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Isolation and characterization of an arildone-resistant poliovirus 2 mutant with an altered capsid protein VP1

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Summary

Arildone (4-[6-(2-chloro-4-methoxyphenoxy)hexyl] -3,5-heptanedione is a selective inhibitor of poliovirus uncoating. We have isolated an arildone-resistant poliovirus 2 variant and characterized it. In single cycle growth curve experiments the resistant virus was not influenced by 1 μg/ml of arildone, which completely inhibited the replication of the sensitive virus. In neutralization experiments the rates of inactivation vs time of the parent and the resistant virus proved indistinguishable. The adsorption behavior of the mutant closely resembled that of the wild-type.

Uncoating as measured with neutral red-sensitized arildone-resistant mutant virus was unaffected by arildone, in contrast to the uncoating of the sensitive parent virus. It has been previously shown that arildone stabilizes the poliovirus capsid from alkaline degradation. The arildone-resistant mutant, however, was not markedly stabilized by the drug under these conditions. No difference in thermostability between mutant and parent virus could be detected. In isoelectric focusing the sensitive virus exhibited 2 peaks, but in the presence of arildone only one peak was apparent. In contrast, the arildone-resistant virus remained unaffected by the presence of arildone. Analysis of the capsid proteins of the sensitive parent and the resistant virus in SDS-polyacrylamide gels revealed no difference in the patterns; however, peptide mapping showed clear differences in VP1.

Arildone; Poliovirus; Uncoating; Arildone-resistant mutant; Alkaline degradation

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Introduction

Arildone (4-[6-(2-chloro-4-methoxyphenoxy)hexyl] -3,5 heptanedione) (Diana et al., 1977) has been shown to selectively inhibit poliovirus replication in cell culture by preventing virion uncoating (McSharry et al., 1979). The drug also prevents physical and biological alterations of the virus particle induced by alkaline pH or heat in vitro (McSharry et al., 1979; Caliguiri et al., 1980). Thus, it is believed to interact directly with the viral capsid (for a review see McSharry and Pancic, 1982). Mutants resistant to the action of arildone and mutants requiring the presence of the drug for replication have been isolated (Schrom et al., 1982). These mutants, however, are stabilized by arildone against heat inactivation to the same degree as is the parental drug-sensitive virus. This suggests that the drug, although not inhibiting infectivity, still interacts with the arildone-resistant variants to increase their thermal stability. Consequently, stabilization of the drug-sensitive parent virus per se may not be sufficient to explain inhibition of viral replication. No differences in the capsid proteins of the mutants could be detected by SDS-polyacrylamide gel electrophoresis and by *Staphylococcus aureus* V8 finger printing.

In this communication we describe the isolation and characterization of a different type of arildone-resistant poliovirus mutant. This variant is not stabilized by arildone against alkaline degradation to the same extent as is the arildone-sensitive parent virus. Furthermore, we show directly that cell-mediated uncoating of the mutant virus takes place in the presence of arildone. Comparison of the virion particles of arildone-resistant and -sensitive poliovirus by isoelectric focusing revealed characteristic differences. By fingerprinting analysis using *S. aureus* V8 protease we could demonstrate that these differences correlate with a mutational change in capsid protein VP1.

Materials and Methods

Virus and cells

Poliovirus type 2 (P712-ch-2ab) was originally supplied by A.B. Sabin (Eggers and Tamm, 1961). The GMK cell line, a continuous line derived from African green monkey kidney cells, was kindly given to us by H. Lennartz (Hamburg, F.R.G.). Virus stocks were prepared and quantitated by plaque assay in GMK cell monolayers according to procedures described previously (Caliguiri et al., 1965; Bablanian et al., 1965; Rosenwirth and Eggers, 1978a).

Chemicals and other materials

Most of the chemicals were described before (Eggers 1977; Rosenwirth and Eggers, 1978a,b). Arildone was a gift of Sterling-Winthrop Research Institute, Rensselaer, New York; it was dissolved in dimethyl sulfoxide (DMSO) and then diluted in minimum essential medium (MEM).

Methods

Growth curve experiments were carried out at 37°C in a constant temperature room as described previously (Rosenwirth and Eggers, 1978a).

Neutral red-sensitized virus was prepared, handled, assayed and irradiated following Kato and Eggers (1969) and Eggers and Waidner (1970).

[^{35}S]Methionine-labeled poliovirus 2 particles were prepared and purified by isopycnic centrifugation in CsCl three times as described in detail for echovirus 12 (Rosenwirth and Eggers, 1978a).

