Biochemical characterization including amino acid and carbohydrate compositions of canine and human brain Thy-1 antigen

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Human and canine brain Thy-1 antigens were solubilized in deoxycholate and antigen activity was followed both by conventional absorbed anti-brain xenosera of proven specificity and by mouse monoclonal antibodies to canine and human Thy-1. It is shown that >80% of Thy-1 activity in the dog and man binds to lentil lectin, that the mobility on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of canine and human Thy-1 is identical with that of rat Thy-1 and that the Stokes radius in deoxycholate of canine and human brain Thy-1 is 3.0 nm and 3.25 nm respectively. Both lentil lectin affinity chromatography followed by gel-filtration chromatography on the one hand and monoclonal antibody affinity chromatography on the other gave high degrees of purification of the brain Thy-1 molecule in the dog and man, resulting in single bands staining for both protein and carbohydrate on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (except for a slight contaminant of higher molecular weight staining for protein but not carbohydrate with human Thy-1 purified by lentil lectin and gel-filtration chromatography). Analysis of canine and human brain Thy-1 purified by monoclonal antibody affinity chromatography with additional gel filtration through Sephadex G-200 showed that these molecules had respectively 38% and 36% carbohydrate. The amino acid and carbohydrate compositions were similar to those previously reported for Thy-1 of the rat and mouse, the main point of interest being the presence in canine and human brain Thy-1 of *N*-acetylgalactosamine, which has been reported in rat and mouse brain Thy-1 but not in Thy-1 from other tissues.

The Thy-1 molecule, originally described as a brain-thymus antigen in the mouse (Reif & Allen, 1964), has more recently been studied in several species, and extensive biochemical studies, including amino acid sequence (Campbell *et al.*, 1981), have been done on rat brain Thy-1. The Thy-1 molecule shows a variety of interesting features. First, within any species it is present on an unusual array of tissues, such as brain and thymus (Acton *et al.*, 1974), fibroblasts (Stern, 1973) and myoblasts (Lesley & Lennon, 1977). Secondly, it shows remarkable species differences in its tissue distribution being absent, for example, from peripheral T-lymphocytes in the rat (Acton *et al.*, 1974) and from most thymocytes in man (Dalchau & Fabre, 1979). Finally, there are differences in the carbohydrate composition between brain and thymus Thy-1 of the rat (Barclay *et al.*, 1976).

These characteristics of the Thy-1 molecule are interesting when considering possible functions for

the Thy-1 molecule, and it has been suggested on the basis of the carbohydrate differences that the functions of Thy-1 might vary from tissue to tissue and be mediated by the carbohydrate moiety (Barclay *et al.*, 1976). For these reasons, and more generally to compare homologous molecules in different species, we have examined some biochemical characteristics including the carbohydrate compositions of brain Thy-1 of the dog and man, and compared them with the data available in the rat and mouse.

Materials and methods

Antisera

Immunoabsorbent purified rabbit F(ab')₂ anti-[mouse F(ab')₂] and immunoabsorbent-purified goat F(ab')₂ anti-[rabbit F(ab')₂] were prepared as described previously for GAR (Dalchau & Fabre,

1979). Mouse immunoglobulins were pepsin-degraded at pH 4.1.

The preparation and specification of the mouse monoclonal antibodies to the canine (McKenzie & Fabre, 1981b) and human (McKenzie & Fabre, 1981a) homologues of Thy-1 have been described previously. Both lines were obtained by fusing spleen cells from immunized Balb/c mice with the myeloma line NS-1, kindly provided by Dr. C. Milstein.

The liver-absorbed rabbit anti-(dog brain serum) and rabbit anti-(human brain serum) were the same batches as those used in previous studies (Dalchau & Fabre, 1979).

Assay for antigen activity

Antigen activity was measured by inhibition of indirect radioactive binding assays (Morris & Williams, 1975). For canine Thy-1, the assay systems involved either the monoclonal antibody F3-20-7 (McKenzie & Fabre, 1981b) or dog-liver-absorbed rabbit anti-(dog brain serum) at $10\times$ dilution assayed on glutaraldehyde-fixed (Williams, 1973) dog thymocytes, an assay that has been shown to be directed at canine Thy-1 (Dalchau & Fabre, 1979). For human Thy-1, the monoclonal antibody F15-42-1 was used with brain homogenate targets (McKenzie & Fabre, 1981a) or rat-liver-absorbed rabbit anti-(human brain serum) at $8\times$ dilution and assayed on freshly prepared rat thymus cells, an assay directed at the human-rat cross-reactive component of Thy-1 (Dalchau & Fabre, 1979). Glutaraldehyde-fixed rat thymus cells were found not to be suitable in this assay system, as the determinant recognized appeared to be denatured by the fixation procedure.

