Amino Acid Residues on Human Poliovirus Receptor Involved in Interaction with Poliovirus*

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Junken Aoki‡§, Satoshi Koike‡, Iku Ise‡, Yasuko Sato-Yoshida‡§, and Akio Nomoto‡§

From the ‡Department of Microbiology, the Tokyo Metropolitan Institute of Medical Science, Honkomagome, Bunkyo-ku, Tokyo 113, Japan and the §Department of Microbiology, Institute of Medical Science, the University of Tokyo, Shirokanedai, Minato-ku, Tokyo 108, Japan

We have previously demonstrated that the N-terminal immunoglobulin-like domain (domain 1; 115 amino acids) of human poliovirus receptor (hPVR) is essential for poliovirus binding and infection to cells. To identify amino acids involved in the interaction with poliovirus, we constructed a number of cDNAs encoding mutant hPVRs whose domain 1 was partially derived from mouse PVR (mPVR) homolog, which does not serve as a binding site for poliovirus. Poliovirus binding and infection assays were performed on mouse L cells that express these chimera cDNAs. Anti-hPVR monoclonal antibodies were employed to confirm the presence of mutant PVRs on the surface of mouse cells and to know conformational alteration of these PVRs. A significant decrease in efficiency of both poliovirus binding and infection to the cells was observed when one or a few amino acids of hPVR at Gln32, Ser74, Gln75, Leu88-Glu102, or Gln130-Glu132 were substituted by the corresponding amino acids of mPVR. Similar results were obtained when a 2-amino acid insertion of mPVR, which was missing in hPVR, was introduced at the corresponding site (between Arg38 and Leu39) of hPVR. These amino acids were highly conserved in functional PVRs of primates but not in unfunctional PVRs of rodents. These results indicate that the amino acids identified may have important roles in interaction of PVR with poliovirus that leads to the establishment of the virus infection. In the three-dimensional model of the domain 1 of hPVR, these amino acids are located on one side of the molecule. This suggests that the interaction with poliovirus occurs on this side of the domain 1.

Poliovirus, known to be the causative agent of poliomyelitis, is a human enterovirus that belongs to the Picornaviridae. Poliovirion, an icosahedral nonenveloped particle, is composed of 60 copies each of four capsid proteins, VP1, VP2, VP3, and VP4, and a single-stranded RNA genome of positive polarity (1). A precise three-dimensional structure of the poliovirus has been elucidated by an x-ray crystallographic study (2, 3). Depressions, called "canyon," were observed on the surface of the virion and were suggested to be attachment sites for specific cellular receptors (4).

Poliovirus infection is initiated by binding of the virus to a specific cell surface molecule that serves as a poliovirus receptor (PVR).1 Interaction between poliovirus and PVR destabilizes the virion particle (5). Indeed, the binding leads to the formation of "A-particles" that do not contain the capsid protein VP4. These A-particles are considered to be intermediates during the virus-uncoating process. Thus, PVR appears to have an important role in the uncoating process of the virus as well as in recognition and binding of the virus. Elucidation of the interaction of poliovirus with PVR, therefore, must provide insights into mechanisms of early infection processes of poliovirus.

The genomic and complementary DNAs for human PVR (hPVR) have been isolated from HeLa cells (6, 7). Human PVR, a member of the immunoglobulin (Ig) superfamily, has three extracellular Ig-like domains, followed by a membrane-spanning domain and a cytoplasmic domain. Some hPVR mRNA isoforms, generated by alternative splicing, lack a nucleotide sequence encoding a transmembrane-spanning domain, and therefore encode soluble types of hPVR. Only membrane-bound forms (hPVRa and hPVRb) are functional receptors for poliovirus (7). Antibodies against PVR detected them as membrane glycoproteins of approximately 75 kDa, although molecular masses of hPVRa and hPVRb calculated from the deduced amino acid sequences were approximately 45 and 43 kDa, respectively. There are eight putative N-linked glycosylation sites in the extracellular domain (6, 7). Recent molecular genetic analysis has revealed that the poliovirus binding site resides in the N-terminal Ig-like domain (domain 1) (8, 9) and that sugar moieties possibly attached to this domain are dispensable for the virus-receptor interaction leading to the infection (10, 11). These results suggest that protein moiety of the domain 1 is important for the interaction between poliovirus and PVR.

