

Infectious cDNA Clones of Echovirus 12 and a Variant Resistant against the Uncoating Inhibitor Rhodanine Differ in Seven Amino Acids

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Determination of the complete sequences of echovirus 12 and a rhodanine-resistant variant revealed seven amino acid deviations and two additional exchanges not confirmed in all clones. In rhodanine sensitivity assays with infectious cDNAs, it was shown that the biological markers of the original viruses are maintained.

We present the first sequence of echovirus 12 prototype Travis, a human enterovirus, and of a variant resistant against the uncoating inhibitor rhodanine. Despite their clinical importance, little is known about the pathogenic mechanisms and molecular biology of echoviruses. In order to study early events in picornavirus infection, antiviral compounds stabilizing the virus by interacting with the capsid turned out to be a valuable tool. Rhodanine (2-thio-4-oxothiazolidine) blocks selectively the uncoating of echovirus 12, whereas virus adsorption, penetration, virus macromolecular synthesis, and assembly are not affected (2, 4).

Echovirus 12 (Travis) RNA was cloned and sequenced (Fig. 1). Excluding the poly(A) tract, the RNA genome is 7,422 nucleotides (nt) in length and encodes a single polyprotein of 2,193 amino acids. The genome comprises a 5' noncoding region of 741 nt, an open reading frame of 6,579 nt, and a 3' noncoding part of 102 nt. Comparison with other picornaviruses exhibited a close relationship to echovirus 11 and the coxsackie B viruses. Capsid proteins VP1, VP2, and VP3 were separated as described previously (6) and sequenced at their N termini in order to determine the posttranslational cleavage sites of the P1 region (Fig. 1).

To generate a rhodanine-resistant variant of echovirus 12, we isolated plaques appearing on plates containing 100 µg of rhodanine per ml in the overlay. After five passages in cell culture with rhodanine-containing medium, we selected a virus stock with infectious titers of 8×10^7 PFU/ml without and 6×10^7 PFU/ml in the presence of rhodanine. RNA of this variant was cloned, and the sequence was determined. The polyprotein of the resistant variant differs from that of the wild type in at least 7 amino acids (Fig. 2). Six differences are located in the capsid proteins: two in VP2, one in VP3, and three in VP1. The seventh amino acid exchange is located in the P3 region close to the 3' end of the virus. Furthermore, we found an additional exchange at position 2748 (Fig. 2, no. 5a) in four of six clones and in one of the remaining two clones an exchange at position 2650 (Fig. 2, no. 4a), both located in VP1.

A full-length wild-type cDNA clone (pT7E12wt) was constructed by ligating a PCR fragment spanning the very 5' end of the genome to the longest cDNA clone. The RNA transcribed *in vitro* was identical to the viral genome except for three indispensable G's at the 5' end due to the internal starting point of the T7 promoter and four additional nucleotides (GGGC) flanking the poly(A) tail of 109 adenine residues.

A rhodanine-resistant clone (pT7E12rhod-res) containing the seven nucleotide exchanges present in all clones tested and two additional clones (pT7E12rhod-res4a and pT7E12rhod-res5a) including the additional exchanges 4a and 5a (Fig. 2) were constructed by exchanging corresponding restriction fragments of pT7E12wt.

In vitro-transcribed RNA copies of the four clones described (Fig. 3) were shown to be infectious. The resulting recombinant viruses were neutralized by echovirus 12-specific antiserum to the same titer as the original virus.

Rhodanine sensitivity assays of the virus stocks were done as follows: confluent GMK cells were infected with 100 50% tissue culture infective doses and every 12 h the cytopathic effects were evaluated (Fig. 3) (4). Both the wild-type and the resistant recombinant viruses respond to rhodanine as their original counterparts (Fig. 3). It is shown that seven amino acid exchanges are sufficient to turn the sensitive virus into a resistant one. A further exchange (labelled 5a), a Val-to-Ala substitution found in four of six clones, does not influence the growth characteristics of the virus in the presence or absence of rhodanine. Another point mutation (labelled 4a), found in only one of the six clones investigated, introduces a drug-dependent character (Fig. 3).

Because X-ray crystallographic studies have not yet been performed, the precise basis for rhodanine resistance or dependence is still not known. Except VP4, all capsid proteins of wild-type echovirus 12 differ from those of the rhodanine-resistant variant. The fact that six of seven amino acid substitutions are located in P1 supports the concept that rhodanine stabilizes the virion by direct interaction with the capsid (2), as has also been demonstrated with radioactively labelled rhodanine (3).