Isoelectric focusing of virus particles was performed essentially as outlined by Mandel (1971) and as described in detail for echovirus 9 (Rosenwirth and Eggers, 1982).

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using the discontinuous buffer system described by Laemmli (1970). Peptide mapping of viral proteins was carried out according to Cleveland et al. (1977) and as described in detail for echovirus 9 (Rosenwirth and Eggers, 1982). Additional details are given in the legends to the Figures.

Results

Isolation of arildone-resistant mutants

Poliovirus type 2 (P712-ch-2ab) replication in GMK cell monolayers is selectively inhibited by arildone at a concentration of 1 μg/ml. Under plaque assay conditions typically 10^2- to 10^3-fold lower titers than in the control are observed when the overlay contains 1 μg/ml of arildone. Several plaques grown in the presence and in the absence of arildone were isolated, plaque purified, and virus stocks were prepared in the presence and absence of the drug, respectively. The variants obtained were titrated by plaque assay in the presence or absence of 1 μg/ml of arildone. A mutant stock (A^{res}) showing a titer of 1.2×10^8 plaque forming units (pfu) per ml in the presence of arildone as compared to 1.5×10^8 pfu/ml in the absence of the drug was selected for further study. A control stock (A^{scns}) having titers of 3.5×10^8 pfu/ml in the absence and 1.0×10^6 pfu/ml in the presence of arildone was used for comparison.

Single cycle growth in the absence and presence of arildone

GMK cell monolayers were infected with a multiplicity of infection (20 pfu/cell) sufficient to ensure synchronous replication of the virus. Under these conditions typical single cycle growth curves are obtained (Fig. 1). Obviously, replication of the wildtype virus is completely inhibited in the presence of 1 μg/ml of arildone. A resistant subpopulation cannot grow to high enough titers within the 8 h of the experiment to become measurable against the background of the inoculum virus. In contrast to the sensitive virus, the growth curves of the arildone-resistant mutant are nearly identical in the absence or presence of arildone.

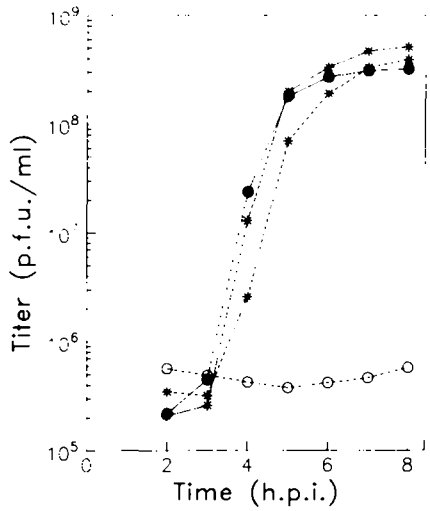

Fig. 1. Time course of mutant and wildtype poliovirus 2 replication in the absence and presence of arildone. Confluent GMK cell monolayers were inoculated with virus at a multiplicity of 20 pfu/cell in the absence or presence of 1 μg/ml arildone. The drug was present from 0.5 h before infection on throughout the experiment. After adsorption the inoculum was removed and cell culture medium with or without arildone was added. Aliquots of the infected cultures were harvested at different times post infection. Lysates were prepared and quantitated by plaque assay in the absence of arildone. (●—●) A^{sens} virus, without arildone; (○---○) A^{sens} virus, with 1 μg/ml arildone; (×—×) A^{res} virus, without arildone; (×---×) A^{res} virus, with 1 μg/ml arildone.

Genetic stability of arildone resistance

The arildone-resistant mutant was passaged five times in the presence of absence of 1 μg/ml of arildone. In parallel, the control stock was passaged in the absence of arildone. The virus lysates thus obtained were compared with their parent stocks by plaque assay in the presence or absence of arildone (Table 1). The results clearly demonstrate the genetic stability of the variant A^{res} in the presence or absence of the selecting drug, as well as the stability of the sensitive phenotype.

Neutralization kinetics

There was no detectable difference between the neutralization kinetics of the arildone-resistant mutant and the -sensitive virus, when an antiserum against poliovirus wildtype was used (Fig. 2). Thus, a mutation altering arildone sensitivity and presumably leading to changes in capsid protein, does not necessarily affect the antigenic determinants recognized by neutralizing antibodies.

Adsorption of viral particles of GMK cells

GMK cells were exposed to [^{35}S]methionine-labeled virus particles at 20°C in the absence or presence of arildone. After various times the amount of non-attached virus was determined by measuring the radioactivity in the supernatant (Fig. 3). The differences between the radioactivity initially present and the values after var-

TABLE 1

Genetic stability of arildone-resistance*.