The host range of most poliovirus strains is restricted to primates. This host range restriction appears to be determined by cellular receptors accessible to poliovirus. Indeed, the mouse PVR (mPVR) homolog was proved not to serve as a functional PVR (12). It is therefore possible that experiments involving "homolog scanning mutagenesis" identify the amino acids important for the interaction between poliovirus and its cellular receptor.

Here, we describe the identification of several amino acid residues on hPVR as important residues for the virus-receptor interaction. All of these amino acids are located on one side of the three-dimensional structure proposed for the domain 1 of hPVR. The key amino acids identified here are highly conserved in primates but not in rodents. This suggests that these

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1 The abbreviations used are: PVR, poliovirus receptor; mPVR, mouse poliovirus receptor homolog; mAb, monoclonal antibody; PBS, phosphate-buffered saline; hPVR, human poliovirus receptor; PCR, polymerase chain reaction.
amino acids are involved in determination of species specificity of poliovirus.

**Experimental Procedures**

Cloning of mPVR cDNA—A cDNA library was prepared from poly(A)+ RNA of the brain of mouse strain C57BL/6, using vector pgt10 as described previously (7), except that the NotI and BamHI EcoRI adapter (Takara Shuzo Co.) was employed. An AatII-EcoRV fragment of hPVR cDNA (nucleotide positions 82–406) labeled with 32P was used as a probe. The cloned plasmid DNA was digested with HindIII and EcoRI (7). The subcloned fragments were hybridized to the probe and were selected by their restriction endonuclease maps (8). The selected cDNA clones were analyzed by dideoxy method (13) using Sequenase Version 2.0 kit (U. S. Biochemical Corp.). In some cases, synthetic oligonucleotides were used as primers for the dideoxy method. The nucleotide sequence data reported in this paper will appear in the GSDS, DDBJ, EMBL, and NCBI nucleotide sequence data bases with the following accession number D26107.

Cloning of PVR Gene from Various Animal Species—The genomic DNA segment encoding a part of the domain 1 of the PVR or its homolog was amplified by PCR from DNAs isolated from cynomolgus monkey (Macaca fascicularis), white-tailed tamarin (Saguinus labiatus), and Western rat. Cloning of the gene from cynomolgus monkey was performed as described previously (7). Polymerase chain reaction (PCR) primers employed for the amplification of the cDNA fragment of the rat PVR were 5'-GACGTCGTCGTGCAGGCGCCCAC-3' (antisense sequence of positions 349–372 of hPVR) or 5'-CGGGTACGAGT-CCTCTACATG-3' (sense sequence of positions 410–430 of hPVR) or 5'-CGGGTACGAGT-CCTCTACATG-3' (antisense sequence of positions 410–430 of hPVR) or 5'-CGGGTACGAGT-CCTCTACATG-3' (antisense sequence of positions 410–430 of hPVR) (9).

Recombinant PVR cDNAs of hPVR and mPVR—To construct a series of cDNAs encoding recombinant molecules between hPVR and mPVR with regard to the domain 1, restriction enzyme sites were introduced in both hPVR and mPVR cDNAs. As shown in Fig. 2, a fragment of hPVR cDNA nucleotides 82–406 was replaced by the BglI site in SalI and AatII sites and EcoRV and BamHI sites at 5' and 3' ends, respectively. Removal of the BglI site at nucleotide position 212 generated SalI and AatII sites and EcoRV and BamHI sites at 5' and 3' ends, respectively. Removal of the BglI site at nucleotide position 212 generated SalI and AatII sites and EcoRV and BamHI sites at 5' and 3' ends, respectively. Removal of the BglI site at nucleotide position 212 generated SalI and AatII sites and EcoRV and BamHI sites at 5' and 3' ends, respectively. Removal of the BglI site at nucleotide position 212 generated SalI and AatII sites and EcoRV and BamHI sites at 5' and 3' ends, respectively.

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**Results**

Cloning of mPVR cDNA—A cDNA clone that hybridized to the hPVR cDNA probe was obtained from 5 × 10⁵ plaques of a mouse DNA library. A 2.4-kilobase cDNA insert of this phage clone was subcloned into a plasmid vector pBluescript KS. Nucleotide sequence analysis revealed that the cDNA had a nucleotide sequence encoding a peptide of 530 amino acids (Fig. 1A) followed by a part of the 3' noncoding region of 874 base pairs. The nucleotide sequence in the first 1015 nucleotides of this cDNA was identical to that previously reported (8). However, the nucleotide sequence downstream of position 1016 was unique (Fig. 1A), resulting in the loss of a region encoding the putative membrane-spanning domain (Fig. 1B). The coding sequence of this cDNA carried 4 additional exons (exons 7–10) in the downstream of exon 6 of the mPVR gene (data not shown). The branch point of the sequence divergence was one of the splicing sites (12). Northern blot analysis revealed that a probe...
Mutational Analysis of Poliovirus Receptor

