Protein Engineering vol.14 no.11 pp.875–880, 2001

Homology modelling and molecular dynamics studies of human placental tissue protein 13 (galectin-13)

Balázs Visegrády[1], Nándor G.Than[2], Ferenc Kilár[1], Balázs Sümegi[2], Gábor N.Than[3,4] and Hans Bohn[5]

[1]Central Research Laboratory, [2]Department of Biochemistry and [3]Department of Obstetrics and Gynaecology, Medical School, University of Pécs, H-7624 Pécs, Hungary and [5]Behringwerke AG, Postfach 1140, D-35041 Marburg/Lahn, Germany

[4]To whom correspondence should be addressed

The primary structure of the newly sequence analysed placental tissue protein 13 (PP13) was highly homologous to several members of the β-galactoside-binding S-type lectin (galectin) family. By homology modelling, the three-dimensional structure of PP13 was built based on high-resolution crystal structures of homologues and also their characteristic 'jellyroll' fold was found in the case of PP13. Our model has been deposited in the Brookhaven Protein Data Bank. By multiple sequence alignment and structure-based secondary structure prediction, we underlined the structural similarity of PP13 with its homologues. The secondary structure of PP13 was identical with 'prototype' galectins consisting of a five- and a six-stranded β-sheet, joined by two α-helices, and galectins' highly conserved carbohydrate-recognition domain (CRD) was also present in PP13. Of the eight consensus residues in the CRD, four identical and three conservatively substituted were shared by PP13. By docking simulations PP13 possessed sugar-binding activity with highest affinity to *N*-acetyllactosamine and lactose typical of most galectins. All ligands were docked into the putative CRD of PP13. Based on several lines of evidence discussed in this paper demonstrating that PP13 is a novel galectin, PP13 was also designated galectin-13. These computational results provide some new insights into the possible role and importance of PP13 in various processes of the human body and can be of help in the initial steps of further functional research.

Keywords: β-galactoside binding/carbohydrate-recognition domain/galectin/molecular modelling/placental protein 13

Introduction

Placental protein 13 (PP13) is the member of the so-called 'pregnancy-related protein' family, which consists of 56 different proteins diverging either in their structure or function and grouped earlier on the basis of their increased expression in placenta, in some maternal tissues, especially in the liver or in the fetus during pregnancy. Expressions of these proteins are repressed after delivery, but they may be re-expressed in different tumour tissues. All these proteins were summarized in our earlier book (Than *et al.*, 1993). The structural and functional characteristics of these proteins and their possible role in cell and tissue development and cell differentiation are in the focus of interest.

PP13 was purified and characterized physico-chemically in 1983. Its molecular mass was 30.3 kDa by ultracentrifugation and 29.0 kDa by SDS–PAGE. PP13 was composed of two identical 16 kDa subunits held together by disulfide bonds as it could be reduced to half of its original size with mercaptoethanol. The carbohydrate content of PP13 was the lowest of the placental proteins, 0.6%. On average, human term placenta contained 3.7 mg of PP13 (Bohn *et al.*, 1983).

In our molecular biological studies, expression of PP13 was investigated in 26 types of normal adult and fetal and some adult tumorous human tissues by Western and Northern blot assays. PP13 was mainly found in placenta, but some PP13 expression was also detected in healthy spleen, kidney and bladder tissues and in liver adenocarcinoma, neurogen tumour and malignant melanoma. Neither in previous PP13 RIA measurements nor in the present PP13 Western blots could we find detectable quantities of PP13 in maternal or fetal serum or in amniotic fluid (Than *et al.*, 1986, 1999). By cloning and sequence analysis, PP13 turned out to be a 16.118 kDa, 139 residue-long protein (GenBank accession no.: AF117383) (Than *et al.*, 1999). Its primary structure showed 69% homology to the 16.5 kDa human eosinophil Charcot–Leyden crystal protein (galectin-10), a unique dual-function protein, a member of the widespread and growing β-galactoside-binding galectin family (Barondes, 1984; Leffler *et al.*, 1989; Barondes *et al.*, 1994), which also possesses weak lysophospholipase activity (Ackerman *et al.*, 1993; Leffler, 1997). PP13 was also highly homologous (50%) to several other galectins and therefore we concluded that it was a new member of this protein family. Previously weak lysophospholipase activity of PP13 was confirmed by ^{1}H and ^{31}P NMR measurements (Than *et al.*, 1999) and now its carbohydrate-binding properties are under investigation.

