RESEARCH COMMUNICATION

Different molecular forms of rat kidney gp330, the dominant autoantigen of active Heymann nephritis

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The primary structure, consisting of 1650 amino acid residues, of the C-terminal end of the dominant autoantigen of active Heymann Nephritis, gp330, from rat kidney was obtained by cloning and sequencing of cDNA clones. Comparison of this sequence with the previously published sequences of fragments of the C-terminal end of gp330 [Raychowdhury, Niles, McCluskey and Smith (1989) *Science* **244**, 1163–1165] revealed

certain differences in their primary structures. These differences included several single amino acid substitutions, replacement of a stretch of 15 amino acid residues by a different stretch of six amino acid residues, and different lengths of cytoplasmic domain (188 versus 213 amino acid residues). These findings of two different primary structures of gp330 provide direct evidence for the existence of two molecular forms of gp330.

INTRODUCTION

Active Heymann Nephritis is an autoimmune glomerular disease and has served as an important model for the study of membranous glomerulonephropathy, a common glomerular disease in humans. Morphologically, the disease is characterized by the formation of glomerular immune-deposits which ultrastructurally appear electron-dense and are exclusively located in the sub-epithelial region of glomerular basement membrane. On direct immunofluorescence the deposits stain for IgG and complement, and give a characteristic fine to coarse granular appearance outlining the glomerular capillaries. The dominant autoantigen (gp330) of active Heymann Nephritis, a single polypeptide receptor involved in endocytosis, was first recognized by us as a mannose- or glucose-containing renal glycoprotein (Makker, 1979, 1980), with molecular mass approx. 600 kDa (Makker and Singh, 1982, 1984). The primary structure of fragments of gp330 was reported by Raychowdhury et al. (1989) and revealed similarity to epidermal-growth-factor precursor (EGFp), low-density-lipoprotein (LDL) receptor (LDLr) (Yamamoto et al., 1984) and LDLr-related protein (LRP)/ α_2 -macroglobulin receptor (Herz et al. 1988; Strickland et al., 1990; Kristensen et al., 1990). Immunohistochemical studies with a polyclonal antibody to a C-terminal peptide and a monoclonal antibody to gp330 have lead to a proposal that there may be two forms of gp330 on the cell surface (Bachinsky et al., 1993). In this paper we report the sequence of 1650 amino acid residues of the C-terminal end of gp330 and compare this sequence with the sequence of fragments of gp330 reported previously by Raychowdhury et al. (1989). The comparison provides direct evidence for the existence of two molecular forms of gp330 differing in their primary structure.

MATERIALS AND METHODS

gp330 clone 3A was obtained by immunoscreening of a rat kidney lgt11 expression library (Clontech, Palo Alto, CA, U.S.A.) with a monospecific polyclonal antibody prepared against homo-

geneous rat gp330 by using standard protocols (Sambrook et al., 1989). Preparations of gp330 protein from rat kidney and of polyclonal antibody to gp330 have been described previously (Kanalas and Makker, 1993). Clones TA9, TA28 and 2E were obtained by 5' RACE (Rapid Amplification of cDNA Ends) procedure by PCR using appropriate oligonucleotide primers. 5' RACE (Frohman et al., 1988) was performed using a commercial kit (Gibco-BRL) according to instructions from the supplier. Sequencing was performed by the dideoxy method (Sanger et al., 1977) using Sequenase (USB-Amersham).

RESULTS AND DISCUSSION

The map of cloned cDNAs is shown in Figure 1, and the deduced amino acid sequence is shown in Figure 2. The amino acid sequence of the clones encompassing the C-terminal part of