ATGCCCGGCGCCGATCTCCTCCCGGCTCCAGATGCTACACGCAGCTGCCTGTCTGCGCTCTACGCTCTGCAGGAACAGGAG

GCCCAGATGGGCTAGGCCTAGGCTAACCCTGGCCAGCTTGCTGGGACGACATGCTCTGAGTAAGCTTGGGACAGCTGCTAGTT

ACCGAGGCCGCTGCTAGGCTAACCCTGGCCAGCTTGCTGGGACGACATGCTCTGAGTAAGCTTGGGACAGCTGCTAGTT

FIG. 1. Structure of the soluble form of mPVR cDNA. A, nucleotide sequence and the deduced amino acid sequence for the coding region of soluble mPVR are shown. The first and second lines indicate nucleotide and the deduced amino acid sequences, respectively. Nucleotide and amino acid positions are shown at the right side of the figure. The nucleotide sequences specific for the soluble form of mPVR are underlined. The arrowhead indicates the splicing site at which two forms of mPVR diverge. The putative signal peptide region is indicated by a dashed line below the amino acid sequence. B, schematic representation of expected structures of soluble type (above) and membrane-bound type (below) (12) of mPVR. Open bar shows an amino acid sequence that is specific for the soluble type of mPVR, and the hatched box indicates the membrane-spanning domain of the membrane-bound type of mPVR. Numbers indicate amino acid positions.
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Fig. 2. Comparison of amino acid sequences of hPVR and its homologs and strategy for constructing human/mouse PVR chimera cDNAs. A, amino acid sequences of hPVR and its homologs. The amino acid sequences of PVR domain 1 deduced from the DNA sequences were compared with human, mouse, African green monkey (AGM1 and AGM2), cynomolgus monkey (CYN1 and CYN2), white-lipped tamarin (tamarin), and Wister rat. In the case of cynomolgus monkey, tamarin, and rat, partial amino acid sequences obtained are shown. Numbers at the right of the amino acid sequences indicate the amino acid positions of hPVR. Dots represent the amino acids that are identical to those of hPVR. Dashes indicate the positions of deletions. The positions of the possible nine β-strands and regions used in the construction of chimeric PVRs were shown below the sequences. B, strategy for construction of chimeric PVR cDNAs. An AatII-EcoRV fragment of human PVR cDNA (shadowed box) was amplified, and BstEII and/or BglI sites were introduced by PCR as described under “Experimental Procedures.” A corresponding region of mPVR (open box) was amplified by PCR to generate AatII and EcoRV sites at the 5' and 3' ends, respectively. The BglI site at position 212 was removed without changing the amino acid sequence, and Apal and/or EcoRI sites were introduced by PCR as described under “Experimental Procedures.” The AatII-EcoRV fragments of hPVR and mPVR thus obtained were subcloned into pUC 119. Human/mouse chimera PVR cDNAs were constructed by using the common restriction enzyme sites. Numbers in parentheses indicate nucleotide positions of restriction enzyme sites.

Regions of hPVR Influencing Interaction with Poliovirus—Amino acid sequences in domain 1 of hPVR and mPVR are shown in Fig. 2A. In this region of mPVR, 55 amino acid substitutions, two deletions, and two insertions are observed compared with that of hPVR, resulting in 52% sequence homology in this domain between hPVR and mPVR. To identify regions of the first 1015 nucleotide sequence hybridized both to 2- and 3-kilobase bands (data not shown). A probe that had nucleotide sequence 1016–2464 hybridized only to the 3-kilobase band. These data strongly suggest that cDNA obtained in this study corresponds to mRNA for a soluble form of mPVR, generated by alternative splicing from the primary transcript.
Mutational Analysis of Poliovirus Receptor

