Glyco-Forum section

Letter to the Glyco-Forum

Controversial iduronate ring conformation in dermatan sulphate

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Glycosaminoglycans are linear polymers of alternating acidic (β-D-glucuronic acid or α-L-iduronic acid; just β-D-galactose in keratan sulphate) and basic (2-amino-2-deoxy-β-D-galactosamine or 2-amino-2-deoxy-β-D-glucosamine) monosaccharides. The repeating disaccharide unit is often N-acetylated or N-sulphated and/or O-sulphated (except in hyaluronic acid). Glycosaminoglycans constitute the carbohydrate part of proteoglycans which have been shown to take part in a wide range of biological functions. For example, they are involved in forming the extracellular matrix with collagen to bind to growth factors with a high degree of specificity and regulate the growth factor activity, and are also shown to have anticoagulant, antiplatelet, antiangiogenic and antitumour activities (Casu, 1985; Casu et al., 1988; Hardingham and Bayliss, 1989; Hardingham and Fosang, 1992). Hence an understanding of the structure and conformation of glycosaminoglycans is of utmost importance to understand their biological functions.

Although a great deal of information is available about the chemical structure of glycosaminoglycans, the conformation of some of them, particularly dermatan sulphate which consists of alternating N-acetyl-β-D-galactosamine (β-D-GalNAc) and α-L-iduronic acid (α-L-IdUA) residues, has been the subject of much debate. While the conformation of β-D-GalNAc is well established (C1) (Takai et al., 1972; Virudachalam and Rao, 1976), there are conflicting views about the conformation of α-L-IdUA. Such an uncertainty about the conformation of α-L-IdUA has led to controversy about the chain conformation of dermatan sulphate. Mitra et al. (1983) considered a C4 conformation for α-L-IdUA in order to satisfy the repeat distance of the helical chain derived from X-ray diffraction studies. However, Ragazzi et al. (1990) found that all the three ring conformations, C1, S0 and C4 (Figure 1), are compatible with the unit axial rise observed by Mitra et al. (1983). Based on the X-ray diffraction and 13C NMR studies, Winter et al. (1986) showed that α-L-IdUA in dermatan sulphate assumes very similar conformations both in solid state and solution with trans-diaxial orientation of hydroxyl groups as in the C4 conformation. Rees et al. (1985) suggested that α-L-IdUA assumes predominantly a C4 conformation based on low coupling constant values and circular dichroism studies. They also suggested that a small fraction of the C1 conformation is in equilibrium with the predominant C4 conformation to explain the periodate oxidation studies. However, such an equilibrium cannot explain the higher value for JH2,H3 compared to JH3,H4 (Gatti et al., 1979; Table 1). On the other hand, Casu et al. (1986) suggested that the S0 skew boat conformation also explains the susceptibility of the α-L-IdUA to periodate oxidation. Recently, Venkataraman et al. (1994) reported that the C4 conformation of α-L-IdUA does not provide the required repeat length and suggested yet another conformation, S2 (Figure 1d), as a likely candidate for α-L-IdUA in dermatan sulphate. However, the JH2,H3 and JH3,H4 values calculated by these authors for α-L-IdUA in either the C1 or C4 conformations are approximately equal, in contrast to the experimental values where JH2,H3 is much higher than JH3,H4 (Table I). The JH,H values calculated for S2 conformation are also quite different and have least agreement with the experimental values. Moreover, this conformation is least favoured energetically among the four conformations that have been considered by the earlier workers. Although the calculated JH,H values of α-L-IdUA in S0 conformation show similar

Fig. 1. Stereo diagram showing the possible conformations for the pyranose ring in α-L-iduronic acid. (a) C1; (b) S0 (also referred to as S3); (c) C4; (d) S2 (also referred to as S3).

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gauche (H4, H5e), the coupling protons are in orientation, (4.37 Hz) is low compared to the experimental value (5.7 Hz).

3 and, for a-D-xylopyranosides, the calculated pyranose ring geometry. However, this equation is useful for from the experimental values by as much as 2 Hz (Table II).

Negative atom/groups on the values. For example, for the HiH HiH values (Karplus, bond length) may have large effects on the \( V \) values for the torsion angles and hence the deviations in the values since a small change in the geometry (bond angle and however, cannot be reproduced in quite a few cases by

3 values for a number of HiH HiH J values (1980) were used to calculate the \( J_{HH} \) values for various saccharide geometries. These were compared with the experimental \( J_{HH} \) values to arrive at a pyranose ring conformation without testing the validity of this equation for pyranosides. The calculated \( J_{HH} \) values reported for \( \alpha-L-IdUA \) by different authors for the same conformation also differ significantly (Table I).

