

# Rat Thy-1 antigen has a hydrophobic segment at the carboxyl terminus

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We have isolated a cDNA clone coding for rat Thy-1 antigen from a rat thymocyte cDNA library. The 549 base pairs insert includes the complete coding region of a mature Thy-1 polypeptide of 142 amino acids, 31 amino acids longer than the previously reported glycoprotein sequence. These 31 amino acids contain an extremely hydrophobic region of 26 uncharged amino acids which apparently represent the transmembrane segment that allows for integration within the membrane. The presence of this additional protein segment will solve most of the enigmas regarding the properties of the C-terminal region of Thy-1 antigen.

*Thy-1 antigen    T lymphocyte    Brain    Surface antigen    Nucleotide sequence*

## 1. INTRODUCTION

Thy-1 is a membrane glycoprotein found predominantly on the cell surface of thymocytes and brain cells [1]. Originally defined in mice, Thy-1-like proteins appear to be present in many species [2-4] although the distribution of Thy-1 among hematopoietic cells seems to vary [2-5]. A Thy-1 protein isolated from rat brains has been characterized and shown to consist of an immunoglobulin-like domain of 111 amino acids with 3 attached carbohydrate chains [6]. The most unusual aspect of this structural data is the absence of a hydrophobic segment which would allow for integration within the lipid bilayer of the membrane. This has prompted speculation that Thy-1 is covalently linked to a membrane phospholipid which anchors the Thy-1 molecule. We report here the isolation of a cDNA clone prepared from rat thymocyte mRNA that code for a Thy-1 molecule consisting of 142 amino acids, 31 amino acids longer than the previously reported protein sequence. Within these 31 amino acids there is an extremely hydrophobic region which we believe represents the transmembrane segment.

## 2. MATERIALS AND METHODS

### 2.1. Enzymes and chemicals

T4 polynucleotide kinase and restriction endonucleases were purchased from Bethesda Research Laboratories. Calf intestinal alkaline phosphatase was a product of Boehringer, Mannheim. [ $\gamma$ -<sup>32</sup>P]ATP was obtained from ICN Chemical and Radioisotope Division.

### 2.2. cDNA synthesis and clone identification

The cDNA library was constructed using mRNA from W/Fu rat thymocytes as in [7]. Plasmid pT86 was identified by colony hybridization with a nick-translated probe derived from first Thy-1 clone pT64.

### 2.3. Restriction mapping and sequence determination

A restriction map was constructed based on the size of DNA fragments obtained after restriction endonuclease digestion. The entire DNA of pT86 was cleaved at selected sites with restriction endonucleases and fragments corresponding to the insert were purified by acrylamide gel electro-

phoresis and electroelution. Purified fragments were treated with calf intestinal alkaline phosphatase,  $^{32}\text{P}$ -labeled at both 5' ends with T4 polynucleotide kinase plus  $[\gamma\text{-}^{32}\text{P}]\text{ATP}$  as described [8], and cleaved secondarily to generate subfragments with only one labeled end. The subfragments were separated by acrylamide gel electrophoresis, electroeluted, and subjected to partial chemical degradation sequence analysis as in [8].

Hydrophilicity plot data was obtained by using a computer program GENETYX (SDC GENETYX, Tokyo).

### 3. RESULTS AND DISCUSSION

We have previously isolated a rat Thy-1 cDNA clone pT64, which prematurely ends at amino acid 103 due to a polyadenylation signal within the coding sequence [7]. pT64 was used to screen a new rat thymocyte cDNA library and a new Thy-1 cDNA clone pT86 was isolated from approx. 10000 colonies. The insert of pT86 comprises 549 base pairs. The restriction map and sequence strategy are shown in fig.1. Starting from the last nucleotide, A, of the leader sequence at position -1, there is a continuous stretch of 427 nucleotides in an open reading frame, encoding 142 amino acids. Following the stop codon, TGA, there is a 119 nucleotide 3'-untranslated sequence. The main nucleotide sequence of the pT86 insert and its translation into amino acids are shown in fig.2. The nucleotide sequence analysis of this clone has shown the presence of codons for an additional 31 amino acids linked to the C-terminus (Cys 111) of the previously reported glycoprotein sequence [6]. Studies on the genomic sequence of rat Thy-1 gene (to be published) have shown that Thy-1 gene encodes 3 exons and this extra 31 amino acid sequence is encoded in the third exon. The genomic DNA sequence is completely consistent with the pT 86 cDNA sequence and, therefore, the extra 3 amino acids are not a cloning artifact. This additional sequence includes a stretch of 26 uncharged amino acids which extends from amino acid 112 to 137. This stretch is hydrophobic and includes a very hydrophobic core of 6 consecutive leucine residues (fig.2,3). As is the case for other integrated membrane proteins [9,10], this hydrophobic sequence is surrounded by charged residues (lysine and aspartic acid). We believe that the