Table I. Poliovirus binding and infection of mPVR mutants

| Mutants | Alanine in PVR domain 1 | Poliovirus binding | Poliovirus infection | 4C6 binding |
|---------|------------------------|-------------------|----------------------|-------------|
|         | region(s) |                  | % of wild type | Log(P/0) | % of wild type |                  |                |             |
| wild type | 1 2 3 4 5 | 100                  | 5.4               | 100       |                  |                  |                |             |
| R 1234   |             | 60                   | 5.3               | 98        |                  |                  |                |             |
| R 1235   |             | 43                   | 4.3               | 84        |                  |                  |                |             |
| R 1245   |             | 4                    | 4.7               | 128       |                  |                  |                |             |
| R 1345   |             | 3                    | 3                  | 93        |                  |                  |                |             |
| R 2345   |             | 2                    | 3.0               | 55        |                  |                  |                |             |
| R 123    |             | 2                    | 2                  | 56        |                  |                  |                |             |
| R 234    |             | 2                    | 4.4               | 59        |                  |                  |                |             |
| R 345    |             | 1                    | 1                  | 81        |                  |                  |                |             |
| R 125    |             | 2                    | 2                  | 97        |                  |                  |                |             |
| R 145    |             | 0                    | 0                  | 98        |                  |                  |                |             |
| R 12     |             | 3                    | 3                  | 34        |                  |                  |                |             |
| R 23     |             | 1                    | 1                  | 65        |                  |                  |                |             |
| R 34     |             | 0                    | 0                  | 79        |                  |                  |                |             |
| R 45     |             | 1                    | 1                  | 78        |                  |                  |                |             |
| R 15     |             | 0                    | 0                  | 85        |                  |                  |                |             |
| R 1      |             | 0                    | 0                  | 63        |                  |                  |                |             |
| R 2      |             | 1                    | 1                  | 50        |                  |                  |                |             |
| R 3      |             | 2                    | 2                  | 86        |                  |                  |                |             |
| R 4      |             | 2                    | 2                  | 56        |                  |                  |                |             |
| R 5      |             | 3                    | 3                  | 26        |                  |                  |                |             |
| M        |             | 0                    | 0                  | 59        |                  |                  |                |             |
| Mock     |             | 0                    | 0                  | 0         |                  |                  |                |             |

Fig. 3. PVR activities of human/mouse chimera PVRs. Structures of chimeric PVR domain 1 (corresponding to the AaII-EcoKI fragment) are shown by the combination of segments of hPVR (shaded boxes) and mPVR (open boxes). Poliovirus and mAb 4C6 bound are shown by percentages of those to cells carrying wild type hPVR. Virus titers of infectious particles recovered were shown in logarithmic values of plaque-forming units (log10 plaque-forming units) measured in AGMK cells. Dashes mean that the virus was not detected. The mutant PVRs are designated RX, in which X represents the region number(s) of hPVR segment(s) within the domain 1 (see Fig. 1).

critical for the virus-receptor interaction, allele replacement experiments were carried out with regard to 5 regions in domain 1 (Fig. 2, A and B). This domain is classified into V-type (19) and is composed of nine antiparallel β-strands. According to the consensus sequence of the V-type domain, it is roughly estimated that region 1 corresponds to A and B strands, region 2 to C and C', region 3 to C, region 4 to D and a part of E, and region 5 to the rest of E, F, and G (Fig. 2).

Mouse L cells transfected with chimera cDNAs were examined for their capacity to find poliovirus and their susceptibility to the virus. Various mAbs against hPVR were used to examine for the existence of mutant PVRs on the cell surface and their conformation. The results are summarized in Fig. 3. Binding of mAbs (4C6 and 4E12) was observed in all mouse cell cultures transfected with chimera cDNAs used, indicating that these chimeras were expressed on the surface of mouse L cells.

When all 5 regions of hPVR were replaced with those of mPVR (M, in Fig. 3), neither poliovirus binding nor infection was detected as expected previously (12). Similar results were obtained for chimera PVRs in which two or more regions were replaced by those of mPVR except for R234 (Fig. 3). Mouse L cells carrying R234 showed both binding activity to poliovirus and susceptibility to the virus, albeit to a lesser extent as compared with those carrying wild type hPVR (Fig. 3). This suggests that regions 2, 3, and 4 of hPVR harbor important sites for the virus-hPVR interaction.

As for chimera PVRs in which only one region of hPVR was replaced by that of mPVR, only R1234 seems to have receptor function with regard to both binding and infection of poliovirus. A relatively low amount of progeny virus was recovered from cells carrying R1235, R1245, or R2345. However, a significant amount of bound poliovirus was not detected in these cells (Fig. 3). This indicates that these chimeras have low activities as a poliovirus receptor. Cells carrying R1345 showed neither binding activity nor susceptibility to poliovirus. Thus region 2 of hPVR seemed to be most important for maintaining receptor function, although every other region influences the function.