Using Haasnoot's equation \( \text{equation (8)} \) in Haasnoot et al. (1980), we have calculated the \( J_{HH} \) values for a number of saccharide derivatives whose NMR data are available and compared these with the experimental values (Figure 2, Table I). Generally, a high value (7–11 Hz) is expected for the coupling constant when the coupling protons are in axial–axial orientations, whereas a low value of 1–5 Hz is expected when they are in axial–equatorial or equatorial–equatorial orientations. Although there is a general agreement between the calculated and experimental coupling constants, the observed values, however, cannot be reproduced in quite a few cases by Haasnoot's equation. However, these differences in \( J_{HI} \) values may not signify substantial differences in the ring conformations since a small change in the geometry (bond angle and bond length) may have large effects on the \( J_{HI} \) values (Karpplus, 1963). Thus, Haasnoot's equation does not predict 'accurate values' for the torsion angles and hence the deviations in the pyranose ring geometry. However, this equation is useful for predicting qualitatively the orientational effects of electronegative atom/groups on the \( J \) values. For example, for the various galactopyranosides, the calculated \( J_{HIH} \) values differ from the experimental values by as much as 2 Hz (Table II) and, for \( \alpha-D-xylopyranosides \), the calculated \( J_{HIH} \) value (4.37 Hz) is low compared to the experimental value (5.7 Hz).

In both these cases, i.e. \( \beta-D-\text{galactose} \) (H4, H5S) and \( \alpha-D-\text{xylose} \) (H4, H5e), the coupling protons are in gauche orientation, but the electronegative atoms (O4, O5) are in gauche (B-D-galactose) and trans (\( \alpha-D-\text{xylose} \)) orientations (Figure 2). Such a difference in the orientation of the electronegative atoms with respect to the coupling protons will have opposing effects on the \( J \) values: in \( \beta-D-\text{galactose} \), it will have a decreasing effect (trans-coplanar effect) (Abraham and Gatti, 1969) and in \( \alpha-D-\text{xylose} \) it will have an increasing effect. In the case of \( \alpha-D-\text{glucose} \) and \( \alpha-D-\text{mannose} \), even though the electronegative atoms are similarly oriented with respect to the coupling protons (Figure 2), the \( J \) values are different due to the axial 2-OH in mannose which affects the H1-C1-C2-H2 torsion angle (Rao, 1974).

In the case of \( \alpha-L-\text{IdUA} \) (\( \text{C}_{4} \)), the relative orientations of the coupling protons with respect to the electronegative atoms at C5→C4, C4→C3/C2→C2 and C2→C1 are similar to that in \( \beta-D-\text{galactose} \) (C5→C4), \( \alpha-D-\text{xylose} \) (C5→C4) and \( \alpha-D-\text{mannose} \) (C2→C1), respectively (Figure 2). In view of this, one expects coupling constants values of ~1.7 (\( J_{H1H2} \)), 5.5 (\( J_{H2H3}, J_{H3H4} \)) and 0.9–1.6 (\( J_{H4H5S} \)). Although these expected values differ significantly from the observed values, they are nevertheless closer to these values than to the calculated values for \( 2S_{0} \) or \( 2S_{2} \) conformations (Table I). In \textit{in vacuo} molecular dynamics studies, it was found that the disaccharide \( \text{D-GalNAc}1,4\text{a-L-IdUA} \) occasionally accesses a conformation in which the \( \alpha-L-\text{IdUA} \) ring deformed slightly from its \( \text{C}_{4} \) geometry, resulting in a slight increase in the O3-GalNAc to O1-IdUA distance. In these conformations, the calculated coupling constants not only show the trends where \( J_{H2H3} > J_{H3H4} \approx J_{H4H5S} \approx J_{H1H2} \), but are also close to the experimental values (Table I).

Based on the above, it is suggested that in solution \( \alpha4-L-\text{IdUA} \) in dermatan sulphate exists predominantly in a 'slightly distorted' \( \text{C}_{4} \) conformation. However, a small fraction of these residues