Elucidation of structural determinants of binding specificity for each galectin is important in order to understand their interaction with glycoconjugates at physiological concentrations and their biological function. To gain a better understanding of structure–function relationships in the case of PP13, as currently no crystal structure data are available, we modelled its three-dimensional (3D) structure based on the available structures of the homologues proteins.

Methods

Computational methods of 3D model building

All computations were done on an Indy workstation (Silicon Graphics). Energy minimization, molecular dynamic and docking studies were carried out using the molecular modelling package SYBYL (version 6.5, Tripos, St. Louis, MO). The 3D model of PP13 was built from its primary amino acid sequence by homology modelling based on six high-resolution crystal structures of homologous proteins. Swiss-Model (version 3.5) protein modelling server (Peitsch, 1995, 1996; Guex and Peitsch, 1997) was used for selecting the 3D models of the closest homologues available in the Brookhaven Protein Data Bank (PDB) for creating our own protein database. The 3D conformation of PP13 was built using the Composer

Table I. Data for the closest homologue galectins of PP13 with known 3D structures obtained with the Swiss-Model server

PDB code	Protein	Chain	Authors	Identity to PP13 (%)
1LCL	Human Charcot–Leyden crystal protein/galectin-10		Leonides *et al*., 1995	54.0
1BKZ	Human galectin-7	A, B	Leonides *et al*., 1998	28.8
1A3K	Human galectin-3		Seetharaman *et al.*, 1998	25.9
1QMJ	CG-16 chicken agglutinin	A, B	Varela *et al.*, 1999	24.5

in Biopolymer module of SYBYL. Conformations of loop sequences were automatically generated by the program. We used a four-step energy minimization within SYBYL, which was mainly based on the manual, but modified in detail. First, hydrogen atoms were added to the structure and their position was refined using a 1000-step minimization, while the rest of the structure was kept fixed. Second, 1000 steps of energy minimization were performed, with structurally conserved regions (SCRs) remaining fixed. Third, 1000-step minimization of the structure was done by fixed backbone of SCRs. Finally, 2000 steps of geometrical optimization were performed without any fixed region. Energy minimization of the PP13 model was carried out using the Tripos force field, a distance-dependent dielectric constant of 4.0 R and a non-bonded atom cut-off of 25 Å, by the Powell method (Powell, 1977). All hydrogen atoms were included during the calculation. The model was subjected to molecular dynamics study, as the temperature was raised to 300 K for 10 ps in 1 fs steps and unconstrained pressure, with Boltzmann initial velocities and random number seed. We used the same force field setup as that used for energy minimization. Snapshots were taken every 10 fs. No solvent was included in the model. Validation of the model was carried out using Ramachandran plot calculations computed with the PROCHECK program (Laskowski *et al.*, 1993). Colour figures were generated by SYBYL.

Multiple sequence alignment and secondary structure prediction

PP13 amino acid sequence was first aligned with all non-redundant sequences and databases using the MaxHom dynamic multiple sequence alignment program (Sander and Schneider, 1991). Structure-based multiple sequence alignment of PP13 and its selected homologues was carried out with the CLUSTALW package (Thompson *et al.*, 1994). Automatic alignments were critically analysed and compared. Secondary structural elements of the aligned proteins were determined by the DSSP program (Kabsch and Sander, 1983).

Substrate docking

Five carbohydrate ligands (*N*-acetyllactosamine, lactose, mannose, *N*-acetylgalactosamine and galactose) were built and geometrically optimized by the Spartan molecular modelling software (Wavefunction, USA) and used for two different molecular docking calculations.