R234 retained the receptor function to some extent as mentioned above and also retained binding capacity for mAbs that recognized hPVR in a conformation-dependent manner (data not shown). R2345 showed receptor activity lower than R234 both in virus binding and infection. Furthermore none of conformation-dependent mAbs used recognized R2345. It is therefore possible that exchange of region 1 caused global alteration in the conformation of domain 1 and that an additional exchange of region 5 provides the initial conformation to some extent to the domain. Thus regions 1 and 5 appear to interact with each other. Indeed the two regions closely exist in a three-dimensional model of hPVR (Fig. 4A).

Similar results were obtained from experiments using Sabin 1, Lansing, Sabin 2, Leon, and Sabin 3 strains of poliovirus (data not shown). These results suggest that most poliovirus strains recognize similar parts of hPVR.

Amino Acids Critical for Virus-Receptor Interaction—Single or several amino acid substitutions were introduced in domain 1 of hPVR to identify the amino acid residues involved in the virus-receptor interaction. Because region 2 of hPVR seemed to be most important for both poliovirus binding and infection, a number of single amino acid substitutions were introduced in region 2. Poliovirus and mAb binding assays and poliovirus infection assay were performed, and the results are summarized in Table I.

All mutant PVRs were present on their cell surface as judged by binding of mAbs 4C6 and 4E12 (Table I). Eight mutants, i.e. S1, S5, S11, S17, S21, S24 (S21 and S24, insertion mutants), S25, and S28, showed significantly reduced activities in poliovirus binding (Table I) (to approximately <50% of wild type hPVR). The S12 mutant also showed a significant reduction of virus binding activity (63% of hPVR). The results suggest that the amino acids replaced in these mutants are important in virus-receptor interaction. However, considerable amounts of virus production were observed in cells transfected with every mutant cDNA used. It was impossible to find mutant PVRs that did not mediate the infection but bound to poliovirus. Thus poliovirus binding activity appears to be always associated with functional PVR activity.

Amino acid substitutions in the nine mutants described above might be critical contact residues for poliovirus. It is possible, however, that the substitutions significantly alter the conformation of the binding site recognized by the virus. Five mAbs with different epitopes that recognized hPVR in a conformation-dependent manner were employed to examine the possibility (Table I). Significant reduction of binding of all five groups of mAbs was observed in the case of S1, S5, and S21 (Table I). Thus these mutations seemed to induce gross conformational changes in mutant PVRs. In addition, it is impossible to identify contact amino acid residues for poliovirus from the experiment involving mutant S24, because this mutant is an insertion mutant of hPVR. Consequently, mutated amino acids in five mutants S11, S12, S17, S25, and S28 are thought to be directly involved in the interaction with poliovirus. It should be noted that no individual mutations can completely abolish poliovirus binding. It is therefore possible that the sum of the plural mutations in mPVR may result in a loss of interaction with poliovirus. This possibility is supported by an observation that the S30 mutant PVR shows no receptor function for poliovirus, although mutants S21 and S24 retain binding activity to some extent and confer cell-permissiveness for the poliovirus infection (Table I).

Key Amino Acids on Three-dimensional Model of PVR—A three-dimensional structure of PVR domain 1 has been pre-
dected by computer modeling by using the coordinates for Ig V-type domain as a reference protein (5). Nine antiparallel β-strands of Ig V-type and possible C-α trace of PVR domain 1 are shown in Fig. 4, A and B, respectively. The location of mutated amino acids in mutants S11, S12, S17, S24 (an insertion mutant), S25, and S28 are shown in Fig. 4B. Interestingly, these amino acid residues were located on one side of this three-dimensional model. The observation supports that PVR interacts with poliovirus on this side. This is consistent with our computer-aided prediction of how the domain 1 of PVR

![Diagram](https://via.placeholder.com/150)