The amino acid changes in VP1 are of special interest. By X-ray diffraction investigations it was shown that the two antiviral compounds WIN 51711 and WIN 52084 as well as the

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110 UAAAAACAGC CUGUGGGUUG UCCCCACCCA CAGGGCCAC UGGGCGCUAG CACACUGGUA UCCCGUACC UUUGUGCGCC UGUUUUAU AUCCUCCCU CAGUAACCUA
 220 GAAGUUAUC ACAAUGAUC AAUAGUUAGC UCAACAAACC AGUUGAGCCU AGAUCAAGCA CUUCUGUAC CCCGGGUGA GUUAUAAU GCUGUUGACA CGGCUGAAGG
 330 AGAAAACGCC CGUUACCCGA CCAGCUACU CGGAGAACCU AGUAUACCA UAGAGGUUGC GUAGCGUUUC GCUCCGCACA ACCCCAGUGU AGAUCAGGUC GAUGAGUCAC
 440 CGCGUCCCC ACAGCGGACU GUGGCGGUGG CUGCGUUGGC GGCCUGCCCA UGGGGUJACC CAUGGGACGC UUCAUACUG ACAUGGUGUG AAGAGUUGAC UGAGCUAGCU
 550 GGUAGUCCUC CGGCCCCUGA AUGCGGCUAA UCCUAACUGU GGAGCAAGUG CCCACAACCC AGUGGGUGGC UUGUCGUAU GGGCAACUCU GCAGCGGAAC CGACUACUUU
 660 GGGUGACCGU GUUUCUUCUU AUUCUUAUUA UGGGUGCUUA UGGUGACAUA CUCAGAGUUG UUAACCAUUA GCUAUUGGUU UGGCCAACCA GUGACUACA GAGCAAUUU
 741 AUAUCUUUUC AUUGGAUUUA UACCUAUAUA CAACACCAGU UAUACCACUU UGUGUUACAU UAUUGGCUUG AACUCGAAAA G-----
 831 AUG GGA GCG CAA GUA UCA ACA CAA AAA ACU GGG GCA CAC GAG ACU GGC CUG AGU GCU AGU GGA AAU UCC AUC AUC CAC UAU ACC AAC AUU
 M G A Q V S T Q K T G A H E T G L S A S G N S I I H Y T N I
 921 AAU UAC UAC AAA GAU GCG GCA UCC AAU UCA GCA AAC AGG CAG GAU UUC ACC CAA GAC CCC GGG AAA UUC ACC GAG CCA GUG AAG GAU AUC
 N Y Y K D A A S N S A N R Q D F T Q D P G K F T E P V K D I
 1011 AUG AUA AAA UCG AUG CCU GCG UUA AAC UCC CCA ACA GCA GAA GAA UGU GGG UAC AGC GAU AGA GUC AGA UCA AUC ACC CUG GGG AAC UCU
 M I K S M P A L N S P
 VP4 ↑ VP2
 1101 ACC AUC ACC ACG CAG GAG UGU GCC AAU GUG GUG GUG GGA UAU GGC ACG UGG CCU GAU UAU CUA CAU GAU GAU GAG GCC ACU GCA GAG GAC
 T I T T Q E C A N V V V G Y G T W P D Y L H D D E A T A E D
 1191 CAA CCA ACA CAA CCG GAU GUG GCG ACG UGU AGG UUU UAC ACA CUU GAA UCC AUU CAG UGG CAG AAG ACA UCA GAU GGG UGG UGG UGG AAG
 Q P T Q P D V A T C R F Y T L E S I Q W Q K T S D G W W W K
 1281 UUU CCG GAA GCA UUG AAA GAC AUG GGA CUG UUC GGC CAA AAC AUG CAC UAU CAU UAU CUU GGC AGG UCG GGU UAC ACC AUC CAC GUC CAA
 F P E A L K D M G L F G Q N M H Y H Y L G R S G Y T I H V Q