Virus stock	Plaque formation under overlay containing:	
	No arildone	1 μg/ml arildone
A^{sens}, no passage	4.3×10^7	1.3×10^5
A^{sens}, 5th passage without arildone	3.5×10^7	9.7×10^4
A^{res}, no passage	5.3×10^7	5.4×10^7
A^{res}, 5th passage without arildone	4.8×10^7	3.9×10^7
A^{res}, 5th passage with arildone	5.4×10^7	4.9×10^7

*Virus stocks were diluted 1:4 and inoculated onto GMK cell monolayers in the absence or in the presence of 1 μg/ml arildone. At 8 h post infection the cells were harvested and lysates were prepared by three cycles of freezing and thawing. The procedure was repeated five times, and the plaque forming capacity of the lysates was measured in the absence and presence of arildone.

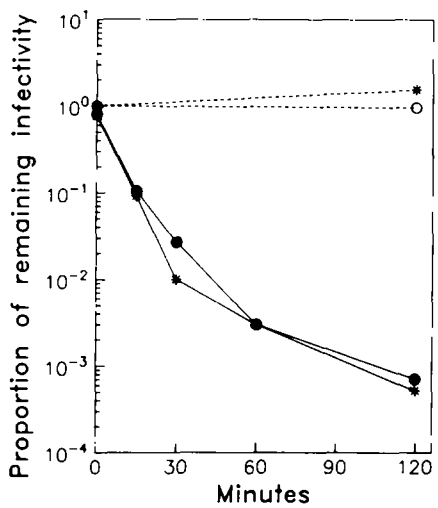

Fig. 2. Neutralization kinetics of mutant and wildtype poliovirus 2.Antiserum against wildtype poliovirus 2 (P712-ch-2ab) was prepared in rabbits. A 1:10 dilution of virus was diluted further 1:10 in diluent (PBS with 1% fetal calf serum) or in antiserum diluted 1:1600. Virus-diluent or virus-serum mixtures were held in ice. Aliquots were removed after various times, diluted further immediately and inoculated onto GMK cell monolayers to determine the non-neutralized fraction by plaque assay. The values obtained are expressed relative to the control (= diluent) at time 0. (•—•) A^{sens} virus plus antiserum; (o---o) A^{sens} virus plus diluent; (×—×) A^{res} virus plus antiserum; (×---×) A^{res} virus plus diluent.

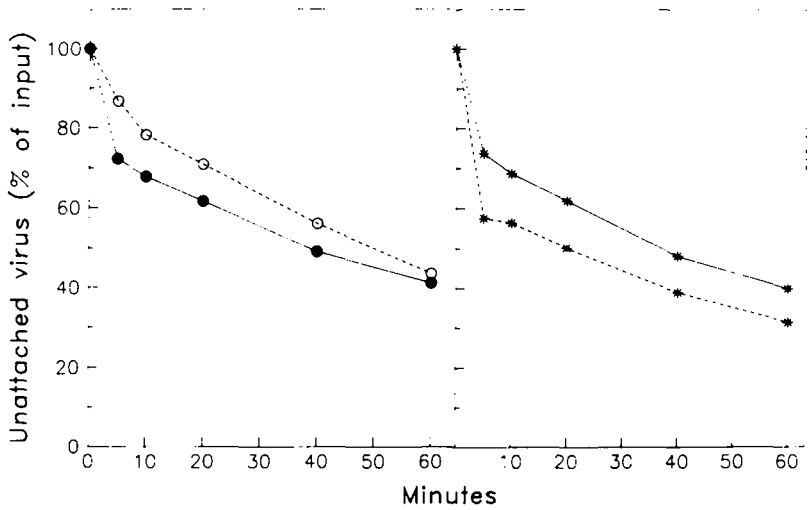

Fig. 3. Adsorption of mutant and wildtype poliovirus 2 to GMK cells in the absence and presence of arildone. Confluent GMK cell monolayers in 50 mm plastic Petri dishes were inoculated at a multiplicity of 100 pfu/cell with 0.4 ml of [^{35}S]methionine-labeled virus particles in MEM without or with 1 μg/ml arildone. After various times of adsorption at 20°C aliquots of 20 μl were removed from the supernatant and assayed for acid-precipitable radioactivity. The values obtained are expressed relative to the amount of radioactivity initially present in the inoculum. (●—●) A^{sens} virus, without arildone; (○---○) A^{sens} virus, with 1 μg/ml arildone; (×—×) A^{res} virus, without arildone, (×---×) A^{res} virus, with 1 μg/ml arildone.

ious times of interaction with the cells were considered to represent attached virus. The adsorption behavior of A^{res} and A^{sens} virus in the absence of arildone was indistinguishable. However, in the presence of arildone, the A^{sens} virus adsorbs slightly less efficiently, while the A^{res} mutant seems to adsorb to the cells even better.