**Table 1**

| Constructs | Mutations | Region Possible location on 3-D structure | Poliovirus-bound | Poliovirus-produced | mAb bound |
|------------|-----------|-------------------------------------------|------------------|---------------------|-----------|
| Wild type (human) | | | | | |
| Mouse | | | | | |
| Mock | | | | | |
| S1 | 30VR, 32Q/R, 33AV, 34P/L, 35T/P, 36Q/E | 1 | A strand | 100 | 5.4 | 100 | +++ ++ ++ ++ ++ ++ + + |
| S2 | 38P/R, 40V/F | 1 | A strand | 90 | ND | 59 | -- -- -- -- -- |
| S3 | 43Q/G, 44S/L, 46T/F | 1 | B strand | 0 | ND | 0 | -- -- -- -- -- |
| S4 | 50Y/H, 52Q/L, 53V/P, 55N/T, 56M/T | 1 | B-C loop | 123 | 4.4 | 56 | ++ ++ ++ + + |
| S5 | 58V/F, 59T/-, 60P/R | 1 | B-C loop | 37 | 3.9 | 51 | + + -- -- |
| S6 | 67T/Q | 2 | C strand | 92 | 5.5 | 109 | +++ ++ ++ ++ + + |
| S7 | 69H/L | 2 | C strand | 79 | 5.5 | 74 | ++ ++ ++ ++ + + |
| S8 | 70G | 2 | C strand | 75 | 5.4 | 76 | +++ ++ ++ ++ + + |
| S9 | 71D/E | 2 | C strand | 99 | 5.4 | 122 | +++ ++ ++ ++ + + |
| S10 | 72S/G | 2 | C'-C' loop | 96 | 5.3 | 41 | ++ ++ ++ ++ ++ |
| S11 | 73G/T | 2 | C'-C' loop | 53 | 5.5 | 68 | ++ ++ ++ ++ |
| S12 | 74S/V | 2 | C'-C' loop | 63 | 5.5 | 63 | ++ ++ ++ ++ |
| S13 | 75M/V | 2 | C' strand | 106 | 5.5 | 97 | +++ ++ ++ ++ |
| S14 | 77V/A | 2 | C' strand | 109 | 5.4 | 100 | +++ ++ ++ ++ |
| S15 | 80Q/P | 2 | C' strand | 79 | 5.4 | 101 | +++ ++ ++ ++ |
| S16 | 81T/S | 2 | C' strand | 123 | 5.5 | 74 | +++ ++ ++ ++ |
| S17 | 82Q/F | 2 | C' strand | 50 | 4.9 | 71 | ++ ++ ++ + |
| S18 | 84P/V | 2 | C' strand | 112 | 5.4 | 107 | +++ ++ ++ ++ |
| S19 | 85S/D, 86Y/F | 3 | C''-C'' loop | 74 | 5.2 | '41 | ++ ++ ++ + |
| S20 | 87S/V, 88E/N | 3 | C''-C'' loop | 73 | 5.4 | 100 | +++ ++ ++ + |
| S21 | 4 amino acids insertion | 3 | C''-C'' loop | 20 | 5.2 | 65 | ++ ++ ++ |
| S22 | 90K/D | 3 | C'' strand | 110 | 5.5 | 132 | ++ ++ ++ + |
| S23 | 93E/F, 96A/R | 3 | C'' strand | 110 | 5.5 | 90 | ++ ++ ++ + |
| S24 | 2 amino acids insertion | 4 | C''-D loop | 22 | 4.4 | 151 | ++ ++ ++ + |
| S25 | 99L/T, 100G/N, 102E/D | 4 | D strand | 25 | 5.2 | 151 | ++ ++ ++ + |
| S26 | 105T/G, 106R/A, 110M/F, 111F/R | 5 | E strand | 85 | 5.5 | 59 | ++ ++ ++ + |
| S27 | 124L/F, 126V/A | 5 | F strand | 99 | 5.2 | 104 | +++ ++ ++ + |
| S28 | 130Q/N, 132S/T | 5 | F-G loop | 37 | 5.3 | 122 | -- ++ ++ ++ |
| S29 | 134S/R, 135V/G | 5 | G strand | 135 | 5.1 | 72 | +++ + ++ + |
| S30 | S21 + S24 | 3, 4 | C' strand, C''-D loop | 0 | ND | 90 | ++ ++ ++ + |

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* Binding of conformation-independent mAbs were indicated by % of binding of wild type human PVR.

* Binding of conformation-dependent mAbs were shown as follows: ++, 75–100%; +, 50–75%; --, 0–9% binding of wild type.