Detergent solubilization of brain Thy-1

The procedure was the same for both dog and human brain, and was carried out by using a preliminary extraction with Lubrol WX followed by solubilization with sodium deoxycholate (Sigma), essentially as described by Barclay *et al.* (1975).

Lentil lectin affinity chromatography

Lentil lectin was prepared essentially as described by Hayman & Crumpton (1972) and coupled to CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) at the ratio of 5 mg of lectin per ml of swollen gel, essentially as recommended by the manufacturers. Sodium deoxycholate-solubilized brain was passed through a 20 ml column at the rate of 15 ml/h, with 5 ml fractions being collected. The column was washed with 4 column volumes of 0.5% sodium deoxycholate in 0.01 M-Tris/0.02% NaN_3 in (pH 8.4) buffer, and bound material was eluted with 1 M- α -methyl D-glucopyranoside, in 1% sodium deoxycholate in the pH 8.4 buffer.

Gel filtration

Upward flowing K26/100 or K16/100 columns packed with G-200 (Pharmacia, Uppsala, Sweden) and equilibrated in 0.5% sodium deoxycholate in the pH 8.4 buffer described above were used.

Monoclonal antibody affinity chromatography

This was performed essentially as described previously (Dalchau *et al.*, 1980). Both monoclonal antibody columns behaved well as immuno-adsorbents, completely depleting the passed fractions of Thy-1, and the Thy-1 could be recovered in good yield.

Protein estimations

Protein was estimated by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. Sodium deoxycholate was included at the appropriate concentration in the standards whenever it was present in the sample to be measured.

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis

These were disc gels run essentially as described by Laemmli (1970). Separating gels were 10% or 12.5% acrylamide, and stacking gels were 3% acrylamide. Gels were stained for protein with Coomassie Blue and for carbohydrate by the periodate/Schiff procedure.

In experiments where Thy-1 antigen activity was to be recovered from the gel, the sample was prepared in 0.1% rather than 4% sodium dodecyl sulphate and it was not boiled. At the end of electrophoresis, the gel was cut into 3 mm slices, each of which was crushed in 0.3 ml of 0.05% sodium dodecyl sulphate/0.01 M-Tris (pH 8.4), and left overnight at 4°C. The gel was removed by centrifugation, and the supernatant was assayed for Thy-1 antigen activity.

Amino acid and carbohydrate

A Locarte mini-analyser fitted with an automatic loading attachment was used for the analysis of amino acids and amino sugars. Details of the elution systems, and the sources of reagents have been given previously (Allen, 1979). Samples of the glycoproteins were hydrolysed at 24, 48 and 72 h in 3 M-toluene *p*-sulphonic acid at 110°C under N_2 for amino acid analysis and at 100°C for 24 h for determination of amino sugars (Allen & Neuberger, 1975). Half-cystine values were obtained for the analysis of cysteic acid residues resulting from the performate oxidation (Hirs, 1956) and subsequent hydrolysis of the protein for 24 h at 110°C in constant-boiling HCl.

Tryptophan was measured spectrophotometrically by measuring A_{280} and A_{288} of solutions after

subtracting background absorbance by extrapolating the trace from the 350 to 325 nm absorbances and also allowing for the contribution of tyrosine and cystine residues. Preparations of both the canine and human brain Thy-1 glycoproteins showed a significant background absorbance (possibly due to bound deoxycholate), which reduced the accuracy of this method. Human Thy-1 was therefore also analysed for tryptophan content by the method of Simpson *et al.* (1976), which involved hydrolysis in 4-M-methanesulphonic acid [containing 0.2% 3-(2-aminoethyl)indole] *in vacuo* at 115°C for 24 h followed by determination on the amino acid analyser. The presence of carbohydrate in hydrolysates is known to bring about a destruction of tryptophan under these conditions. Therefore as a control hen egg-white lysozyme to which had been added 35% (w/w) mannose was hydrolysed under the same conditions and the recovery of tryptophan (56% of theoretical value) was used as a factor in the calculation of the tryptophan content of the Thy-1 hydrolysate.