 1371 UGU AAC GCG UCU AAA UUC CAC CAG GGU UGU UUG CUU GUG GUG UGU GUG CCU GAA GCU GAG AUG GGG UGC GCU ACA GUG GCG AAU GAG GUU
 C N A S K F H Q G C L L V V C V P E A E M G C A T V A N E V
 1461 AAC GCA GCC GCU CUC UCA UCU GGU GAA ACG GCA AAA CAU UUU GCA AAG ACU GGU GCG ACG GGA ACC CAC ACA GUG CAA AGC AUA GUG ACA
 N A A A L S S G E T A K H F A K T G A T G T H T V Q S I V T
 1551 AAC GCC GGC AUG GGG GUG GGC GUG GGC AAU CUG ACA AUA UUC CCA CAU CAG UGG AUA AAC CUC CGU ACC AAC AAC AGC GCG ACU AUU GUC
 N A G M G V G V G N L T I F P H Q W I N L R T N N S A T I V
 1641 AUG CCU UAC AUA AAC AGU GUG CCC AUG GAU AAC AUG UUC AGA CAC UAC AAU UUC ACC CUA AUG AUC AUC CCC UUC GUC CCA CUG GAC UUC
 M P Y I N S V P M D N M F R H Y N F T L M I I P F V P L D F
 1731 ACA GCU GAG GCA UCU ACG UAU GUA CCA AUC ACU GUA ACA GUA GCU CCC AUG UGC GCC GAG UAC AAC GGG CUA CGC CUG GCC UCG CAU CAA
 T A E A S T Y V P I T V T V A P M C A E Y N G L R L A S H Q
 VP2 ↑
 1821 GGU UUG CCG ACA AUG AAC ACC CCU GGU AGU AAC CAA UUC CUC ACA UCA GAU GAC UUU CAA UCA CCA UCA GCC AUG CCA CAA UUC GAU GUC
 G L P T M N T P G S N Q F L T S D D F Q S P S A M P Q F D V
 VP3
 1911 ACA CCC GAG CUC AGG AUU CCA GGG GAG GUA AAG AAC UUA AUG GAA AUA GCC GAA GUU GAC UCC GUA GUG CCU GUG AAC AAU ACA CAG GAC
 T P E L R I P G E V K N L M E I A E V D S V V P V N N T Q D
 2001 UCA GUG UAC AAC AUG GAU GUG UAC AAA AUU CCU GUU AGU GGA GGC AAC CAA CUG UCA ACU CAA GUC UUC GGG UUC CAA AUG CAA CCA GGG
 S V Y N M D V Y K I P V S G G N Q L S T Q V F G F Q M Q P G
 2091 CUA AAU AGU GUG UUC AAA AGG ACA UUA CUA GGU GAG AUA CUG AAC UAC UAU GCA CAC UGG UCU GGU AGU GUA AAG CUC ACC UUU GUG UUC
 L N S V F K R T L L G E I L N Y Y A H W S G S V K L T F V F
 2181 UGU GGA UCG GCC AUG GCA CUG GCC AAA UUU CUG CUG GCA UAU UCC CCU CCA GGC GCA GAC CCC CCA AAG UCU AGG AAA GAA GCA AUG CUU
 C G S A M A L A K F L L A Y S P P G A D P P K S R K E A M L
 2271 GGU ACU CAC GUG AUA UGG GAU AUA GGA CUU CAG UCA AGC UGC GUC UUG UGC GUA CCG UGG AUC AGC CAA ACA CAU UAU AGA UUG GUU CAG
 G T H V I W D I G L Q S S C V L C V P W I S Q T H Y R L V Q
 2361 CAG GAU GAA UAU ACG AGU GCA GGA UAU GUA ACU UGC UGG UAU CAA ACU AGU UUG GUU GUC CCG CCC GGC GCU CCC GCC ACG UGU GGA GUC
 Q D E Y T S A G Y V T C W Y Q T S L V V P P G A P A T C G V