An automatic docking study was performed using the FlexX module of SYBYL (Böhm, 1992, 1994; Klebe, 1994; Rarey *et al.*, 1996). The whole PP13 protein was used as receptor without any constraint during the calculations. The FlexX algorithm determined the binding site for all dockings, resulting in different ligand conformations positioned at the binding site. Final protein–ligand conformations were obtained after 1000 steps of energy minimization with the same parameters as used for 3D model building. All docked carbohydrate conformations at the binding site were characterized by their binding energies (Huang *et al.*, 1999).

Docking with prepositioned ligands were computed with SYBYL based on the X-ray diffraction data for related galectin–sugar complexes. As the highly conserved triptophan residue in galectins' carbohydrate-recognition domain (CRD) has a key role in orienting sugar rings to the binding site, at the start, sugar rings were placed to the same position in PP13 CRD in relation to the conserved Trp72 as it was in other galectin–sugar complexes. Final protein–ligand conformations were obtained after 200 steps minimization using the Tripos force field and a non-bonded atom cut-off of 25 Å, by the Powell method (Powell, 1977). Since stacking interactions between aromatic rings and pyranose rings account for the most of binding energy in galectins besides H-bond interactions, characteristic van der Waals energies, H-bonds and H-bond forming residues in PP13–ligand complexes were analysed and compared. Strict comparison between the predicted ligand positions obtained by docking and the sugar bound in related galectins was also performed.

Results

3D model building of PP13

The 3D structure of PP13 was built by homology modelling based on the known 3D structures of its closest homologues. We selected those models of the homologue galectins, which were not in complexes with ligands and had the highest identity (54.0–24.5%) to PP13 (Table I). In addition to their significant homology, all selected structures (human CLC protein, human galectin-7, human galectin-3 and chicken agglutinin CG-16) possessed highly similar overall structural folds to each other (Leonidas *et al.*, 1995, 1998; Seetharaman *et al.*, 1998; Varela *et al.*, 1999).

In the first step, we constructed different models of PP13 based on varying numbers of the six preselected homologue structures. Since there was no significant improvement in the folding of the models based on fewer but higher identity score homologues, we therefore used the model based on all six pre-selected structures. There were no inserted gaps in the sequence and altogether 10 structurally conserved regions (SCR) and 11 loops were built in this model. Loop sequences were generated automatically by the program using its default protein database. We obtained the minimum energy of –198.412 kcal/mol and RMS force value of 0.111 kcal/mol.Å of PP13 structure after the four-step energy minimization procedure. The topology of PP13 structure showed very close similarity to galectin-7 and CLC protein, with the characteristic 'jellyroll' motif resulting from a tight association between a five- and six-stranded β-sheet joined by two α-helices at the two ends (Figure 1A and B).

