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A CLONED RAT CD38-HOMOLOGOUS PROTEIN AND ITS EXPRESSION IN PANCREATIC ISLETS †

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Cyclic ADP-ribose recently has been suggested to be an important intracellular signal for insulin secretion. CD38, which was originally isolated from human lymphocytes as a surface marker, is active in the synthesis of cyclic ADP-ribose. We report here the cloning of a rat CD38-homologous protein (CD38H) which is expressed in pancreatic islets. The deduced amino acid sequence shows that rat CD38H is a protein of 303 amino acids (Mr, 34.4 kD) and contains one possible membrane-spanning domain, consistent with a type II transmembrane glycoprotein. The overall identity and similarity of the amino acid sequences between the human CD38 and the rat CD38H are 58% and 76%, respectively. RNA blot analysis showed a strong signal of 3.4 kb in rat brain, duodenum, and heart. CD38H also is shown to be expressed in pancreatic islets by the RT-PCR procedure, but its expression is not significantly different in Wistar and GK rats, a genetic model of non-insulin-dependent diabetes mellitus. The presence of rat CD38H in the pancreatic islets suggests that CD38H may be involved in insulin secretion by synthesizing cADP-ribose.

A rise in cytoplasmic Ca^{2+} concentration is known to play a critical role in the insulin secretion from pancreatic β cells in response to various stimuli including glucose (1). Three

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major mechanisms have been shown to be responsible for the increase of intracellular Ca^{2+} concentration. One is the voltage-dependent calcium channel system (2). Opening of this calcium channel following closure of the ATP-sensitive potassium channel induces an inward calcium current. The second is the inositol triphosphate (IP_3) system (1). Stimulation of some G protein-coupled receptors activates phospholipase C, which hydrolyses phosphatidylinositol-2-phosphate to IP_3 and diacylglycerol. IP_3 then binds to its specific receptor in the endoplasmic reticulum and mobilizes Ca^{2+} stored in the endoplasmic reticulum. The third is the recently proposed pathway dependent on cyclic ADP-ribose (cADPR) production (3). cADPR is synthesized by glucose stimulation, serving as a second messenger for Ca^{2+}-mobilization from the endoplasmic reticulum by acting on a ryanodine-sensitive Ca^{2+} release channel distinct from the IP_3 receptor.

cADPR is a metabolite of NAD^+. ADP-ribosyl cyclase, the enzyme that synthesizes cADPR from NAD^+, has been cloned from *Aplysia* ovotestis (4) and shows considerable homology with the human lymphocyte antigen CD38 (5). CD38 is a type II transmembrane protein expressed in lymphoid and myeloid cells. Several studies then showed that CD38 has not only a cADPR-synthesizing activity but also a cADPR-hydrolyzing activity (6-8).

In this study we have cloned and characterized a rat homologue of CD38. We also have examined its expression in pancreatic islets of GK rats, a spontaneous model of non-insulin-dependent diabetes mellitus (9).

MATERIALS AND METHODS

General Methods

Standard methods were carried out as described in Sambrook et al. (10). Sequencing of both DNA strands was performed by the dideoxy chain-termination method.

Isolation of Rat CD38-Homologous Protein cDNA Clone

Human brain cDNA was amplified by polymerase chain reaction (PCR) using a pair of human CD38-specific oligonucleotides (sense, 5'-ACTCTGTCTTGGCGTCAGTA-3'; antisense, 5'-GTGCAAGATGAATCCTCAGG-3', corresponding to 138-157 and 940-959 of the human CD38 cDNA). The PCR product was labeled by nick-translation and used to screen a rat brain cDNA library (Clontech, Palo Alto, CA). Hybridization was carried out at 42°C in 50% formamide, 5 x SSC (1 x SSC = 0.15 M sodium chloride/0.015 M sodium citrate), 2 x Denhardt's solution, 0.1% SDS, 20 mM sodium phosphate (pH 7.0), 100 μg/ml of salmon sperm DNA and 10% dextran sulfate. Membranes were washed in 0.1 x SSC and 0.1% SDS at 50°C.

RNA Blotting Analysis

Total RNA was extracted from various rat tissues by the guanidinium isothiocyanate-cesium chloride procedure, denatured with glyoxal, electrophoresed on 1% agarose gels, and transferred onto nylon membranes. Hybridization and washing conditions were the same as those described above.