### Table I. Calculated vicinal coupling constants (Hz) for \( \alpha-L-\text{iduronate} \)

<table>
<thead>
<tr>
<th>( J_{HIH} )</th>
<th>( J_{HIJ} )</th>
<th>( J_{HIK} )</th>
<th>( J_{HIH} )</th>
<th>Reference</th>
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<tr>
<td>( ^{3}C_{1} )</td>
<td>7.7</td>
<td>10.1</td>
<td>9.9</td>
<td>4.9</td>
</tr>
<tr>
<td>7.9</td>
<td>9.9</td>
<td>9.7</td>
<td>4.5</td>
<td>4.9</td>
</tr>
<tr>
<td>( ^{3}C_{4} )</td>
<td>2.1</td>
<td>2.8</td>
<td>3.0</td>
<td>0.4</td>
</tr>
<tr>
<td>3.1</td>
<td>4.5</td>
<td>4.5</td>
<td>0.0</td>
<td>Venkataraman et al., 1994</td>
</tr>
<tr>
<td>( ^{2}S_{0} )</td>
<td>5.8</td>
<td>9.5</td>
<td>6.1</td>
<td>3.6</td>
</tr>
<tr>
<td>5.1</td>
<td>9.4</td>
<td>5.4</td>
<td>4.3</td>
<td>Venkataraman et al., 1994</td>
</tr>
<tr>
<td>( ^{2}S_{2} )</td>
<td>1.0</td>
<td>4.5</td>
<td>0.9</td>
<td>8.6</td>
</tr>
</tbody>
</table>

Distorted \( ^{3}C_{4} \):

1 | 2.1 | 4.6 | 1.7 | 1.8 |
2 | 3.2 | 4.6 | 2.8 | 1.8 |
3 | 2.1 | 4.7 | 1.9 | 2.1 |
4 | 2.4 | 4.2 | 3.4 | 0.7 |

Dermatan sulphate:

\( ^{3}C_{4} \) | 3.0 | 6.0 | 3.5 | 3.3 | Gatti et al., 1979 |

*Calculated using Haasnoot's equation (equation (8), Haasnoot et al. (1980)). Electronegativity values were taken from Huggins (1953)).

*Experimental values.

**Fig. 2.** Plot of observed versus calculated coupling constants. The values and the saccharide names are listed in Table II. Calculation of the coupling constants is described in the legend to Table I. Oligosaccharide structures were built using the Biopolymer module of Biosym's InsightII package, energy minimized using CVFF force field (\( \epsilon = 4\sigma \)) and conjugate gradient minimization algorithm until the maximum gradient was <0.001 kcal/mol/\( \text{Å} \).
The recent proposal that α-L-IdUA is in a $S_1$ conformation (Venkataraman et al., 1994) is less likely as it satisfies neither the NMR data nor energy criteria. It should be recalled that this skew conformation was proposed based on the assumption that the $^{13}C_4$ conformation cannot satisfy the fibre repeat value reported by the X-ray diffraction studies.

Acknowledgements

The authors thank Prof. R.Chandrasekaran, Purdue University, for providing the n and h values for dermatan sulphate.

References


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Table II. Observed and calculated vicinal coupling constants (Hz) for some monosaccharides and their derivatives

<table>
<thead>
<tr>
<th>a</th>
<th>$J_{H_2,H_3}$</th>
<th>$J_{H_3,H_4}$</th>
<th>$J_{H_2,H_3}$</th>
<th>$J_{H_3,H_4}$</th>
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</thead>
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<td>Exp/Calc</td>
<td>Exp/Calc</td>
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<td>2</td>
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<td>10.5/10.0</td>
<td>2.5/1.8</td>
<td>2.5/1.1</td>
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<td>3.0/2.0</td>
<td>3.5/3.4</td>
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<td>4</td>
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<td>2.5/1.9</td>
<td>9.7/9.5</td>
<td>9.0/1.0</td>
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<td>2.0/1.9</td>
<td>10.9/9.7</td>
<td>10.0/1.0</td>
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<td>6</td>
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<td>2.0/1.9</td>
<td>10.9/9.6</td>
<td>10.0/1.0</td>
</tr>
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<td>1.00/6</td>
<td>2.4/2.0</td>
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<td>9.6/10.3</td>
<td>9.6/1.0</td>
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<td>10.5/9.0</td>
<td>3.5/3.3</td>
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</tr>
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<td>13</td>
<td>4.0/4.5</td>
<td>10.5/9.0</td>
<td>3.0/3.3</td>
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<td>14</td>
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<td>9.6/8.8</td>
<td>3.5/3.3</td>
<td>0.0/1.4</td>
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<tr>
<td>15</td>
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<td>9.3/10.3</td>
<td>9.2/11.0</td>
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<td>3.83/7</td>
<td>10.0/10.4</td>
<td>9.5/9.0</td>
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<td>1.0/1.8</td>
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<td>NA</td>
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<td>10.4/10.4</td>
<td>9.0/9.0</td>
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<td>9.9/9.8</td>
<td>3.1/2.9</td>
<td>1.5/1.3</td>
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<td>24</td>
<td>NA</td>
<td>NA</td>
<td>11.6/11.5</td>
<td>10.5/10.0</td>
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</tbody>
</table>

*See legend to Table I.