Figure 1 Maps of cloned gp330 cDNA fragments and similar amino acid repeats

RCVRQSFRCD	RRNDCGDYSD	ERGCSYPPCH	ANQFTCQNGR	CIPRFFVCDE	DNDCGDSDE	60
QEHLCHTPEP	TCPLHQFRCD	NGHCIEMGRV	CNHVDDCSDN	SDEKGCGINE	CLDSSISRCD	120
HNCTDTITSF	YCSCLPGYKL	MSDKRSCVDI	DECKESPQLC	SQKCENNVGS	YICKCAPGYI	180
REPDGKSCRQ	NSNIEPYLIF	SNRYIIRNLT	TDGSSYSLIL	QGLGNVVALD	FDRVEKRLYW	240
IDAEKQIIER	MFLNKTNRET	IINHRLRAE	SLDVDWVSTK	LYWLDAILDC	LFVSDLEGRH	300
RKMIAQHCV	ANNTFCFEHP	RGIVLHPQRG	HVYWTDWGVH	AYIGRIGMDG	TNKSVIISTK	360
IEWPNAITID	YTNDLLYWAD	AHLGYIEFSD	LEGHHRHTVY	DGSLPHPFAL	TIFEDTVFWT	420
DWNRTRVEKG	NKYDGSGRVV	LVNTTHKPF	IHVYHPYRQP	IMSNPCGTNN	GGCSHLCLIK	480
AGGRGFTCAC	PDDFQTVQLR	DRTLCPMPCS	SQQFLCGNNE	KCIPIWWKCD	GQKDCSDGSD	540
EPDLCPHRFC	RLGQFQCRDG	NCTSPQALCN	ARQDCADGSD	EDRVLCEHHR	VSHECESNEW	600
QCANKRCIPQ	SWQCDSVND	LDNSDEDTSH	CASRTRCPGQ	FKCNGRCIP	QSWKCDVDND	660
CGDYSDEPID	ECTTAAYNCD	NHTEFSCKTN	YRCIPQWAVC	NGFDDCRENS	DEQGCEVPC	720
HPSGDFRCAN	HHCIPLRWKC	DGTDDCGDNS	DEENCVPREC	SESEFRCADQ	QCIPSRWVCD	780
QENDCGDNSD	ERDCMKTC	PEHFQCTSGH	CVPKALACDG	RADCLDASDE	SACPTRFPNG	840
TYCPAAMFEC	KNHVCIQSFW	ICDGENDCVD	GSDEEIHLCF	NIPCESPQRF	RCDNSRCVYG	900
HQLCNGVDDC	GDGSDEKEEH	CRKPTHKPC	DTEYKCSNGN	CISQHYVCDN	VNDCGDLSD	960
TGCNLGDNRT	CAENICEQNC	TQLSSGGFIC	SCRPGFKP [*] ST	SDKNSCQDIN	ECEEFGICPQ	1020
SCRNSKGSYE	CFCVDGFKSM	STHYGERCAA	DGSPPLLLP	ENVRIKYNT	SSEKFSEYLE	1080
EEEHITIDY	DWDPEHIGLS	VVYYTVLAQG	SQFGAIKRAY	IPNFESGSNN	PIREVDLGLK	1140
YLMQPDGLAV	DWVGRHIYWS	DA [*] KSQRIEVA	TLDGRYRNWL	ITTQLDQPR ^{**} S	YV ^{***} LDDQ GKQP	1200
KIESAWMNGE	HRSVLVSENL	GWPNGLSIDY	LNDDRVIWSD	SKEDVIEAIK	YDGTDRRLII	1260
NEAMKPFSLD	IFEDKLYWVA	KEKGEVWRQN	KFGKENKEKV	LVVNPWLTV	RIFHQLRYKQ	1320
SVSNPCKQVC	SHLCLLRPGG	YSCACPQGS	FVTGSTVQCD	AASELPVTMP	PPCRCMHGNG	1380
CYFDENELPK	CKCSSGYSGE	YCEVGLSRGI	PPGTT ^{MAVLL}	TFVIVIIVGA	LVLVGLFHYR	1440
KTGSLLP ^{TL} P	KLPSLSSLAK	PSENGNGVTF	RSGADVNMID	GVSPFGPETI	IDRSMAMNEH	1500
FVMEVGKQPV	IFENPMYAAK	DNTSKVALAV	QGPSTGAQVT	VPENVENQNY	GRPIDPSEIV	1560
PEPKPASPGA	DEIQGKKWNI	FKRKPKQTTN	FENPIYAEMD	SEVKDAVAVA	PPSPSLPAK	1620
ASKRNLTPGC	TATEDTFKDT	ANLVKEDSDV	Stop			1650