Reverse Transcription (RT)-PCR Analysis

Male GK rats (8w) and age-matched Wistar rats were used to isolate islets by collagenase digestion followed by purification on Ficoll gradients (11). Total RNA was extracted as described above.

cDNA was synthesized from 1 μg total RNA of freshly isolated rat islets, RINm5F cells and control rat brain with 100 ng of oligo(dT) primer in a solution that contained 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM dithiothreitol, 500 μM each deoxynucleotide (dNTP) and 200 U MMLV reverse transcriptase (Superscript, GIBCO BRL, Gaithersburg, MD) for 1 h at 37°C followed by 30 min at 42°C. cDNA derived from 10 ng total RNA was combined with 1 μM oligonucleotide primers specific for the rat CD38H cDNA sequence (sense, 5'-TTGCAGAAGATGCCTGTGGT-3'; antisense, 5'-ACAGGTCGGTAGTTATCCTG-3', corresponding to 601-620 and 840-859 of the rat CD38H cDNA) in 10 μl of a solution containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% TritonX-100, 2 mM $MgCl_2$, 200 μM of each dNTP, and 0.25 U Taq DNA polymerase (TOYOBO, Osaka, Japan). The reactions were carried out in a DNA thermal cycler (9600-R, Perkin Elmer, Norwalk, CT). After an initial denaturation at 94°C for 3 min, the samples were subjected to 40 cycles of amplification under the following conditions: denaturation at 94°C for 15 s, annealing at 50°C for 15 s and extension at 72°C for 30 s. The PCR products were electrophoresed on a 2% agarose gel.

The levels of CD38H mRNA in islets from GK and control rats were quantified as described previously (12). Briefly, PCR was carried out with 2.5 μCi of [^{32}P]dCTP (3000 Ci/mmol, Amersham, Bucks, UK). The samples were subjected to 38 cycles, at which point the amplification was in the exponential phase of the amplification curve (data not shown). The ^{32}P-labeled PCR products were electrophoresed on a 5% polyacrylamide gel and the radioactivity was measured by the Fuji Bio-Imaging Analyzer (Tokyo, Japan).

RESULTS AND DISCUSSION

Two positive clones were obtained by screening approximately 6 x 10^5 clones of a rat brain cDNA library with the ^{32}P-radiolabeled human CD38 cDNA as a probe. Because these two clones showed an overlapping restriction map, the longer clone (λRBC-1) was examined further. A 1.5 kb DNA fragment isolated from λRBC-1 was sequenced. This fragment contains a long open reading frame encoding a protein of 303 amino acids with an estimated molecular mass of 34,413 and is designated CD38 homologous protein (CD38H, Fig. 1). Hydrophobic analysis of the deduced amino acid sequence of rat CD38H displayed one

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                                                                    cggca

  1 Met Ala Asn Tyr Glu Phe Ser Gln Val Ser Glu Asp Arg Pro Gly Cys Arg Leu Thr Arg
    atg gcc aac tat gaa ttt tcc cag gtg tct gag gac aga cct ggc tgc cgc ctc act agg

 21 Lys Ala Gln Ile Gly Leu Gly Val Gly Leu Leu Leu Leu Val Ala Leu Val Val Val Val
    aaa gcc cag atc ggt ctt gga gtg ggt ctc ctg ctc ctg gtc gcc ttg gta gtg gtc gtg

 41 Val Ile Val Leu Trp Pro Arg Ser Pro Leu Val Trp Lys Gly Lys Pro Thr Thr Lys His
    gtc ata gtt ctg tgg ccg cgc tca ccc ctg gtg tgg aaa ggg aag cct acc acg aag cac

 61 Phe Ala Asp Ile Ile Leu Gly Arg Cys Leu Ile Tyr Thr Gln Ile Leu Arg Pro Glu Met
    ttc gct gac atc atc ttg gga cgc tgc ctg atc tat act caa atc ctc cgg ccg gag atg

