structural homology between meIA-2 and SEA could be related to the primary structure. This provides a new perspective in the study of the evolution, structure, and function of these domains.

The only two structures of SEA domain previously known, mucins MUC1 and MUC16 (36, 37), were solved by NMR and lack the necessary details to hint at possible associations modes between monomers. Now, the intermolecular interactions of the homodimeric meIA-2 structure immediately suggest two potential ways by which this module might function in oligomerization. The feasibility of dimerization and higher order association was confirmed by the hydrodynamic behavior of meIA-2. In addition, MDS strongly suggests that these structures are energetically favored even in the absence of crystal lattice forces.

It remains to be seen whether the crystallographic dimers correspond to the actual dimers in solution and, even more important, if they have real significance in vivo. However, the details revealed by the x-ray structure should allow the design of experiments to answer this question, not only for IA-2 but also for many other extracellular molecules carrying SEA domains. The two observed dimerization modes (Fig. 1, a and b) and the conformational switch identified for one of the monomers by MDS (supplemental Fig. 2a) highlight the potential plasticity of meIA-2 for mediating adhesive interactions with the like molecules of the extracellular matrix and even with membranes from different cells. By structure-aided design of probes, tagged analogues, and site-directed mutations, it should be possible to achieve the molecular characterization of these interactions in vivo and in vitro.

Proteolytic release of an extracellular fragment or shedding has been described for an increasing number of transmembrane proteins: several mucins (36, 41), some PTP (2), Ig-hepta (42), and serine proteases matriptase and ephitin (43). This processing has been implicated in cell-to-cell communication, apoptosis, signaling, sensing of chemical or mechanical stress, defense against infections and chemical damage, and cancer (37, 43, 44). Many of the above mentioned proteolytic events occur at SEA modules (37, 42), and it was hypothesized that all membrane-residing proteins containing a SEA module will undergo cleavage as part of a regulatory mechanism based in receptor alliances (45). In some cases, shedding is due to specific membrane proteases (45). In other cases, autoproteolysis takes place. The typical cleavage signal G↓SVVV of SEA-containing proteins (45) is missing in meIA-2. However, the unexpected finding that meIA-2 becomes slowly proteolyzed at room temperature leads one to wonder whether this is relevant in...
Structure of IA-2 Mature Ectodomain

in vivo. SDS-PAGE multiple bands of IA-2, which are not fully explained by glycosylation, have been repeatedly reported in the literature. It has been hypothesized that altered electrophoretic mobility after extensive enzymic deglycosylation may be due to the sulfation of tyrosine in the ectodomain (46), but proteolysis was not dismissed as the cause of the heterogeneity. Although in vitro proteolysis of meIA-2 is slow, it is much faster than the spontaneous hydrolysis of peptide bonds. Moreover, in vivo enhancing factors cannot be discarded a priori. One enhancing factor could be the presence of ROS, particularly because of the known high susceptibility of beta cells to those noxious agents (47). H2O2 is involved in insulin and its receptor regulates PTP activity (48), and it would not be a surprise if H2O2 were also involved in an oxidative shedding of the ectodomain. Clearly, further work is justified to establish whether meIA-2 is excised from the membrane in vivo, by which mechanism, and what would be the physiological meaning of such event.

When designing further investigations aimed to unveil its physiological role, the possibility of a concerted functioning of the entire IA-2 receptor must be considered. Particularly, meIA-2 might be involved in signaling, by experiencing conformational changes upon binding of a yet unknown moiety of the SG or the extracellular matrix. Those conformational changes might alter the oligomerization state of the receptor or be transmitted via the transmembrane domain to the cytoplasm. In this way, effective delivery of the cargo to the extracellular space might be sensed by the secretory cells, and the subsequent intracellular events might be modified accordingly. Another exciting possibility is that meIA-2 might act as an adhesive module for the spatial ordering of the newly exposed membrane during exocytosis. The variety of interaction modes envisaged from the crystal arrangement permits speculation as to whether, by homo- or heterodimerization, meIA-2 would participate in reorganizing the membrane after secretion.

Also, a participation of meIA-2 in the condensation of the secretory granule and conditioning of the proteins inside should be considered. Preliminary studies gave no evidence of a significant in vitro interaction between meIA-2 and the secreted proteins. However, the issue deserves a deeper investigation.

In summary, the x-ray structure of meIA-2 adds to that of the intracellular domains of PTP (15), providing a solid platform for further studies on the biochemistry of PTP and making it possible in the near future to model the receptor structure as a whole and to assess the conformational changes associated with its function. Also, the classification of meIA-2 among the SEA domains should be of use in the evolutionary study of transmembrane proteins in general. Finally, a number of testable hypotheses can be derived from the dimerization mode and the scissile nature of meIA-2, covering a broad range of biological effects in normal and pathological conditions.

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