* N.D.: not detected.
Key Amino Acids during Evolution—To examine if species specificity of poliovirus infection can be explained by the key amino acids, PVR homologs from other mammals were examined for conservation of the key amino acids. We have already reported two types of cDNAs that encode functional PVRs of African green monkey (Ceropithecus aethiops) (cDNAs for AGMx1 and AGMx2) (10). In addition, genomic DNA clones for PVR domain 1 of the cynomolgus monkey, white-lipped tamarin, and Wister rat were prepared as described under “Experimental Procedures.” Amino acid sequences of hPVR and PVR homologs from various animal species are shown in Fig. 2A. Two types of PVR genes were cloned from the cynomolgus monkey. This may be due to the gene duplication that occurred only in old-world monkeys as described (10). Of these species, human, African green monkey, and cynomolgus monkey are susceptible to poliovirus, while mice and rats are not. Although susceptibility of tamarin to poliovirus is not known, a chimera PVR cDNA in which nucleotide sequence of positions 108–251 and the remaining sequence are derived from tamarin PVR homolog and hPVR, respectively, confer mouse L cells permisiveness for poliovirus (data not shown). This strongly suggests that tamarin is also susceptible to poliovirus.

Amino acid sequence homology in domain 1 among PVRs of human, African green monkey (AGMx1 and AGMx2), cynomolgus monkey (CYN1 and CYN2), and tamarin were 92, 89, 88, 87, and 73%, respectively. All key amino acids identified on hPVR were conserved among PVRs of primates examined, except for amino acid position 73 in a tamarin PVR homolog where Gly is replaced by Arg. A number of substitutions other than the key amino acids observed in tamarin PVR homolog did not seem to affect the PVR function as described above.

On the other hand, rat PVR homolog and hPVR showed a 47% identity in the amino acid sequence of domain 1. The rat sequence had two deletions at positions 58–59 and 70, and two insertions at positions between 89 and 90, and 98 and 99 of hPVR just as mPVR. The amino acid sequences of rat PVR homolog and mPVR showed a 96% identity. Amino acid residues at positions 73, 74, and 82, which corresponded to the key amino acid positions of hPVR, were identical to those of mPVR (Fig. 2). These results support the idea that the host range of poliovirus infection may be determined by the key amino acids identified in this study. Thus accessibility of PVRs to poliovirus may be influenced by multiple amino acid residues on domain 1 of PVRs including the key amino acids proposed here.

**DISCUSSION**

The mouse PVR homolog gene reported previously (12) has been considered to encode equivalent cellular molecules to hPVR, because 1) nucleotide sequence of the cDNA is most homologous to that of hPVR cDNA, 2) the gene organization was identical to that of the hPVR gene, and 3) the mouse gene was mapped to the locus in the proximal region to centromere of the mouse chromosome 7, which includes the corresponding region of q13.1–13.2 of the human chromosome 19, the locus of the hPVR gene (7). We demonstrated here the presence of the soluble form of PVR homolog in mouse as well as in humans. Tissue distribution of mPVR mRNA detected by Northern blot hybridization was similar to that of hPVR mRNA in human tissues (20). These two additional observations further support the assumption that this gene encodes a mouse homolog for PVR.

In experiments involving homolog scanning mutagenesis performed in this study, mAbs that recognized hPVR in a conformation-dependent manner were effectively used to detect conformational alterations of PVR induced by mutations. Thus mutations in S1, S5, and S21, which appeared to cause conformational change in PVR, were eliminated from the key amino acids, although we had no evidence to deny that amino acid residues mutated in these mutants were contact residues for poliovirus. Consequently, amino acids Gly72, Ser74, Gln82, Leu84–Glu102, and Gln130–Ser132 of hPVR were proposed as possible contact residues for poliovirus. It should be noted that there may be some other amino acid residues directly involved in the virus-hPVR interaction in amino acid residues conserved between hPVR and mPVR.

PVR has a dual function, which is binding to poliovirus and destabilization of the virus (initiation of uncoating). Mason et al. (21) and Aarnes et al. (22) showed that infection does not occur when poliovirus bound to the cell surface without PVR. This supports the fact that two functions of PVR are necessary for poliovirus infection. It might be possible to separate the two functions if the PVR has two catalytic regions. However, no mutants were obtained that have poliovirus binding activity without conferring the permissiveness to the cells, suggesting that both binding and uncoating activities reside on the same site in domain 1. Thus binding and initiation of uncoating failed to be separated by this strategy.