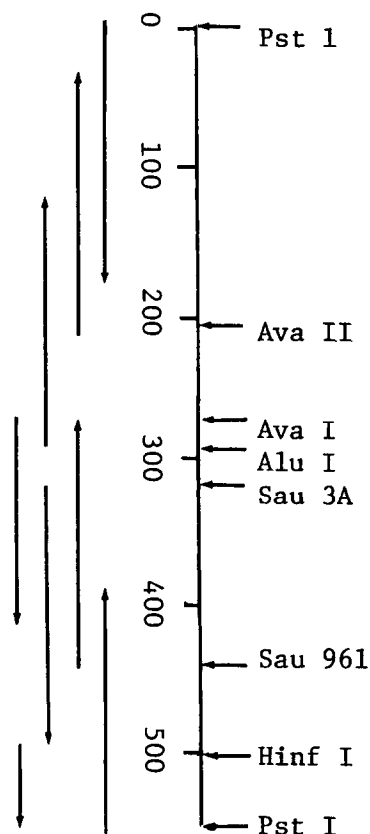


Fig.1. Partial restriction map and strategy for sequencing Thy-1 cDNA clone, pT86. Restriction endonuclease sites used in sequence determination are indicated. Nucleotide residues are numbered in the 5'-3' direction. Horizontal arrows represent the number of nucleotides sequenced from each  $^{32}\text{P}$ -labeled 5'-end.

stretch of 26 uncharged amino acids is the trans-membrane portion of the membrane-bound Thy-1 molecule. There are several explanation why authors in [6] failed to demonstrate the presence of the hydrophobic segment. One is that the hydrophobic segment is cleaved off, either physically or enzymatically, from the glycoprotein during isolation. The other is that the extra amino acids are present initially, but are cleaved off later in a manner similar to the cleavage of N-terminal signal peptides [11], although this explanation is less likely since most other related membrane integral proteins, i.e., major histocompatibility antigens [9] and surface immunoglobulin [10], remain intact. The authors in [6] speculated that Thy-1 without

1										10						
Gln	Arg	Val	Ile	Ser	Leu	Thr	Ala	Cys	Leu	Val	Asn	Gln	Asn	Leu	Arg	
CAG	AGG	GTG	ATC	AGC	CTG	ACA	GCC	TGC	CTG	GTG	AAC	CAG	AAC	CTT	CGA	
20										30						
Leu	Asp	Cys	Arg	His	Glu	Asn	Asn	Thr	Asn	Leu	Pro	Ile	Gln	His	Glu	
CTG	GAC	TGC	CGT	CAT	GAG	AAT	AAC	ACC	AAC	TTG	CCC	ATC	CAG	CAT	GAG	
40										50						
Phe	Ser	Leu	Thr	Arg	Glu	Lys	Lys	Lys	His	Val	Leu	Ser	Gly	Thr	Leu	
TTC	AGC	CTG	ACC	CGA	GAG	AAG	AAG	AAG	CAC	GTG	CTG	TCA	GGC	ACC	CTG	
60										70						
Gly	Val	Pro	Glu	His	Thr	Tyr	Arg	Ser	Arg	Val	Asn	Leu	Phe	Ser	Asp	
GGG	GTT	CCC	GAG	CAC	ACT	TAC	CGC	TCC	CGC	GTC	AAC	CTT	TTC	AGT	GAC	
80										90						
Arg	Phe	Ile	Lys	Val	Leu	Thr	Leu	Ala	Asn	Phe	Thr	Thr	Lys	Asp	Glu	
CGC	TTT	ATC	AAG	GTC	CTT	ACT	CTA	GCC	AAC	TTC	ACC	ACC	AAG	GAT	GAG	
100										110						
Ser	Asn	Lys	Thr	Ile	Asn	Val	Ile	Arg	Asp	Lys	Leu	Val	Lys	Cys	Gly	
TCC	AAT	AAA	ACT	ATC	AAT	GTG	ATC	AGA	GAC	AAG	CTG	GTC	AAG	TGT	<u>GGT</u>	
120										130						
Gly	Ile	Ser	Leu	Leu	Val	Gln	Asn	Thr	Ser	Trp	Leu	Leu	Leu	Leu	Leu	
GGC	ATA	AGC	CTG	CTG	GTT	CAA	AAC	ACT	TCC	TGG	CTG	CTG	CTG	CTC	CTG	
140										150						
Leu	Ser	Leu	Ser	Phe	Leu	Gln	Ala	Thr	Asp	Phe	Ile	Ser	Leu	*		
CTT	TCC	CTC	TCC	TTC	CTC	CAA	GCC	ACG	GAC	TTC	ATT	TCT	CTG	TGA		

Fig.2. The nucleotide sequence of the cDNA insert of pT86 and the predicted amino acid sequence of mature Thy-1 antigen. The termination codon is indicated by an asterisk.

the hydrophobic C-terminus remains associated with the membrane by being covalently linked to lipid through the C-terminus Cys 111. In view of our new finding, however, it appears Thy-1 is attached to the membrane by means of its hydrophobic C-terminus segment as with other cell surface glycoproteins.