Figure 2 Amino acid sequence of the gp330 C-terminal end deduced from cDNA

The membrane-spanning domain is shown as doubly underlined letters. Differences in sequence compared with the sequence reported by Raychowdhury et al. (1989) are designated by * above the letters. The sequence of the peptide used by Bachinsky et al. (1993) is shown as singly underlined letters.

gp330 revealed similarity with the repetitive, as well as the non-repetitive, regions of LRP, LDLr and EGFp proteins. There were seven Cys-rich EGFp repeats, 14 Cys-rich LDLr ligand-binding repeats, and two non-repetitive regions containing LDLr- and EGFp-like short repeats, YWXD. The structure of similar repeats is shown schematically in Figure 1. The membrane-spanning domain consists of 22 amino acid residues (Figure 2) and separates the C-terminal cytoplasmic tail (213 amino acid residues long) from the extracellular domain. The organization of various repeats in gp330 (Figure 1) and LRP in C-terminal parts is similar. However, the number of EGFp-like repeats adjacent to the membrane spanning domain is fewer in gp330 (two versus seven) (Herz et al., 1988).

In the region of non-repetitive similarity to LDLr and EGFp (amino acid residues from 1145 to 1311), we found a difference in our sequence from the sequence of the same region reported by Raychowdhury et al. (1989). A stretch of six amino acid residues (numbers 1189–1194 in Figure 2, designated by * above the letters) in our sequence substitutes for another stretch of 15 amino acid residues in the sequence reported by Raychowdhury et al. (1989). Also, scattered throughout the sequence are several

single amino acid substitutions (S-999 → L, K-1163 → N, N-1178 → K, F-1591 → I) in the C-terminal part of gp330. The F-1591 → I substitution is located within the putative internalization signal directing receptors to clathrin-coated pits. There is also a difference in the length of cytoplasmic tails of gp330. Unfortunately, Raychowdhury et al. (1989) did not present the amino acid sequence of the C-terminal cytoplasmic tail, but stated that it was 188 amino acid residues long. In the primary structure obtained by us, the length of the cytoplasmic tail is 213 amino acid residues, i.e. considerably longer. Taking all these differences into account, we conclude that we have cloned and sequenced another molecular form of gp330, which is different in primary structure from the previously reported gp330, providing direct evidence for the existence of at least two forms of gp330.

Previously, using antibodies against the extracellular domain and against a peptide deduced to be in the cytoplasmic domain, Bachinsky et al. (1993) showed that, immuno-ultrastructurally, the anti-(cytoplasmic peptide) antibody reacted only with structural components at the base of microvilli of proximal renal tubular cells, whereas the anti-(ecto-domain) antibody reacted with components at both the base and the surface of microvilli.

On the basis of these results, they proposed that there were two forms of gp330 on the cell surface. One form (gp330-I) is restricted to the base of microvilli and has a cytoplasmic domain, whereas the other form (gp330-II) is present over the base and surface of microvilli and lacks the cytoplasmic domain.

We found differences in primary structures in the C-terminal part of gp330, showing that our form (gp330-III) is different from gp330-I. It needs to be pointed out that, although there are differences in the primary structures of gp330-I and gp330-III, both share the same peptide (Figure 2) that was used to produce anti-(cytoplasmic peptide) antibody by Bachinsky et al. (1993). Because of the differences in the primary structures of cytoplasmic domains of gp330-I and gp330-III, it is possible that these two forms have different conformations in their cytoplasmic domains, and therefore anti-peptide antibody may react with gp330-I and not with gp330-III. In that case, in the experiments performed by Bachinsky et al. (1993) gp330-III could have behaved as gp330-II. A test of this possibility, however, will require sequencing the various forms of gp330.

Whether these forms are products of different genes, or whether they result from alternative splicing, needs to be studied. It is possible that the structure of the C-terminal cytoplasmic domain plays an important role in the cellular compartmentalization of gp330 and its functions.

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