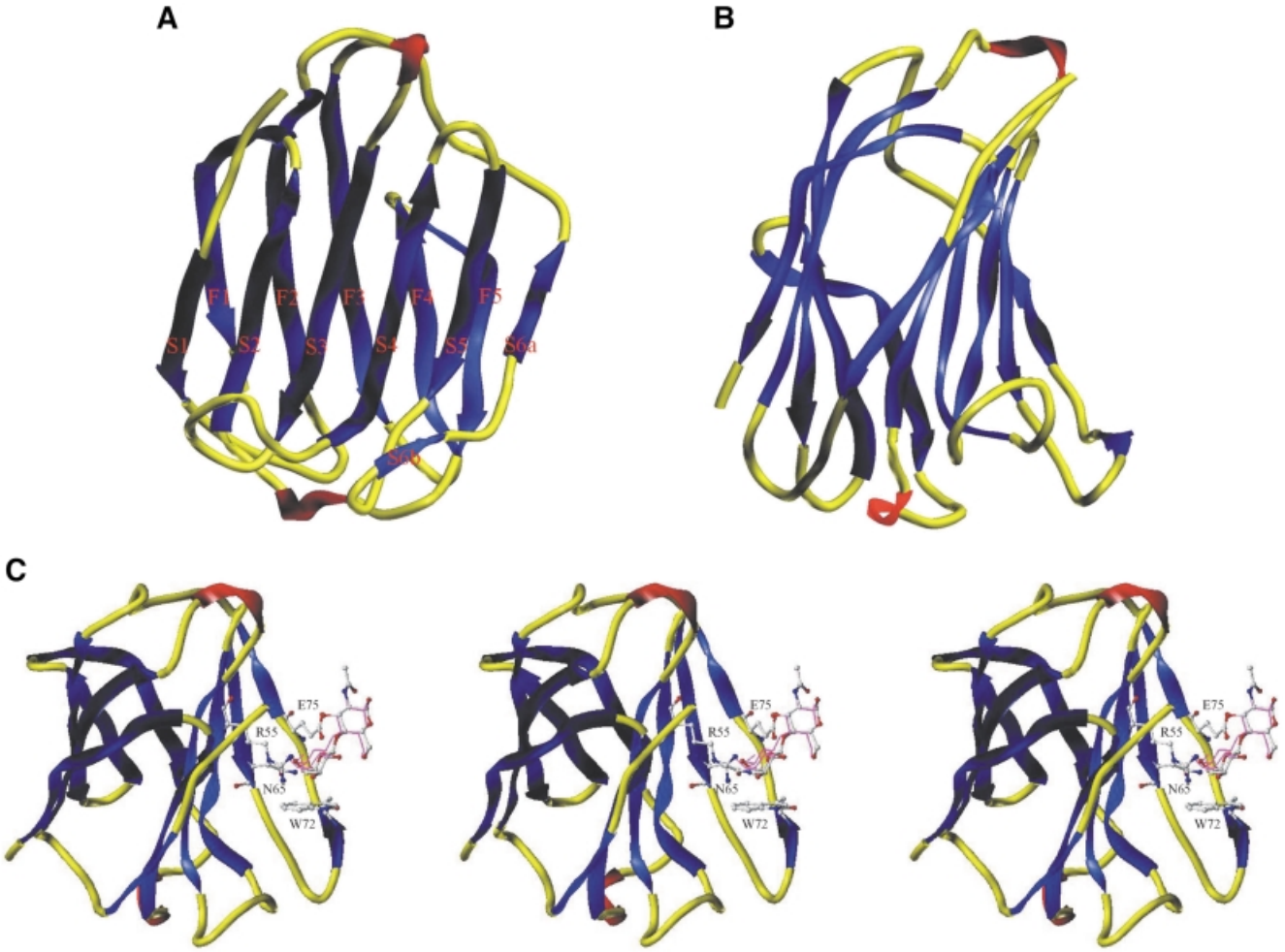

Fig. 1. (**A**, **B**) Orthogonal views of PP13 molecule. The 3D model of PP13 was coloured by its secondary structure. The characteristic 'jellyroll' structure comes from the tight association between a five-stranded (F1–F5) and six-stranded β-sheet (S1–S6a/S6b) joined by two α-helices at the two ends. (**C**) Stereoscopic view of PP13 with the bound *N*-acetyllactosamine. Conserved residues interacting in carbohydrate binding (Arg55, Asn65, Gln75) and the triptophan ring (Trp72) playing a key role in stacking interactions are shown by a ball-and-stick model. The orientation of *N*-acetyllactosamine in the galectin-7 complex is shown with pink line drawing.

Table II. Ramachandran plot calculations on 3D model of PP13 computed with the PROCHECK program

% of residues in most favoured regions	73.0
% of residues in additional allowed zones	26.2
% of residues in generously allowed regions	0.0
% of residues in disallowed regions	0.8
% of non-glycine and non-proline residues	100.0

The r.m.s. deviation for covalent bonds relative to the standard dictionary is 0.021 Å and for the covalent angles is 2.2°.

Validation of PP13 structure

We performed the Ramachandran structure validation test in the PROCHECK program. The ϕ and φ distributions of the Ramachandran plots of non-glycine, non-proline residues are summarized in Table II. The r.m.s. deviation for covalent bonds relative to the standard dictionary was 0.021 Å and for the covalent angles was 2.2°. Altogether 99.2% of the residues were in favoured and allowed regions. After successful structural validation, the PP13 model has been deposited in the Brookhaven Protein Data Bank with the accession code 1F87.