FIG. 1. Complete nucleotide sequence of echovirus 12 genome. The first 15 nt (italics) have not been sequenced but are assumed from the highly conserved 5' end of enteroviruses sequenced so far. Cleavage sites of the viral capsid proteins determined by amino acid sequencing are indicated; the cleavage site between VP1 and P2 (in parentheses) has not been analyzed but is deduced from the coxsackievirus genome.

2451
 UUG UGC UUA GCA UCG GCG UGC AAU GAU UUC UCU GUG AGA AUG UUG AGA GAC ACA CCU UUU AUC GAG CAA AAA CAG CUA CUC CAA GGA GAU
 L C L A S A C N D F S V R M L R D T P F I E Q K Q L L Q G D
vp3 ↑ **vp1**

2541
 GUG GAG GAG GCC GUG AAC AGA GCC GUU GCA CGG GUG GCG GAU ACA CUA CCC ACA GGG CCG AGA AAU UCA GAG AGC AUC CCC GCA CUA ACA
 V E E A V N R A V A R V A D T L P T G P R N S E S I P A L T

2631
 GCA GCA GAA ACU GGG CAU ACA UCU CAG GUG GUU CCU GGG GAU ACC AUG CAA ACC CGU CAU GUG AAG AAU UAC CAC UCC AGA ACU GAA UCG
 A A E T G H T S Q V V P G D T M Q T R H V K N Y H S R T E S

2721
 UCU GUU GAA GAC UUC UUA UGC CGA GCA GCA UGU GUG UAU AUU ACA AAG UAC AAG ACC AAG GAC AGU GAU CCA GUG CAA AGG UAC GCC AAC
 S V E D F L C R A A C V Y I T K Y K T K D S D P V Q R Y A N

2811
 UGG CGG AUC AAC ACC CGU CAA AUG GUU CAG CUA AGG AGA AAA UUU GAG UUG UUU ACA UAC CUA AGG UUU GAU AUG GAA GUU ACU UUU GUG
 W R I N T R Q M V Q L R R K F E L F T Y L R F D M E V T F V

2901
 AUC ACC AGU UCA CAA GAU GAU GGC ACG CAG UUG GCG CAG GAC AUG CCA GUA UUA ACA CAC CAA GUC AUG UAU AUA CCA CCU GGA GGG CCA
 I T S S Q D D G T Q L A Q D M P V L T H Q V M Y I P P G G P

2991
 GUG CCG AAU AGU GUU ACU GAU UUU GCG UGG CAA UCG UCU ACC AAC CCU AGC AUC UUC UGG ACG GAG GGA AAC GCU CCA GCU AGG AUG UCC
 V P N S V T D F A W Q S S T N P S I F W T E G N A P A R M S

3081
 AUC CCC UUU AUC AGU AUA GGC AAC GCG UAU AGC AAU UUC UAU GAU GGU UGG UCA CAU UUC ACC CAA GAU GGA GUA UAU GGU UUC AAC UCC
 I P F I S I G N A Y S N F Y D G W S H F T Q D G V Y G F N S

3171
 CUC AAU AAC AUG GGC UCC AUC UAC AUU AGG CAU GUC AAU GAA CAA AGC CCG UAU GCA AUU ACG AGC ACA GUC AGA GUC UAU UUC AAA CCA
 L N N M G S I Y I R H V N E Q S P Y A I T S T V R V Y F K P

3261
 AAA CAC GUG CGG GCC UGG GUG CCA AGA CCA CCU AGG CUU UGU GCA UAU GAG AAA UCA AGC AAC GUG AAU UUC AAA CCA ACA GAU GUG ACU
 K H V R A W V P R P P R L C A Y E K S S N V N F K P T D V T

3351
 ACC UCC CGA ACA UCC AUC ACA GAA GUC CCU AGU CUU AGA CCA UCA GUG GUA AAU ACU GGA GCU UUC GGU CAG CAA UCA GGA GCG GCU UAU
 T S R T S I T E V P S L R P S V V N T G A F G Q Q S G A A Y
 (**vp1** ↑ **p2**)

3441
 GUG GGA AAC UAU AGA GUG GUU AAU AGA CAC CUA GCC ACU CAU GUU GAC UGG CAG AAC UGU GUG UGG GAG GAC UAU AAC AGG GAC CUC CUU
 V G N Y R V V N R H L A T H V D W Q N C V W E D Y N R D L L

3531
 GUA AGC ACC ACC ACA GCU CAU GGG UGU GAC ACC AUA GCC AGA UGC CAA UGC ACA ACA GGC GUG UAC UUC UGU GCA UCG AGG AAU AAA CAC
 V S T T T A H G C D T I A R C Q C T T G V Y F C A S R N K H

3621
 UAC CCA GUC AGU UUU GAA GGA CCA GGU CUG GUA GAA GUC CAG GAG AGC GAG UAC UAC CCC AGG AGG UAU CAA UCA CAC GUC UUG CUG GCU
 Y P V S F E G P G L V E V Q E S E Y Y P R R Y Q S H V L L A

3711
 GCG GGA UUU UCA GAG CCA GGA GAU UGU GGA GGG AUU CUC AGA UGU GAG CAC GGU GUC AUC GGU CUA GUU ACU AUG GGU GGC GAA GGC GUU
 A G F S E P G D C G G I L R C E H G V I G L V T M G G E G V

3801
 GUC GGA UUC GCU GAU GUA CGC GAC CUG UUA UGG CUU GAA GAU GAC GCC AUG GAA CAA GGA GUC AAG GAU UAC GUG GAA CAA CUA GGG AAC
 V G F A D V R D L L W L E D D A M E Q G V K D Y V E Q L G N