Neutral sugars and sialic acids were measured by g.l.c. after methanolysis and trimethylsilylation of the protein samples (Chambers & Clamp, 1971) with a Perkin-Elmer F.11 gas chromatograph fitted with dual columns. Sugar analyses were related to the amino acid analyses by adding internal standards (mannitol for neutral sugars and *p*-fluorophenylalanine for amino acids and amino sugars) to samples taken from the same stock solution of protein.

Results

Binding of canine and human brain Thy-1 to lentil lectin

The picture obtained on lentil lectin affinity chromatography of sodium deoxycholate-solubilized brain was the same in both dog and man, and the results for A_{280} and Thy-1 activity with dog brain are given in Fig. 1. It can be seen that the bulk of Thy-1 activity was retained by the lentil lectin column, and could be eluted on addition of 1 M- α -methyl D-glucopyranoside. Quantitative absorptions established that >80% of the Thy-1 activity was retained by the column, and 50% of the original antigen activity was recovered with 5% of the original protein in the sodium deoxycholate extract, giving a 10-fold purification of the antigen. For human sodium deoxycholate-solubilized brain, >80% of Thy-1 activity was retained on the column, and 37% of the activity was eluted with 6% of the protein, giving a 5.9-fold purification.

Stokes radius of canine and human brain Thy-1 in deoxycholate

The results of a typical experiment measuring A_{280} and Thy-1 activity of sodium deoxycholate-solubilized human brain Thy-1 chromatographed on Sephadex G-200 are given in Fig. 2. The material loaded on to the column was that which had been partially purified by lentil lectin affinity chromatography. It can be seen that the human Thy-1 activity was eluted as a single peak behind most of

Fig. 1. *Lentil lectin affinity chromatography of deoxycholate-solubilized dog brain*

Deoxycholate-solubilized dog brain (250 ml) was passed through a 20 ml lentil lectin column, which was washed and then eluted with 1 M- α -methyl D-glucopyranoside. Canine Thy-1 antigen activity was followed by inhibition of an absorbed rabbit anti-(dog brain xeno)serum assayed on glutaraldehyde-fixed dog thymocytes, an assay of proven specificity for Thy-1 (Dalchau & Fabre, 1979). ●, A_{280} ; ■, Thy-1 antigen activity of starting extract; ○, Thy-1 antigen activity during chromatography.

Fig. 2. Gel filtration in deoxycholate of lentil lectin extract of human brain

Lentil lectin-purified deoxycholate extract of human brain (4 ml) was chromatographed on an upward-flowing K16/100 column packed with Sephadex G-200. Thy-1 antigen activity was measured in this experiment by inhibition of liver-absorbed rabbit anti-(human brain serum) assayed on fresh rat thymocytes, an assay of proven specificity for Thy-1 (Dalchau & Fabre, 1970). Precisely similar results were obtained by inhibition of mouse anti-(human Thy-1) monoclonal antibody assayed on glutaraldehyde-fixed human brain. □, A_{280} ; ●, Thy-1 antigen activity. Standards were as follows: BD, blue dextran; BSA, bovine serum albumin; Oval, ovalbumin; Myo, myoglobin.

the protein, and just ahead of the ovalbumin standard. The Stokes radius of human Thy-1 in deoxycholate was measured at 3.25 nm (mean of four determinations, range 3.20–3.30 nm). The degree of purification from the lentil lectin-eluted material was 11.2-fold, in that 76% of the antigen activity was recovered with 6.8% of the protein.

The results with dog Thy-1 were very similar, except that as with rat brain Thy-1, it was eluted in the same position as ovalbumin. The Stokes radius for dog brain Thy-1 in deoxycholate was measured at 3.0 nm (mean of four determinations, range 2.95–3.05 nm). The degree of purification by the gel-filtration step of the lentil lectin-eluted material was 6.9, in that 43% of the antigen was recovered with 6% of the protein.