 81 Arg Asp Gln Asp Cys Lys Lys Ile Leu Ser Thr Phe Lys Arg Gly Phe Ile Ser Lys Asn
    aga gat cag gac tgc aag aag ata cta agt aca ttc aaa aga ggg ttt att tcc aag aat
                *
101 Pro Cys Asn Ile Thr Asn Glu Asp Tyr Ala Pro Leu Val Lys Leu Val Thr Gln Thr Ile
    cct tgc aac atc acc aat gaa gac tac gca cca ctg gtt aaa ttg gtt act caa acc ata

121 Pro Cys Asn Lys Thr Leu Phe Trp Ser Lys Ser Lys His Leu Ala His Gln Tyr Thr Trp
    cca tgt aac aag act ctc ttt tgg agc aag tcc aaa cac ctg gcc cat cag tat act tgg

141 Ile Gln Gly Lys Met Phe Thr Leu Glu Asp Thr Leu Leu Gly Tyr Ile Ala Asp Asp Leu
    atc cag ggg aaa atg ttc acc ctg gag gac aca ctg ctg ggc tat att gca gat gat ctc

161 Arg Trp Cys Gly Asp Pro Ser Thr Ser Asp Met Asn Tyr Asp Ser Cys Pro His Trp Ser
    agg tgg tgt gga gac ccc agt act tcc gat atg aac tat gac tct tgc cca cat tgg agt

181 Glu Asn Cys Pro Asn Asn Pro Val Ala Val Phe Trp Asn Val Ile Ser Gln Lys Phe Ala
    gaa aat tgt ccc aac aac cct gtt gct gtg ttc tgg aat gtg att tcc caa aag ttt gca
                                                  *
201 Glu Asp Ala Cys Gly Val Val Gln Val Met Leu Asn Gly Ser Leu Ser Glu Pro Phe Tyr
    gaa gat gcc tgt ggt gtg gtc caa gtg atg ctc aat ggg tcc ctc agt gag cca ttt tac
          *
221 Arg Asn Ser Thr Phe Gly Ser Val Glu Val Phe Asn Leu Asp Pro Asn Lys Val His Lys
    aga aac agc acc ttt gga agt gtg gaa gtc ttt aat ttg gac cca aat aag gtt cat aaa

241 Leu Gln Ala Trp Val Met His Asp Ile Lys Gly Thr Ser Ser Asn Ala Cys Ser Ser Pro
    cta cag gcc tgg gta atg cat gac att aaa gga act tcc agt aat gca tgt tcg agc ccc

261 Ser Ile Asn Glu Leu Lys Ser Ile Val Asn Lys Arg Asn Met Ile Phe Ala Cys Gln Asp
    tcc ata aat gag ctg aag tcg att gtg aac aaa agg aat atg ata ttt gcc tgc cag gat

281 Asn Tyr Arg Pro Val Arg Phe Leu Gln Cys Val Lys Asn Pro Glu His Pro Ser Cys Arg
    aac tac cga cct gta aga ttt ctt cag tgt gtg aag aat cct gag cat cca tca tgt aga

301 Leu Asn Val OPA
    ctt aat gtg tga aggacttggatctcagaatccccacacacactccgcagcacgcaatgaggtgaaagattcaga

    agcaacactgtcgggaaagcagcagttgtcagggagaggtctctactacaatgctaacatcagagatggaagagcttcc
    caatacattatgacagaacgacacgatcagctcagtgcgtcctgtgtactaccagtaatagtcaagaaaagtcactcca
    taaaccagagttaaggttctatattgttatttcaatgtggtgcttttatattgtttatatttgctagcatccttttttt
    cctctcttttaatgaggtctctctttctctcttaatgaggctgagaatcagacctgagaacttgcacacatggtggata
    agcctatataccactgagctaactcctcagcatggctggatttttattgaaaaatgccaatccaacttcccattggaca
    aagaaaatcaaacatcaagtttatcgaaatgaataaccttatttttttatgttttccg
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Fig. 1. Nucleotide and deduced amino acid sequences of the rat CD38 homologous protein cDNA. Numbers on the left margin indicate the amino acid residues. The putative transmembrane domain is underlined and three potential N-linked glycosylation sites are indicated by asterisks.

hydrophobic region near the amino terminal of the protein. The absence of a signal peptide and the presence of a hydrophobic domain suggest that rat CD38H is a type II transmembrane protein in which the amino terminus of the protein is intracellular. Rat CD38H has three potential *N*-glycosylation sites at the putative extracellular region of the protein (Asn^{103}, Asn^{212} and Asn^{222}). Rat CD38H has 58%, 31%, and 87% amino acid identity with human CD38, Aplysia ADP-ribosyl cyclase, and mouse CD38 (13), respectively (Fig. 2). The