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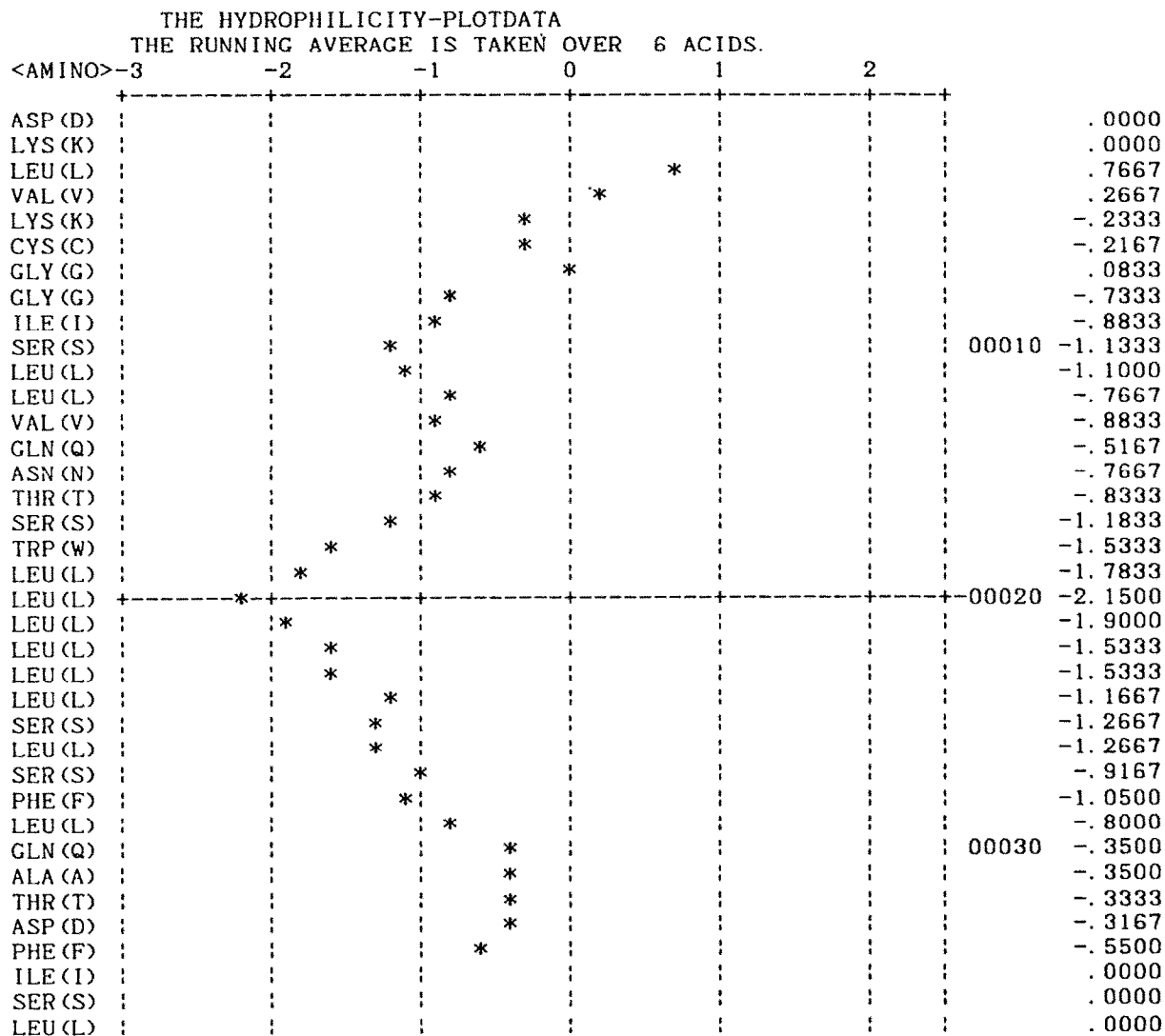


Fig.3. Hydrophilicity plot. Negative values indicate the hydrophobic stretch.

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