Multiple sequence alignment and secondary structure prediction

Amino acid sequences of PP13, human galectin-3, human galectin-7 and human CLC protein were aligned with MaxHom and CLUSTALW algorithms. Given their PDB files, secondary structures were also analysed and compared by the DSSP program (Figure 2). The secondary structure of PP13 showed close similarity to the whole structure of 'prototype' galectins (galectin-7 and CLC protein) and to the structure of the C-terminal carbohydrate-recognition domain of a 'chimaera-type' galectin (galectin-3). All aligned proteins contain a five-stranded (F1–F5) and six-stranded (S1–S6a/S6b) β-sheet, joined by one or two α-helices at their ends. Furthermore, in spite of several amino acid differences in the primary structures of PP13 and CLC protein, their secondary structure turned out to be identical except for sheet F1, which is longer by one residue in PP13. By the alignment, galectins' highly conserved CRD is present in PP13. This consensus and invariant motif is implicated to consist of 13 residues (Oda *et al.*, 1993), of which eight play an essential role in sugar binding (Hirabayashi and Kasai, 1994; Dyer and Rosenberg, 1996). Of these eight, four are identical (Val63, Asn65, Trp72 and Glu75) and

Fig. 2. Multiple sequence alignment of PP13 and its homologues. Structure-based sequence alignment of PP13 (1F87), human Charcot–Leyden crystal protein (galectin-10) (1LCL), human galectin-3 (1A3K), human galectin-7 (1BKZ) and chicken CG-16 agglutinin (1QMJ) was carried out with the CLUSTALW algorithm, secondary structural elements (β-strands F1–F5 and S1–S6b) were determined with the DSSP program and are indicated by boxes. Identical residues between PP13 and its homologues are indicated in boldface; highly conserved residues comprising the carbohydrate-recognition domains are shadowed. Amino acid positions in PP13 are shown above the sequences.

three are conservatively substituted (Arg53, Arg55 and His57) in PP13.

Substrate docking

All five carbohydrate ligands (*N*-acetyllactosamine, lactose, mannose, *N*-acetylgalactosamine and galactose) were docked with two different methods to the surface of the putative CRD of PP13, which lies on the concave face of the S4–S5–S6 β-sheet.

By automatic docking, 15 protein–ligand conformations were analysed for each carbohydrate, from which the best fittings were chosen. The strongest binding interaction, characterized by ligand binding energies, was found in the case of *N*-acetyllactosamine (–26.9 kcal/mol). Galactose (–23.8 kcal/mol), lactose (–22.5 kcal/mol) and mannose (–20.1 kcal/mol) had lower and *N*-acetylgalactosamine (–17.8 kcal/mol) had the lowest ligand binding energy. Although the conformations were minimized, distortions in sugar rings occurred.

By prepositioned docking, we obtained refined PP13–ligand conformations with no distortions in sugar rings. Similarly to automatic docking, the highest van der Waals fitting was found in the case of *N*-acetyllactosamine. A strong stacking interaction with the highly conserved Trp72 residue and three strong H-bond interactions (<2.5 Å) with the conserved Arg55, Asn65 and Gln75 residues in PP13 CRD were detected, resulting in a sugar orientation very similar to that detected in galectin-7 (Figure 1C). In the cases of lactose, *N*-acetylgalactosamine and galactose, smaller van der Waals energy values (lower stacking interactions to Trp72 and fewer H-bond interactions to conserved residues) were detected, resulting in larger differences between sugar orientations in PP13 CRD and CRDs of related galectins. Mannose (natural ligand of CLC protein, the closest homologue of PP13) had the lowest van der Waals fitting to PP13 CRD, with no H-bond interactions detected and an orientation outside the binding region, as a result of a rather weak stacking interaction (Table III).