3891
 GCC UUU GGU UCA GGA UUC ACU AAC CAA AUC UGU GAG CAG GUC AAC CUC CUA AAA GAG UCA CUG GUG GGU CAC GAU UCC AUC CUA GAG AAG
 A F G S G F T N Q I C E Q V N L L K E S L V G H D S I L E K

3981
 UCC CUU AAA GCC CUA GUG AAA AUC AUA UCA GCA CUA GUG AUA GUG GUG AGG AAC CAU GAU GAU UUG AUC ACU GUA ACU GCU ACU CUU GCC
 S L K A L V K I I S A L V I V V R N H D D L I T V T A T L A

4071
 CUC AUU GGU UGU ACC UCC UCU CCA UGG CGG UGG CUC AAG CAC AAG GUG UCA CAA UAC UAC GGG AUA CCC AUG GCA GAG CGC CAA AGC AAC
 L I G C T S S P W R W L K H K V S Q Y Y G I P M A E R Q S N

4161
 GGG UGG CUC AAG AAG UUC ACA GAG AUG ACU AAC GCC UGC AAA GGG AUG GAA UGG AUU GCC AUC AAA AUC CAA AAG UUC AUA GAG UGG CUU
 G W L K K F T E M T N A C K G M E W I A I K I Q K F I E W L

4251
 AAA CUU AAG AUU UUA CCA GAA GUU AAG GAA AAA CAU GAA UUC CUG AAU AGG CUC AAG CAA CUC CCA CUG UUG GAG AGU CAA AUA GCA ACC
 K L K I L P E V K E K H E F L N R L K Q L P L L E S Q I A T

4341
 AUC GAG CAG AGU GCA CCG UCC CAG AGC GAU CAG GAG CAG CUC UUU UCA AAU GUU CAG UAC UUU GCC CAU UAU UGC AGA AAA UAU GCG CCC
 I E Q S A P S Q S D Q E Q L F S N V Q Y F A H Y C R K Y A P

4431
 CUA UAC GCA GCU GAG GCA AAA AGG GUG UUC UCU CUU GAA AAG AAA AUG AGU AAU UAC AUA CAG UUC AAG UCC AAA UGC CGU AUU GAA CCU
 L Y A A E A K R V F S L E K K M S N Y I O F K S K C R I E P

FIG. 1—Continued.

4521
GUA UGC UUG CUA CUA CAU GGG AGC CCC GGA GCA GGG AAA UCA GUU GCU ACC AGC CUC AUU GGG CGA UCA CUA GCU GAA AAA UUG AAU AGC
V C L L L H G S P G A G K S' V A T S L I G R S L A E K L N S

4611
UCA GUG UAC UCC UUA CCA CCA GAC CCA GAU CAC UUU GAU GGU UAC AAG CAA CAA GCU GUA GUG AUC AUG GAC GAU CUG UGC CAA AAU CCA
S V Y S L P P D P D H F D G Y K Q Q A V V I M D D L C Q N P

4701
GAU GGA AAA GAU GUG UCU UUG UUC UGU CAA AUG GUG UCU AGU GUA GAU UUU GUA CCA CCA AUG GCC GCG CUA GAG GAG AAA GGC AAU UUG
D G K D V S L F C Q M V S S V D F V P P M A A L E E K G I L

4791
UUC ACC UCU CCA UUC GUU CUA GCC UCA ACC AAU GCA GGA UCC AUC AAC GCA CCA ACA GUU UCG GAC AGC AGA GCC CUA GCU AGA AGG UUC
F T S P F V L A S T N A G S I N A P T V S D S R A L A R R F

4881
CAC UUU GAC AUG AAC AUU GAA GUU AUC UCC AUG UAC AGU CAA AAU GGA AAA AUA AAC AUG CCC AUG UCA GUC AAA ACA UGU GAU GAG GAG
H F D M N I E V I S M Y S Q N G K I N M P M S V K T C D E E

4971
UGC UGC CCA GUC AAU UUC AAG AGA UGC UGC CCA CUG GUG UGU GGC AAG GCU AUU CAG UUC AUU GAC AGA AGA ACC CAA GUC AGA UAU UCA
C C P V N F K R C C P L V C G K A I Q F I D R R T Q V R Y S

5061
CUG GAU AUG UUA GUC ACC GAG AUG UUC AGG GAG UAC AAC CAC AGG CAC AGU GUG GGC GCC ACC CUC GAG GCU CUG UUC CAA GGU CCA CCG
L D M L V T E M F R E Y N H R H S V G A T L E A L F Q G P P