**NA, not applicable.
Meeting Announcements

Eurocarb VIII

Seville
July 2–7, 1995

Information:
Prof. Manuel Gomez-Guillen
Departamento de Quimica Organica,
Facultad de Correos 553,
41071 Sevilla,
Spain
Fax: +34 54 624952

Biochemical Society Glycobiology Group Colloquia

Manchester
July 18–19, 1995

Two day meeting on ‘Mucins’; information from
Dr J.Sheehan,
Department of Biochemistry,
University of Manchester.

Dublin (University College)
September 13, 1995

One day meeting on ‘Carbohydrate Recognition Proteins’; information from .
Dr M.Taylor,
Glycobiology Institute,
Department of Biochemistry,
University of Oxford.

36th International Conference on the Biochemistry of Lipids (ICBL)

Washington, DC
August 9–11, 1995

The 36th International Conference on the Biochemistry of Lipids (ICBL) will be held in Washington, DC on August 9–11, 1995, on the campus of Georgetown University. The theme of the conference is ‘Lipids as modulators of molecular events’. The Conference Lecture will be given by Michael S.Brown. The sessions are (i) Lipid–Protein Interaction, (ii) Glycosphingolipids and Cell Signalling, and (iii) Newer Approaches in the Treatment of Cardiovascular and Glycolipid Disorders.

Presentations include:

Prenylation, lipid–protein interaction
John Glomset

Protein farnesylation and biological activity
Jay Gibbs

Myristoylation of proteins and biological activity
Alan Adem

The essential role of phosphoinositides in membrane and protein trafficking
Scott Emr

ICBL Distinguished Scientist Lecture

Lipids as modulators of molecular events
Michael S.Brown

Role of sphingoglycolipids in signal transduction
Subroto Chatterjee

Sphingolipid derived products: role in signal transduction and cell regulation
Yusuf Hannun

Gangliosides and sphingolipids as modulators of transmembrane signalling
Sen-Itiroh Hakomori
GLYCO XIII will focus on functional roles of glycoconjugates, in addition to advances in biosynthesis, degradation, and the molecular–genetic basis of these processes. Glycobiology of plants, yeasts, moulds, and bacteria will be covered in view of the remarkable progress in this area. In glycopathology sessions, in addition to covering the functional role of glycosylation in cancer progression, inflammatory, and infectious processes, we will focus on aberrant glycosylation correlated with autoimmune disorders, parasitosis, AIDS progression, and other diseases. Because of the rapid expansion of glycobiology and glycopathology, and the diversity of new research trends, GLYCO XIII will include a greater number of topic sessions (~32) than ever before.

**Mini-symposium sessions**

- Conformational structures of glycoconjugates
- Glycosylation affecting protein structure and function
- Glycomimetics that modify glycosylation
- Carbohydrate–protein interactions
- Dolichol-linked pathways
- Plant glycobiology
- Golgi enzyme targeting
- New methods of glycoconjugate analysis
- Carbohydrate-dependent cell adhesion I and II
- Plant and animal lectins
- Biology of proteoglycans
- Glycosaminoglycan synthesis and molecular–genetic basis
- Glycobiology of yeasts, moulds and bacteria
- Glycosylation regulation through glycosyltransferase genes
- Synthesis and molecular–genetic basis of developmentally regulated carbohydrate epitopes
- Functional role of O-linked glycans with mucin-type domains
- Molecular–genetic basis of sphingolipidosis and other neurological diseases
- Sialic acid, sialidase, and polysialic acid
- Synthesis and degradation of glycosphingolipids
- Glycobiology of immunocytes and immune responses
- Molecular genetics of histo-blood groups and diseases
- Transmembrane signalling control by glycosphingolipids and sphingolipids I, II
- Organization and trafficking of glycosphingolipids in membranes
- Glycosphingolipid interactions and receptor function
- Gangliosides in neurobiology
- Role of glycoconjugates in bacterial and viral infections
- Glycopathology of parasites
- Glycosylation and cancer
- Aberrant glycosylation causing or closely associated with disease processes (autoimmune, AIDS, etc.)