Discussion

Galectins are extensively conserved and distributed in the animal kingdom. Originating more than 800 million years ago derived from an ancestor gene, their common basic molecular properties and affinity for β-galactoside binding have been maintained throughout evolution. They are soluble cytoplasmic proteins, which may be secreted into the cell surface without any signal peptide via a non-classical pathway and act as receptors for poly-*N*-acetyllactosamine sequences on glycoprotein side chains and mediate cognitive interactions between cells and extracellular matrix components. Their specificity for the sugar code provides the cellular selection of binding molecules. Their developmentally regulated expression in a wide variety of cell types and capacity for multiple interactions with carbohydrate ligands make them important factors influencing cell–cell and cell–matrix interactions. Therefore, galectins are thought to mediate such crucial physiological reactions as cell adhesion and migration, cell growth regulation, tissue differentiation and remodelling apoptosis triggering or inhibition and they also may contribute in pathological processes such as neoplastic transformation, tumour progression, invasion and metastasis (Barondes *et al.*, 1994; Inohara and Raz, 1995; Kasai and Hirabayashi, 1996; Perillo *et al.*, 1998).

By cloning and sequence analysis, the previously known PP13 turned out to be very closely related to the CLC protein and other members of the galectin family. Although similarly to CLC protein, PP13 possessed a weak lysophospholipase activity, the possibility of it being a new galectin isolated from the proliferating tissue placenta made this protein attractive to study, as it might be involved in such processes as cell growth regulation and immunomodulation. To understand the structural and functional characteristics, we built its 3D structure by homology modelling and simulated its possible interactions with glucoconjugates by molecular docking.

We found several lines of evidence demonstrating that PP13 is indeed a novel galectin: (1) PP13 was found in soluble form in cells; (2) PP13 was demonstrated to form homodimers as most of the galectins do; (3) reactive SH groups were found in PP13 similarly to other galectins; (4) the primary amino acid sequence of PP13 had high homology to galectins, especially to CLC protein; (5) the secondary structure of PP13 was identical with CLC protein containing the characteristic five- and six-stranded β-sheets and two additional α-helices; (6) the highly conserved galectin CRD was found in PP13, comprising four identical and three conservatively substituted residues out of the eight in the consensus motif implicated to play an essential role in sugar-binding; (7) the overall 'jellyroll' structural fold of PP13 was the same as in 'prototype' galectins; the PP13 model was validated, accepted and deposited in the Brookhaven Protein Data Bank; (8) by docking simulations, PP13 possessed sugar-binding activity with highest affinity to *N*-acetyllactosamine and lactose, main ligands for galectins; (9) all the investigated sugar ligands were docked into the concave face of the S4–S5–S6 β-sheet, which is considered to be the CRD in galectins. H-bonds and stacking interactions were identified at those conserved residues in PP13 CRD (Arg53, Arg55, Asn65, Gln75) that were shown to be involved in sugar binding in related galectins; (10) preliminary data showed that PP13 exhibited, although to a lesser extent than other galectins, considerable sugar-binding properties.

Although its highest sequential homology was to CLC protein, the sugar-binding affinity of PP13 (strongest inter-

Table III. Van der Waals energies of PP13–ligand complexes

Ligand	Van der Waals energy (kcal/mol)	H-bonds	H-bond forming residues
N-Acetyl-D-lactosamine	−765.2	3	Arg55, Asn65, Gln75
Lactose	−759.4	2	Arg55 (2)
N-Acetyl-D-galactosamine	−751.9	2	Arg55 (2)
Galactose	−751.4	3	Arg53 (2), Asn65
Mannose	−741.5	0	–

H-bonds (<2.5 Å) formed between PP13 CRD and its ligands and H-bond forming residues in PP13 CRD were calculated with SYBYL.