As for human immunodeficiency virus and CD4 interaction, a specific amino acid sequence (amino acid positions 41–59 of CD4 receptor plays a major role in human immunodeficiency virus binding (23, 24, 25). On the contrary, several amino acid residues were identified in ICAM-1 as contact residues for a major group of human rhinovirus (26, 27). The interaction of poliovirus with hPVR resembles the latter case. This observation appeared to be consistent with the fact that a canyon structure is involved in the interaction of poliovirus and rhinovirus with their receptors, whereas gp120/160 is involved in the case of human immunodeficiency virus. The important amino acids for poliovirus-hPVR interaction are located in a wide area of one side of domain 1 on the three-dimensional model of the PVR. Thus the interaction seems to occur between wide areas of the canyon and hPVR. Specific conformation of hPVR, therefore, may be essential for recognition by the virus. Because of high species specificity of poliovirus, it appears that the virus has evolved together with primates.

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**REFERENCES**

1. Koch, F., and Koch, G. (1985) The Molecular Biology of Poliovirus, pp. 1–200, Springer-Verlag, Hamburg
2. Hogle, J. M., Chow, M., and Filman, D. J. (1985) Science 229, 1355–1367
3. Filman, D. J., Syed, R., Chow, M., Macadam, A. J., Minor, P. D., and Hogle, J. M. (1988) EMBO J. 8, 1567–1579
4. Rossman, M. G., and Kates, C. C. (1988) Virology 164, 373–382
5. Koike, S., Ise, I., Sato, Y., Ito, K., Horie, H., Umezawa, H., and Nomoto, A. (1982) Semin. Virol. 3, 109–116
6. Mendelsohn, C. L., Wimmer, E., and Racaniello, V. R. (1989) Cell 56, 855–865
7. Koike, S., Horie, H., Ise, I., Okitani, A., Yoshida, M., Iizuka, N., Takeuchi, K., Takegami, T., and Nomoto, A. (1990) EMBO J. 9, 3217–3224
8. Koike, S., Ise, I., and Nomoto, A. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 4104–4108
9. Selinka, H., Zibert, A., and Wimmer, E. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 3698–3692
10. Koike, S., Ise, I., Sato, Y., Yonekawa, H., Gotoh, O., and Nomoto, A. (1992) J. Virol. 66, 7003–7006
11. Zibert, A., and Wimmer, E. (1992) J. Virol. 66, 7368–7373
12. Morrison, M. E., and Racaniello, V. R. (1992) J. Virol. 66, 2807–2813
13. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
14. Iizuka, N., Yonekawa, H., and Nomoto, A. (1991) J. Virol. 65, 4867–4873
15. Kramer, W., and Frits, H. J. (1987) Methods Enzymol. 150, 350–367
16. Tammisto, T., and Miyayama, S. (1980) Int. Immunol. 2, 165–171
17. Spitz, M., Spitz, L., Thorpe, R., and Eugui, E. (1984) J. Immunol. Methods 70, 39–43

2 M. Niwa, H. Yonekawa, J. Aoki, and S. Koike, unpublished observation.
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18. Kawamura, N., Kohara, M., Abe, S., Komatsu, S., Tago, K., Arita, M., and Nomoto, A. (1989) J. Virol. 63, 1302–1309
19. Williams, A. F., and Barcley, A. N. (1988) Ann. Rev. Immunol. 6, 381–405
20. Rothe, S., Aski, J., and Nomoto, A. (1994) Transgenic Mouse for the Study of Poliovirus Pathogenicity, Cold Spring Harbor Press, New York, in press
21. Mason, P. W., Baxt, B., Brown, P., Harber, J., Murdin, A., and Wiener, E. (1993) Virology 192, 568–577
22. Aarnes, C. M., Madshus, I. H., Guilemot, J. C., Sandvig, K., and Olsnes, S. (1997) Exp. Cell Res. 170, 483–490
23. Arthos, J., Deen, K. C., Chaikin, M. A., Sattentau, J. A., Clapham, P. R., Weiss, R. A., McDougal, J. S., Pietropaolo, C., Axel, R., Truneh, A., Maddon, P. J., and Sweet, R. W. (1989) Cell 57, 469–481
24. Clayton, L. K., Hussey, R. E., Steinbrauch, R., Ramachandran, H., Husain, Y., and Remberg, E. L. (1988) Nature 335, 363–366
25. Schockmel, G. A., Samoza, C., Davis, S. J., Williams, A. F., and Healey, D. (1992) J. Exp. Med. 175, 301–304
26. Stanton, D. E., Dustin, M. L., Erickson, H. P., and Springer, T. A. (1990) Cell 61, 243–254
27. Register, B. R., Uncapher, C. R., Naylor, A. M., Lineberger, D. W., and Colonno, R. J. (1991) J. Virol. 65, 6589–6596