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rat       MANYEFSQVSEDRPGCRLTRKAQIGLGVGLLLLVALVVV-VVIVLWPRSPLVWKGKPTTK  59
human     MANCEFSPVSGDKPCCRLSRRAQLCLGVSILVLILVVVL-AVVVPRWRQ--TWSGPGTTK  57
mouse     MANYEFSQVSGDRPGCRLSRKAQIGLGVGLLVLIALVVGIVVILLRPRSLLVWTGEPTTK  60
aplysia   MSPVAIIACV--------------CLAVTLTSISPSEAI--VPTRELEN--VFLGRCKDY  42
          *                      * *           *           *

rat       HFADIILGRCLIYTQILRPEMRDQDCKKILSTFKRGFISKNPCNITNEDYAPLVKLVTQT  119
human     RFPETVLARCVKYTEIH-PEMRHVDCQSVWDAFKGAFISKHPCNITEEDYQPLMKLGTQT  116
mouse     HFSDIFLGRCLIYTQILRPEMRDQNCQEILSTFKGAFVSKNPCNITREDYAPLVKLVTQT  120
aplysia   EI--------TRYLDIL-PRVR-SDCSALWKDFFKAFSFKNPCDLDLGSYKDFFTSAQQQ  92
                  * * * *  *      *  * * ** *       *

rat       IPCNKTLFWSKSKHLAHQYTWIQGKMFTLEDTLLGYIADDLRWCGDPSTSDMNYDSCPHW  179
human     VPCNKILLWSRIKDLAHQFTQVQRDMFTLEDTLLGYLADDLTWCGEFNTSKINYQSCPDW  176
mouse     IPCNKTLFWSKSKHLAHQYTWIQGKMFTLEDTLLGYIADDLRWCGDPSTSDMNYVSCPHW  180
aplysia   LPKNKVMFWSGVYDEAHDYANTGRKYITLEDTLPGYMLNSLVWCGQRANPGFNEKVCPDF  152
           * **   **     **          ****** **    * ***       *   **

rat       SENCPNNPVAVFWNVISQKFAEDACGVVQVMLNGSLSE--PFYRNSTFGSVEVFNLDPNK  237
human     RKDCSNNPVSVFWKTVSRRFAEAACDVVHVMLNGSRSK--IFDKNSTFGSVEVHNLQPEK  234
mouse     SENCPNNPITMFWKVISQKFAEDACGVVQVMLNGSLRE--PFYKNSTFGSLEVFSLDPNK  238
aplysia   -KTCPVQARESFWGMASSSYAHSAEGEVTYMVDGSNPKVPAYRPDSFFGKYELPNL-TNK  210
             *       **   *  *  *   *  *  **          * **  *   *   *

rat       VHKLQAWVMHDIKGTSSNACSSPSINELKSIVNKRNMIFACQDNYRPVRFLQCVKNPEHP  297
human     VQTLEAWVIHGGREDSRDLCQDPTIKELESIISKRNIQFSCKNIYRPDKFLQCVKNPEDS  294
mouse     VHKLQAWVMHDIEGASSNACSSSSLNELKMIVQKRNMIFACVDNYRPARFLQCVKNPEHP  298
aplysia   VTRVKVIVLHRLGEKIIEKCGAGSLLDLEKLVKAKHFAFDCVENPRAVLFLLCSDNPNAR  270
          *      * *         *       *          * *    *   ** *  **

rat       SCRLN------V  303
human     SCTSE------I  300
mouse     SCRLN------T  304
aplysia   ECRLAKRFYRIA  282
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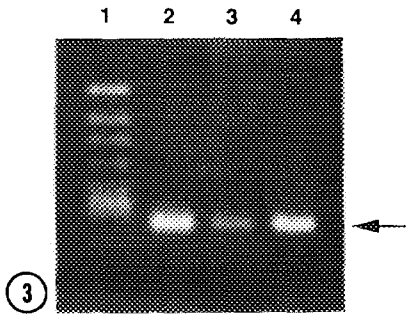
Fig. 2. Alignment of the amino acid sequences of the CD38 family members. Gaps (indicated by dashes) have been introduced to maximize alignment. Amino acid residues which are conserved among the CD38 family members are shown by asterisks.