action with *N*-acetyllactosamine and weakest interaction with mannose) was more similar to galectin-7 (strong interaction with *N*-acetyllactosamine and lactose) (Leonidas *et al.*, 1998) than to CLC protein (strong interaction only with mannose) (Swaminathan *et al.*, 1999). To understand this, we have to take account that in most galectins, conserved arginines in the CRD are indispensable for sugar binding as playing an important role in the interactions between galectins and the glucose moiety of lactose (Lobsanov *et al.*, 1993). In CLC protein, replacement of two arginines found in other galectins (Arg → Cys57 and Arg → Glu77) was proposed to account for its lower binding affinity (Leonidas *et al.*, 1995). Although conserved arginines in other galectins were replaced with hystidine and threonine (Arg → Hys57 and Arg → Thr77) in PP13, conserved hystidine and asparagine in related galectins were also replaced with arginines (Hys → Arg53 and Asn → Arg55). The remaining four conserved CRD residues in other galectins were identical in PP13. Overall, in contrast to CLC protein, the two arginines crucial in sugar binding with a hystidine were kept, but in different positions. This led to a significantly different CRD architecture from CLC protein, but more comparable to other galectin CRDs (Leonidas *et al.*, 1998). Owing to the structural changes, different sets of H-bond interactions and sugar-binding abilities of PP13 occur.

Giving emphasis to our results, parallel with computational studies, the PP13 gene has been identified on chromosome 19 (19q13.1) in a close vicinity between galectin-7 and CLC protein genes, by the collaborators of the International Human Genome Sequencing Consortium (accession no. NT_000966) and was published in *Nature* (International Human Genome Sequencing Consortium, 2001). The coding sequence of PP13 gene is divided into four exons with the entire CRD encoded by the third exon, as in the case of other prototype galectins.

Not only sequence similarity and structural and functional characteristics, but also localization of its gene, probably originating by gene duplication on chromosome 19, demonstrate the relationship of PP13 to related prototype galectins. Since galectins are numbered sequentially (Barondes *et al.*, 1994) and the names for galectin-1–galectin-12 have already been assigned, PP13 has been designated galectin-13.

Being a new galectin, PP13 may have functions similar to those of other galectins in proliferating tissues such as placenta and tumours. Human placentation is a complex biological process involving specifically regulated cell–matrix interactions in which galectins play a crucial role. For example, galectin-1 and galectin-3 were found to bind several placental glycoconjugates such as the basement membrane glycoprotein laminin or fibronectin. Their expression in human placenta is developmentally regulated in an autocrine and paracrine manner and they are possibly involved in many biological events such as embryogenesis, embryo implantation, trophoblast invasion, organization and deposition of the extracellular matrix, etc. (Colnot *et al.*, 1998; Vicovac *et al.*, 1998). In a wide variety of cancer cells, galectin-1 and galectin-3 are regulated in inverse proportion to the laminin receptor, allowing attachment to, and detachment from, laminin as invasion progresses. They are thought to be involved in carcinogenesis and cognitive cellular interactions during transformation and metastasis (Castronovo, 1993; Inohara and Raz, 1995).

Acknowledgements

This work was supported by Grants OTKA T/020622, T/023076, T/025527 and T/029824/1999 and Grants FKFP 1393/1997, 0010/1999, 0320/2000 and 0166/2001.