5151
GUC AUC AGG GAG AUC AAG AUC AGU GUU GCU CCA GAA ACA CCU CCC CCA CCA GCA AUC GCU GAU UUA UUA AAA UCA GUA GAC AGU GAA GCU
V I R E I K I S V A P E T P P P P A I A D L L K S V D S E A

5241
GUG AGA GAG UAC UGC AAG GAA AAA GGC UGG CUU GUA CCG GAA GUU AAC UCC ACC CUA CAG AUU GAG AAG CAC GUC AGC AGA GCA UUU AUC
V R E Y C K E K G W L V P E V N S T L Q I E K H V S R A F I

5331
UGU CUA CAA GCU CUG ACC ACU UUC GUC UCA GUA GCU GGC AUA AUC UAC AUU AUC UAC AAG UUG UUU GCC GGC UUU CAG GGC GCG UAU ACG
C L Q A L T T F V S V A G I I Y I I Y K L F A G F Q G A Y T

5421
GGG AUG CCA AAU CAG AAA CCC AAA GUG CCC ACU CUG AGA CAA GCU AAA GUG CAG GGC CCA GCA UUC GAG UUC GCU GUG GCA AUG AUG AAA
G M P N Q K P K V P T L R Q A K V Q G P A F E F A V A M M K

5511
AGG AAC GCC AGC ACA GUG AAA ACG GAA UAU GGU GAG UUC ACC AUG CUC GGC AUC UAU GAC AGA UGG GCA GUG CUA CCA CAC CAC GCC AAG
R N A S T V K T E Y G E F T M L G I Y D R W A V L P H H A K

5601
CCU GGA CCG ACU AUU UUG AUG AAU GAU CAG GAG AUC GGC GUG UUA GAC GCC AAA GAA UUA GUG GAC AAA GAU GGG ACA AAU CUG GAG UUG
P G P T I L M N D Q E I G V L D A K E L V D K D G T N L E L

5691
ACC CUU UUA AAG CUC AAC CGC AAU GAG AAG UUU AGG GAC AUC AGA GGG UUU CUG GCG AGA GAG GAA GCU GAA GUG AAU GAG GCU GUU CUG
T L L K L N R N E K F R D I R G F L A R E E A E V N E A V L

5781
GCA AUA AAC ACA AGC AAG UUC CCA AAU AUG UAC AUA CCC GUA GGU CAA GUC ACU GAU UAC GGU UUC CUG AAC CUG GGG GGA ACA CCC ACA
A I N T S K F P N M Y I P V G Q V T D Y G F L N L G G T P T

5871
AAG AGG AUG CUC AUG UAC AAC UUU CCA ACU AGA GCA GGG CAA UGU GGU GGG GUA CUC AUG UCA ACA GGG AAA GUU CUU GGU AUA CAU GUA
K R M L M Y N F P T R A G Q C G G V L M S T G K V L G I H V

5961
GGA GGA AAU GGA CAU CAA GGG UUC UCC GCU GCU CUU CUU AGG CAC UAC UUU AAU GAG GAG CAA GGU GAA AUA GAG UUU AUU GAG AGC UCA
G G N G H Q G F S A A L L R H Y F N E E Q G E I E F I E S S

6051
AAG GAU GCA GGA UUC CCU GUG AUC AAC ACC CCC AGC AAA ACA AAA CUG GAA CCA AGC GUG UUU CAU CAG GUG UUU GAA GGC AAU AAA GAG
K D A G F P V I N T P S K T K L E P S V F H Q V F E G N K E

6141
CCA GCA GUC CUU AGA AAU GGU GAU CCG CGU CUU AAA GUA AAC UUU GAA GAA GCC AUA UUU UCC AAG UAC AUC GGG AAC AUC AAC ACA CAU
P A V L R N G D P R L K V N F E E A I F S K Y I G N I N T H

6231
GUG GAU GAA UAC AUG CUC GAA GCA GUG GAU CAC UAC GCA GGG CAA CUG GCU ACU CUU GAC AUU AGC ACU GAA CCC AUG AAA CUA GAA GAU
V D E Y M L E A V D H Y A G Q L A T L D I S T E P M K L E D

6321
GCA GUG UAU GGC ACU GAA GGA CUA GAG GCU CUU GAC UUA ACA ACA AGU GCA GGG UAC CCG UAU GUC GCC AUA GGC AUC AAA AAG AGA GAC
A V Y G T E G L E A L D L T T S A G Y P Y V A I G I K K R D