Purification by use of monoclonal antibody affinity columns

Both of the monoclonal antibodies behaved well as column immunoadsorbents. All Thy-1 activity could be removed from deoxycholate extract passed through the columns, and the activity could be recovered from the columns by; high-pH elution. For human brain Thy-1, preparation of the deoxycholate extract from the washed brain homogenates gave a 2.0-fold purification; 51% of antigen activity was recovered with 25% of the protein. The

monoclonal antibody affinity step gave a 186-fold purification; approx. 100% of the antigen activity was recovered with 0.7% of the protein. For dog Thy-1, preparation of the deoxycholate extract gave a 1.9-fold purification from washed brain homogenate; 40% of the antigen activity was recovered with 21% of the protein. The monoclonal antibody affinity step gave a further 135-fold purification; approx. 100% of the antigen was recovered with 0.7% of the protein.

The dog brain Thy-1 purified by the monoclonal antibody affinity column was passed through Sephadex G-200 gel filtration, but this did not give any further purification.

Mobility on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis

Analysis by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of the Thy-1 preparations purified both by lentil lectin and gel-filtration chromatography and by monoclonal antibody affinity chromatography was performed, both to indicate the purity of the preparations and also measure the apparent molecular weight of canine and human brain Thy-1. Purified rat brain Thy-1 (kindly supplied by Dr. A. F. Williams, Cellular Immunology Unit, Sir William Dunn School of Pathology, Oxford) (Barclay *et al.*, 1975) was run

concurrently to compare the mobilities of rat, dog and human brain Thy-1.

The results obtained by electrophoresing dog and human brain Thy-1 prepared by monoclonal antibody affinity chromatography on 12.5% polyacrylamide gels is given in Fig. 3. It can be seen that with both dog and human brain Thy-1 preparations, a single broad band was obtained when the gels were stained for protein and that the mobility of these bands was the same as that of rat brain Thy-1. This corresponded to a mol.wt. of 24 000. With both dog and human brain Thy-1, the bands were usually seen to be doublets, although this is not clear from the Figure. In all three species staining the gels for carbohydrate gave strong staining in the same position as that seen for the protein, which has been reported previously for rat brain Thy-1 (Barclay *et al.*, 1975) and suggests that the Thy-1 of dog and man contain a large component of carbohydrate.

To be sure that the bands seen on the gels were in

fact Thy-1, the purified dog brain Thy-1 was electrophoresed without boiling in 0.1% sodium dodecyl sulphate and the gel was sliced and assayed for antigen activity as outlined in the Materials and methods section. The results are given in Fig. 4 and show that the Thy-1 activity corresponded precisely to the position of the band on the gel.

The canine and human brain Thy-1 preparations, obtained by lentil lectin and gel-filtration chromatography, when analysed on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, gave bands staining precisely as described above, except that there was one contaminant band of higher molecular weight staining for protein but not carbohydrate with the human preparation, even after two passages through gel filtration. This was probably because of the slightly higher Stokes radius of human Thy-1, which meant that it eluted more closely to the major protein peak (Fig. 2) than canine Thy-1. A similar band was seen after one passage through gel filtration of canine Thy-1 but was eliminated by a second passage.

Amino acid and carbohydrate composition

The canine and human brain Thy-1 purified by

Fig. 3. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of rat, dog and human brain Thy-1 (a) Canine and (b) human brain Thy-1 (20 μ g) purified by monoclonal antibody affinity chromatography were electrophoresed on 12.5% acrylamide disc gels. Pure rat brain Thy-1 (supplied by Dr. A. F. Williams) was run concurrently for comparison. Molecular-weight standards were as follows: A, phosphorylase *b* (mol.wt. 94 000); B, bovine serum albumin (mol.wt. 68 000); C, ovalbumin (mol.wt. 43 000); D, carbonic anhydrase (mol.wt. 30 000); E, soya-bean trypsin inhibitor (mol.wt. 21 000); F, lysozyme (mol.wt. 14 300); G, dye front.

Fig. 4. Inhibition studies after sodium dodecyl sulphate/polyacrylamide-gel electrophoresis

Canine Thy-1 (20 μ g) was electrophoresed on 12.5% polyacrylamide disc gels, without boiling, in 0.1% sodium dodecyl sulphate, and at the completion of electrophoresis the gel was sliced. Each slice was eluted and assayed for canine Thy-1 as described in the Materials and methods section. The diagram of the gel along the abscissa represents the position of the putative Thy-1 band on a similar gel electrophoresed concurrently and stained for protein.