References

Ackerman,S.J., Corrette,S.E., Rosenberg,H.F., Bennett,J.C., Mastrianni,D.M., Nicholson-Weller,A., Weller,P.F., Chin,D.T. and Tenen,D.G. (1993) *J. Immunol.*, **150**, 456–468.
Barondes,S.H. (1984) *Science*, **223**, 1259–1264.
Barondes,S.H., Cooper,D.N.W., Gitt,M.A. and Leffler,H. (1994) *J. Biol. Chem.*, **269**, 20807–20810.
Böhm,H.J. (1992) *J. Comput.-Aided Mol. Des.*, **6**, 61–78 and 593–606.
Böhm,H.J. (1994) *J. Comput.-Aided Mol. Des.*, **8**, 243–256.
Bohn,H., Kraus,W. and Winckler,W. (1983) *Oncodev. Biol. Med.*, **4**, 343–350.
Castronovo,V. (1993) *Invas. Metast.*, **13**, 1–30.
Colnot,C., Fowlis,D., Ripoche,M.A., Bouchaert,I. and Poirier,F. (1998) *Dev. Dyn.*, **211**, 306–313.
Dyer,K.D. and Rosenberg,H.F. (1996) *Life Sci.*, **58**, 2073–2082.
Guex,N. and Peitsch,M.C. (1997) *Electrophoresis*, **18**, 2714–2723.
Hirabayashi,J. and Kasai,K. (1994) *Glycoconj. J.*, **11**, 437–442.
Huang,X., Nagy,P.I., Williams,F.E., Peseckis,S.M. and Messer,W.S.,Jr. (1999) *Br. J. Pharm.*, **126**, 735–745.
Inohara,H. and Raz.A. (1995) *Cancer Res.*, **55**, 3267–3271.
International Human Genome Sequencing Consortium (2001) *Nature*, **409**, 860–921.
Kabsch,W. and Sanders,C. (1983) *Biopolymers*, **22**, 2577–2637.
Kasai,K. and Hirabayashi,J. (1996) *J. Biochem.*, **119**, 1–8.
Klebe,G.J. (1994) *Mol. Biol.*, **237**, 221–235.
Laskowsky,R.A., MacArthur,M.W., Moss,D.S. and Thornton,J.M. (1993) *Appl. Crystallogr.*, **26**, 283–291.
Leffler,H. (1997) *Trends Glysci. Glycotechnol.*, **45**, 9–19.
Leffler,H., Masiarz,F.R. and Barondes,S.H. (1989) *Biochemistry*, **28**, 9222–9229.
Leonidas,D.D., Elbert,B.L., Zhou,Z., Leffler,H., Ackerman,S.J. and Acharya, K.R. (1995) *Structure*, **3**, 1379–1393.
Leonidas,D.D., Vatzaki,E.H., Vorum,H., Celis,J.E., Madsen,P. and Acharya, K.R. (1998) *Biochemistry*, **37**, 13930–13940.
Lobsanov,Y.D., Gitt,M.A., Leffler,H., Barondes,S.H. and Rini,J.M. (1993) *J. Biol. Chem.*, **268**, 27034–27038.
Oda,Y., Herrmann,J., Gitt,M.A., Turck,C.W., Burlingames,A.L., Barondes,S.H. and Leffler,H. (1993) *J. Biol. Chem.*, **268**, 5929–5939.
Peitsch,M.C. (1995) *Biol. Technol.*, **13**, 658–660.
Peitsch,M.C. (1996) *Biochem. Soc. Trans.*, **24**, 274–279.
Perillo,N.L., Marcus,M.E. and Baum,L.G. (1998) *J. Mol. Med.*, **76**, 402–412.
Powell,M.J.D. (1977) *Math. Program.*, **12**, 241–254.

Rarey,M., Kramer,B., Lengauer,T. and Klebe,G. (1996) *J. Mol. Biol.*, **261**, 470–489.
Sander,C. and Schneider,R. (1991) *Proteins*, **9**, 56–68.
Seetharaman,J. Kanigsberg,A., Slaaby,R., Leffler,H., Barondes,S. and Rini,J.M. (1998) *J. Biol. Chem.*, **273**, 13047–13052.
Swaminathan,G.J., Leonidas,D.D., Savaga,M.P., Ackerman,S.J. and Acharya,K.R. (1999) *Biochemistry*, **38**, 13837–13843.
Than,G., Szabó,D., Göcze,P., Arany,A. and Bognár,Z. (1986) *Magy. Nöorv. L.*, **49**, 11–15.
Than,G.N., Bohn,H. and Szabó,D. (1993) *Advances in Pregnancy Related Protein Research*. CRC Press, Boca Raton, FL, pp. 1–333.
Than,N.G., Sümegi,B., Than,G.N., Berente,Z. and Bohn,H. (1999) *Placenta*, **20**, 703–710.
Thompson,J.D., Higgins,J.D. and Gibbons,T.J. (1994) *Nucleic Acids Res.*, **22**, 4673–4680.
Varela,P.F. Solis,D., Diaz-Maurino,T. Kaltner,H., Gabius,H.J. and Romero,A. (1999) *J. Mol. Biol.*, **294**, 537–549.
Vicovac,Lj., Jankovic,M. and Cuperlovic,M. (1998) *Hum. Reprod.*, **13**, 730–755.

Received October 4, 2000; revised May 17, 2001; accepted August 9, 2001