6411
AUC CUA UCC AAA AAG ACU AAA GAU CUG ACU AAA UUG AAG GAA UGC AUG GAC AAG UAC GGU UUG AAC CUG CCG AUG GUG ACU UAU GUG AAG
I L S K K T K D L T K L K E C M D K Y G L N L P M V T Y V K

6501
GAU GAA CUU AGA UCA UCA GAG AAA GUG GCU AAG GGA AAA UCA AGA CUC AUU GAA GCA UCU AGU UUA AAU GAC UCU GUC GCG AUG AGG CAG
D E L R S S E K V A K G K S R L I E A S S L N D S V A M R Q

FIG. 1—Continued.

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6591
ACG UUU GGC AAC UUG UAC AAA ACA UUC CAU CUG AAC CCG GGC AUU GUG ACA GGC AGU GCA GUU GGA UGU GAU CCG GAC CUU UUC UGG AGU
T F G N L Y K T F H L N P G I V T G S A V G C D P D L F W S

6681
AAA AUA CCC GUG AUG UUA GAC GGC CAC CUC AUA GCA UUC GAU UAC UCC GGA UAU GAU GCC AGC CUG AGC CCU GUG UGG UUC GCU UGU CUA
K I P V M L D G H L I A F D Y S G Y D A S L S P V W F A C L

6771
AAA CUG CUA CUC GAA AAA CUC GGG UAC ACG CAC AGA GAG ACA AAC UAC AUC GAC UAC CUA UGC AAC UCU CAC CAC CUG UAC AGA GAC AAA
K L L L E K L G Y T H R E T N Y I D Y L C N S H H L Y R D K

6861
CAC UAC UUU GUG CGU GGU GGC AUG CCA UCA GGG UGC UCC GGU ACC AGC AUU UUC AAU UCA AUG AUC AAU AAC AUC AUA AUC AGG ACA CUG
H Y F V R G G M P S G C S G T S I F N S M I N N I I I R T L

6951
AUG CUC AAA GUG UAC AAG GGA AUU GAC CUG GAC CAG UUU AGA AUG AUU GCA UAU GGU GAU GAU GUA AUU GCA UCC UAC CCU UGG CCU AUA
M L K V Y K G I D L D Q F R M I A Y G D D V I A S Y P W P I

7041
GAC GCU UCA CUA CUU GCU GAA GCU GGG AAG GGU UAU GGA CUG AUU AUG ACA CCA GCA GAC AAA GGG GAG UGC UUC AAU GAG GUU ACC UGG
D A S L L A E A G K G Y G L I M T P A D K G E C F N E V T W

7131
ACC AAU GUC ACC UUC CUA AAG AGG UAC UUU AGA GCU GAU GAG CAG UAU CCU UUC CUG GUU CAU CCA GUU AUG CCC AUG AAG GAC AUC CAU
T N V T F L K R Y F R A D E Q Y P F L V H P V M P M K D I H

7221
GAA UCU AUU AGG UGG ACA AAG GAU CCA AAG AAC ACU CAA GAC CAC GUU CGA UCG UUG UGC UUA UUG GCU UGG CAU AAC GGA GAA CAA GAA
E S I R W T K D P K N T Q D H V R S L C L L A W H N G E Q E

7311
UAU GAG GAA UUC AUC CGA AAG AUC AGA AGC GUC CCG GUU GGG CGC UGU CUG ACU CUC CCC GCG UUU UCA ACU UUA CGC AGA AAA UGG CUG
Y E E F I R K I R S V P V G R C L T L P A F S T L R R K W L

7320
GAU UCC UUU TAAATTAGAG ACAATTTGAA ATAATTTAAA TTGGCTTAAC CCTACTGTAC TAACCGAACT AGACAACGGT GCAGTAGGGG TAAATTCCTCC GCATTCGGTG
D S F
7420
CG
    
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FIG. 1—Continued.