**Session chairs and organizers**

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- D.R.Bundle
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- C.G.Hellerqvist
- R.L.Hill
- O.Hindsgaul
- C.Hirschberg
- R.C.Hughes
- A.Kobata
- R.N.Kolesnick
- S.Kornfeld
- J.Kościelak
Plenary lectures to date

J. Baenziger, Glycosylation affecting protein structure function; K. Bock, Glycopeptides as oligosaccharide scaffolds and mimetics; K. Drickamer, Carbohydrate–protein interaction; R. Gilmore, Dolichol-linked pathways; P. Albersheim, Carbohydrates as plant cell elicitors; G. Warren, Mechanism of Golgi enzyme targeting; M. M. Burger, Sponge cell recognition based on specific glycan-to-glycan interaction; M. Bernfield, Syndecan and related cell-surface proteoglycans; N. L. Shaper, Transcriptional control of glycosyltransferase gene expression; J. Marth, Transgenic approach for testing glycosylation function; G. W. Hart, O-linked GlcNAc controlling nuclear and cytoplasmic function; P. R. Crocker, Sialoadhesin and its gene expression; Y. Inoue, KDN glycans: Their occurrence, structure, biosynthesis, and possible function; K. Sandhoff, Synthesis and degradation of glycosphingolipids and function of metabolites; G. Tettamanti, Metabolic fate and functional implications of exogenous glycosphingolipids; F. Wieland, Organization and trafficking of glycosphingolipids in membranes; K. -A. Karlsson, Cell surface glycoconjugates as attachment sites in the adhesion of microbes to animal tissues; T. Kinoshita, Genetic defect in P1-glycan synthesis causes paroxysmal nocturnal haemoglobinuria; V. Nussenzweig, Liver proteoglycan homing receptors for malaria parasites; R. A. Reisfeld, Anti-cancer therapy based on tumour-associated ganglioside antigens; N. Radin, Effects of ceramide analogues on sphingolipid metabolism and cell growth; Y. Nagai, Ganglioside as a signalling molecule in neural function; A. M. Lefer, Selectins, oligosaccharides and sphingolipid derivatives in reperfusion injury.

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Cellucon—International Cellulose Conference

Stresa—Lake Maggiore
September 10–16, 1995
Information:
Dr B. Focher
Stazione Sperim. Carta Cellulosa,
Piazza Leonardo da Vinci,
20133 Milan,
Italy
Fax: +39 2 2365039

Carbohydrate-Mediated Cell–Cell Interactions in Inflammation and Metastasis

Paris
October 8–12, 1995
Application deadline: 28 April.
Information:
INSERM
Conferences Philippe Laudat
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75654 Paris Cedex 13,
France
Tel.: +33 (1) 44.23.60.89/87
Fax: +33 (1) 44.23.60.89
E-mail: laudat@tolbiac.insERM.fr

Third International Glycobiology Symposium: Current Analytical Methods

San Diego, California, USA
November 29, 1995–December 2, 1995
Scientific Organizing Committee
Chair: R. Reid Townsend, University of California, San Francisco, USA

Members: Jacques Baenziger, Washington University, St Louis, USA; Steven A. Carr, SmithKline Pharmaceuticals, King of Prussia, USA; Harald Conradt, Gesellschaft für Biotechnologische Forschung, mbH, FRG; William Hancock, Hewlett Packard Corporation, Palo Alto, USA; Vincent Hascall, Cleveland Clinic Foundation, Cleveland, USA; Arland Hotchkiss, Jr, US Department of Agriculture, Philadelphia, USA; Adrianna Manzi, University of California, San Diego, USA; Milos Novotny, Indiana University, Bloomington, USA; Stanley Prusiner, University of California, San Francisco, USA; Harry Schachter, University of Toronto, Toronto, Canada; Sandro Sonnino, University of Milan, Milan, Italy; Michael Spellman, Genentech, South San Francisco, USA; Robert Trimble, Department of Health, Albany, New York,
Erratum

Regulation of N-linked glycosylation. Neuronal cell-specific expression of a 5' extended transcript from the gene encoding N-acetylglucosaminyltransferase I

by Jing Yang, Mantu Bhaumik, Yun Liu and Pamela Stanley


During the reproduction process, several of the major bands in Figure 2 of the paper became difficult to discern. An earlier photograph of the same blot is presented below.

![Errata Figure](image_url)

**Fig. 2.** Molecular basis of the difference between the ~2.9kb and ~3.3kb Mgal-1 RNAs. Total RNAs (~15μg) from mouse brain (B) and liver (L) were hybridized to P14 and/or to dT12-18. Following digestion with RNase H, the samples were electrophoresed in a 1.2% glyoxal–agarose gel, transferred and hybridized to probe A or probe B at 65°C overnight. The blots were finally washed in 2 x SSPE and 0.4% SDS at 65°C for 30 min.