monoclonal antibody affinity and gel filtration were examined for their amino acid and carbohydrate compositions. The results are given in Table 1 and compared with published data in the rat (Barclay *et al.*, 1976) and mouse (McClain *et al.*, 1978; Zwerner *et al.*, 1977). It can be seen that, as expected from the heavy carbohydrate staining of Thy-1 after gel electrophoresis, there is a high proportion of carbohydrate in both canine and human Thy-1, the proportion being somewhat higher than that seen with Thy-1 of rat or mouse. The carbohydrate compositions of the canine and human brain Thy-1 molecules are very similar. Like brain Thy-1 of rat and mouse, canine and human brain Thy-1 have small amounts of *N*-acetylgalactosamine, suggesting the presence of an *O*-linked sugar chain. In the rat, thymocyte Thy-1 has larger

amounts of both galactose and sialic acid when compared with brain Thy-1. However, the amounts of galactose and sialic acid on canine and human brain Thy-1 resemble the thymocyte rather than the brain Thy-1 of the rat. Both canine and human brain Thy-1 have larger amounts of fucose when compared with Thy-1 of rat and mouse.

The amino acid composition of canine and human brain Thy-1 glycoproteins do not differ greatly from those reported from rat and mouse brain, although there are some differences in the amounts of aspartic acid (or asparagine), threonine, proline, isoleucine and tyrosine. Rat Thy-1 lacks tryptophan (Campbell *et al.*, 1981) and we have found that human Thy-1 also lacks tryptophan when measured on the amino acid analyser after hydrolysis in methanesulphonic acid. Spectrophotometric methods for the estimation

Table 1. Carbohydrate and amino acid composition of canine and human brain Thy-1, compared with published results of rat and mouse Thy-1*

All analyses were calculated as being equivalent to the mean number of each amino acid residue per 100 amino acid residues (this is also equivalent to residues/17 500-mol.wt. human Thy-1 glycoprotein). The values for each amino acid residue are derived from the average of hydrolyses for 24, 48 and 72 h in 3M-toluene-*p*-sulphonic acid at 110°C, excepting for serine and threonine, which were extrapolated to zero time, valine and isoleucine, for which the 72 h values were taken, and cystine and tryptophan (see the text). Abbreviations used: ND, not determined.

	Content (residues/17 500-mol.wt. glycoprotein)					
	Mouse		Rat		Human brain	Dog brain
	Brain	Lymphoblast	Brain	Thymocyte		
GlcNAc	4.4	5.0	6.3	7.0	8.0	10.7
GalNAc	0.9	0.0	1.0	0.0	1.8	1.2
Fuc	1.5	1.0	1.8	1.0	3.9	4.0
Man	8.8	5.0	11.9	10.6	13.2	14.2
Gal	4.0	3.6	1.8	5.5	5.6	4.3
Sialic acid	0.4	2.0	0.2	1.8	1.3	2.7
Carbohydrate (%) ...	24	22	29	32	36	38
Asx	11.1	12.0	12.7	12.6	7.4	7.5
Thr	7.2	8.3	7.6	8.6	8.3	12.1
Ser	8.5	9.5	7.4	7.1	11.4	9.7
Glx	10.2	10.6	9.1	9.1	8.6	9.7
Pro	4.3	4.1	3.6	3.8	2.2	4.7
Gly	5.4	5.7	6.0	4.9	6.1	5.2
Ala	4.9	4.6	3.3	2.9	4.7	2.2
½Cys	0.8	ND	3.1	3.2	3.4	2.7
Val	7.3	7.8	7.3	7.4	9.7	6.0
Met	1.8	1.5	1.1	0.9	1.3	1.7
Ile	6.7	3.9	3.9	4.1	1.9	3.2
Leu	8.9	9.4	10.4	11.2	8.6	10.2
Tyr	3.5	3.5	2.0	2.0	6.9	4.5
Phe	3.2	3.2	4.0	3.7	3.8	2.7
His	4.0	3.7	4.1	3.8	4.0	2.8
Lys	6.0	6.6	6.9	7.2	7.7	6.9
Trp	ND	ND	0	ND	0	0 or 1
Arg	6.2	5.8	7.5	7.5	4.8	6.9

* Results for mouse Thy-1 were recalculated from McClain *et al.* (1978) and Zwerner *et al.* (1977), and for rat Thy-1 were taken from Barclay *et al.* (1975).

of tryptophan in human Thy-1 proved unreliable and since only spectrophotometric methods were used for the determination of tryptophan in canine Thy-1 it is not possible to say whether tryptophan is absent or that it is present at a low level of about 1 residue/mol of mol.wt. 17500.