homology was observed over the entire protein-coding region. Markedly, 9 of 13 cysteine residues in rat CD38H are conserved among all four proteins, suggesting that the tertiary structures of these proteins are similar. However, the low identity of human and rat CD38 may imply that the human CD38 and the rat CD38H comprise a family of distinct isoforms. To clarify this, we have carried out DNA blot analysis of rat genomic DNA using the human CD38 or the rat CD38H as probes. Since the human cDNA probe revealed an identical pattern of hybridizing signals with a much lower intensity compared with the rat CD38H probe (data not shown), we believe that rat CD38H represents the rat homologue of human CD38.

Tissue distribution of rat CD38H mRNA was examined by RNA blot analysis of total RNA extracted from various rat tissues. Rat CD38H was detected as a single transcript of 3.4 kb (Fig. 3). The CD38H mRNA was abundant in brain, heart, and duodenum. Moderate levels of expression were detected in stomach, liver, and kidney.

Gene expression of rat CD38H also was examined by the RT-PCR procedure. CD38H mRNA was detected in the freshly isolated rat islets and the rat pancreatic β cell-derived cell line, RINm5F, as well as in the rat brain (Fig. 4). To rule out the possibility of the amplification of contaminating genomic DNA, the amplification of total RNA was examined and showed no signal (data not shown). These results suggest that the pancreatic islets, probably the β cells, have CD38H mRNA.

It has been suggested that glucose increases the production of cADPR to stimulate insulin secretion from pancreatic β cells (3). Therefore, altered expression of the synthesizing and degrading enzyme of cADPR may be implicated in the pathogenesis of diabetes. We have examined gene expression of CD38H in the pancreatic islets of diabetic animals (Fig. 5). The



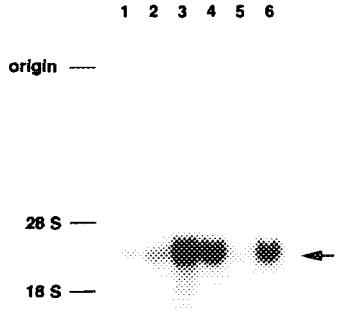


Fig. 3. **Expression of the CD38H in rat tissues.** The CD38H mRNA are 3.4 kilobases in size and indicated by arrow. 1, stomach, 2, liver, 3, duodenum, 4, heart, 5, kidney, 6, brain.

Fig. 4. **Expression of the CD38H in rat pancreatic islets.** Expression of the CD38H mRNA in rat brain (lane 2), rat pancreatic islets (lane 3) and RINm5F cells (lane 4), was examined by the RT-PCR procedure. For size markers (lane 1), HincII-digested ϕX174 was electrophoresed in parallel.

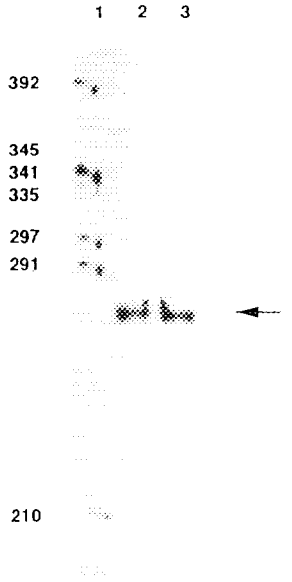


Fig. 5. Comparison of CD38H expression in pancreatic islets between GK and Wistar rats. Expression of the CD38H mRNA in the pancreatic islets was compared between GK rats (lane 2) and control Wistar rats (lane 3). HincII-digested ϕX174 were labeled and used for size markers (lane 1); sizes of the DNA fragments are given in base pairs.

levels of CD38H mRNA in islets from GK and control rats were not significantly different: 2280 ± 304 and 2183 ± 337 arbitrary units, respectively.

The presence of rat CD38H in pancreatic islets suggests that CD38H may be involved in insulin secretion through synthesizing cADP-ribose. Studies utilizing the rat CD38 clone should provide the molecular basis for understanding the role of cADPR synthesis in the pathogenesis of diabetes.

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