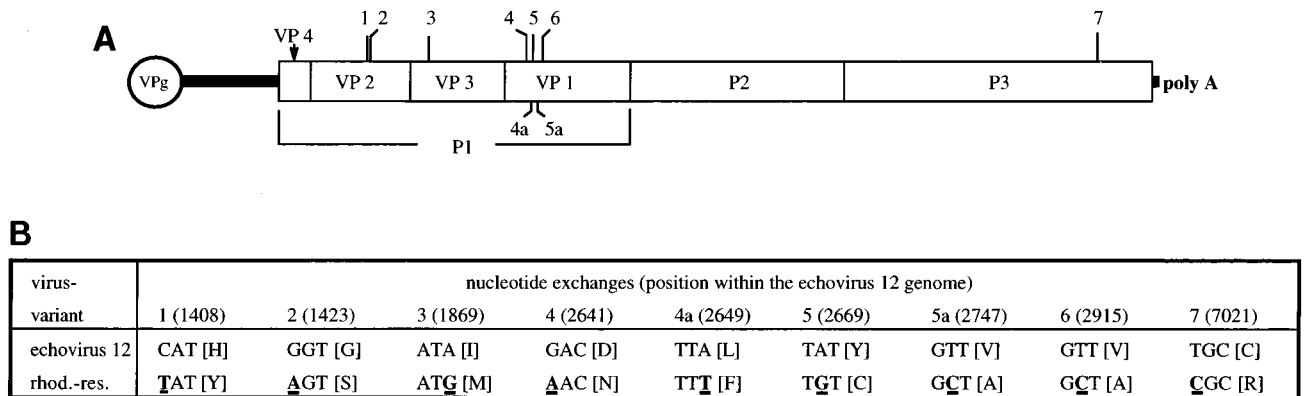


FIG. 2. Sequence deviations of wild-type echovirus 12 and the rhodanine-resistant variant. (A) Schematic drawing of the echovirus genome. The nucleotide exchanges between the wild type and rhodanine-resistant variant are indicated. The translated part of the viral genome is shown as an open bar; the boxes VP (virus protein) 1 to 4 represent the segments coding for the capsid proteins. (B) Nucleotide exchanges are numbered according to panel A, and the position numbers within the echovirus 12 genome are given in parentheses. The codons affected by the exchanges are given, together with the corresponding amino acid (in brackets). The changed bases are underlined.

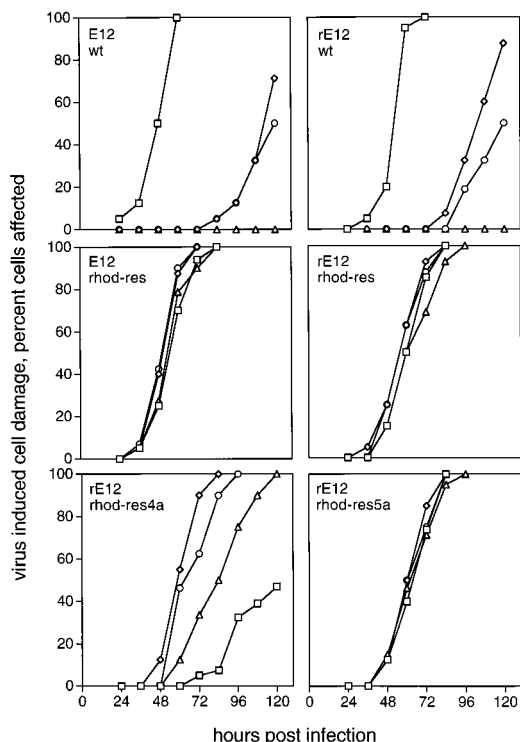


FIG. 3. Rhodanine sensitivity assay (4) of the original viruses (E12-wt and E12-res) and the recombinant ones (rE12-wt, rE12rhod-res, rE12rhod-res4a, and rE12rhod-res5a). Recombinant virus rE12-wt was derived from the plasmid pT7E12-wt, rE12rhod-res was derived from pT7E12rhod-res, rE12rhod-res4a was derived from pT7E12rhod-res4a, and rE12rhod-res5a was derived from pT7E12rhod-res5a (see the text). Cells were infected with 100 50% tissue culture infective doses of the above mentioned viruses in the presence of 0 (□), 50 (◇), 100 (○), and 150 (△) μg of rhodanine per ml. Every 12 h, the percentage of cells exhibiting cytopathic effects was determined.

pyridazinamine derivative R 61837 bind to a hydrophobic pocket located at the base of the canyon within the β -barrel (WIN pocket) of VP1 of rhinoviruses (1, 5).

We are currently investigating in detail which of the seven amino acid differences between echovirus 12, prototype strain Travis, and the rhodanine-resistant variant are responsible for the altered features of the two viruses.

Nucleotide sequence accession numbers. The nucleotide sequences of the wild-type echovirus 12 and the rhodanine-resistant variant have been assigned EBI Data Library accession no. X79047 and X77708, respectively.

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