Discussion

Given the complexity of cell membrane proteins, the establishment of homology between given molecules in different species requires rigorous experimental verification. In a previous study (Dalchau & Fabre, 1979) we established assays detecting the canine and human homologues of Thy-1 using cross-reactivity studies, including inhibitions with pure rat Thy-1 (kindly supplied by Dr. A. F. Williams), to establish homology, and we studied the tissue distribution of Thy-1 in the dog and man. In the present study, we have used these assays to define some biochemical characteristics of canine and human brain Thy-1. We show that in terms of binding to lentil lectin, Stoke's radius and mobility on sodium dodecyl sulphate/polyacrylamide gels, canine and human brain Thy-1 are virtually identical with rat brain Thy-1, except that the Stokes radius in deoxycholate of human brain Thy-1 is slightly but consistently higher than that of canine or rat brain Thy-1. These biochemical findings are further confirmation that our original assays were detecting the canine and human homologues of Thy-1, which is of particular interest in view of the marked species differences we detected in the tissue distribution of Thy-1 (Dalchau & Fabre, 1979). In most instances, identity of tissue distribution is taken as a strong indication of homology, but this does not hold for Thy-1.

Arndt and co-workers (1978) have studied a brain molecule that is almost certainly human Thy-1, although homology with rat or mouse Thy-1 was not clearly established. The Stokes radius of this molecule was shown to be 3.27 nm, which is very similar to our value for human brain Thy-1. More recent data from this group (Hamann *et al.*, 1980) have shown that this molecule migrates on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis with a mobility that corresponds to mol.wt. 27500. Given that the mobility on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of heavily glycosylated molecules like Thy-1 varies with the percentage and degree of cross-linking of acrylamide, the only certain way of establishing that the mobility of rat Thy-1 and those under study is the same is to run them concurrently, which we have done in the present study.

In the original studies on rat Thy-1 (Barclay *et al.*, 1976) lentil lectin followed by Sephadex G-200 gel-filtration chromatography was used for the

preparation of rat brain Thy-1. Using precisely similar approaches, we have obtained a similar degree of purification in terms of antigen activity in relation to total protein, of canine and human brain Thy-1. We have raised mouse monoclonal antibodies to both canine and human Thy-1, using the almost pure preparation of human Thy-1 as the immunogen in the human studies (McKenzie & Fabre, 1981a) and pure dog T-cells as the immunogen in the studies in the dog (McKenzie & Fabre, 1981b). In both cases, the conventionally purified Thy-1 antigens (where purification was monitored using the absorbed xenosera) inhibited the monoclonal antibodies, and the antigens purified from the monoclonal antibody affinity columns (Fig. 3) inhibited the original anti-Thy-1 assays (Dalchau & Fabre, 1979), clearly maintaining the line of homology originally established with rat Thy-1 (Dalchau & Fabre, 1979).

For the studies on amino acid and carbohydrate composition, we used the Thy-1 preparations purified by monoclonal antibody affinity chromatography, since this antigen-specific step in the purification has theoretical and practical advantages over the more conventional approaches. We show that the amino acid and carbohydrate components of both canine and human brain Thy-1 are fairly similar to that published for the rat (Barclay *et al.*, 1976) and mouse (Zwerner *et al.*, 1977; McClain *et al.*, 1978). As it has been suggested that the function of Thy-1 might reside in the carbohydrate side chains (Barclay *et al.*, 1976) it was of interest to see if canine and human brain Thy-1 resembled rat brain Thy-1 more closely than rat thymocyte Thy-1. Canine and human brain Thy-1, like brain and mouse brain Thy-1, had one or two residues of *N*-acetylgalactosamine, suggesting the presence of an *O*-linked sugar chain. However, as regards sialic acid and galactose, the other sugars where differences were seen between brain and thymus Thy-1 of the rat, canine and human brain Thy-1 had amounts similar to rat thymus Thy-1.

From the overall composition of the human and canine brain Thy-1 glycoproteins it would seem likely that there are three or four carbohydrate side chains attached to each monomer. One of these is likely to involve an *O*-glycosidic linkage between *N*-acetylgalactosamine and serine or threonine and the others glycosylamine linkages between *N*-acetylglucosamine and asparagine with chains of both the high-mannose type and the complex type containing *N*-acetylglucosamine, mannose, galactose, fucose and sialic acid.

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