Uncoating of mutant and wildtype poliovirus

In view of the inhibition of uncoating of wildtype poliovirus by arildone we wanted to investigate the uncoating behavior of A^{res} poliovirus directly. As an indicator of uncoating we chose the development of light resistance of neutral red-sensitized virus in infected cells (Eggers and Waidner, 1970; Eggers, 1977).

It can be seen in Table 2 that, in contrast to A^{sens} wildtype virus, uncoating of A^{res} mutant virus is not affected at all by arildone. This experiment also yields quantitatively more precise data on resistance than, e.g., a growth curve, provided uncoating is considered the main site of action of arildone.

Alkaline degradation of mutant and wildtype poliovirus

We have shown that arildone may stabilize the wildtype polio virion against alkaline degradation (see McSharry et al., 1979), as has been demonstrated previously with the inhibitor of uncoating of echovirus 12, rhodanine (Eggers, 1977).

TABLE 2

Uncoating of neutral red-sensitized mutant and wildtype virus*.

Time at 37°C (h)	Irradiation	Infective centers per plate			
		A^{sens}		A^{res}	
		untreated	arildone	untreated	arildone
0	−	2.6×10^{4}	2.5×10^{4}	5.4×10^{4}	3.7×10^{4}
	+	$<0.3\times10^{0}$	$<0.3\times10^{0}$	$<0.3\times10^{0}$	$<0.3\times10^{0}$
3	−	6.1×10^{4}	2.1×10^{4}	4.1×10^{4}	3.3×10^{4}
	+	7.1×10^{4}	5.5×10^{2}	4.6×10^{4}	3.2×10^{4}

*Neutral red wildtype and mutant poliovirus were adsorbed to GMK cells at 0°C for 1 h in the dark in the absence and presence of 1 μg/ml arildone. Some cultures were transferred to 37°C in the dark for 3 h.

In Fig. 4 a similar experiment is shown with A^{res} mutant poliovirus. In contrast to the situation with wildtype virus, arildone protects A^{res} poliovirus at best only partially against alkaline degradation. In a further experiment (data not shown) no protection whatsoever was observed.

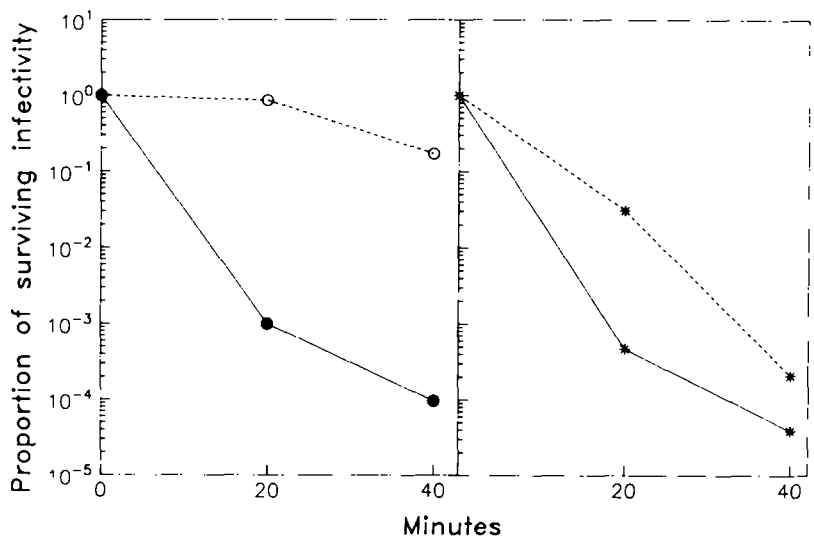

Fig. 4. Alkaline inactivation of mutant and wildtype poliovirus 2 in the absence and presence of arildone. Poliovirus 2 was incubated at 40°C in 0.1 M borate-NaCl buffer, pH 10.5, in the presence or absence of 1 μg/ml arildone. Aliquots were removed after 20 and 40 min, diluted immediately further in MEM and inoculated onto GMK cell monolayers to determine the remaining infectivity by plaque assay. The values obtained are expressed relative to the initial infectivity. (●—●) A^{sens} virus, without arildone; (○---○) A^{sens} virus, with 1 μg/ml arildone; (×—×) A^{res} virus, without arildone; (×---×) A^{res} virus, with 1 μg/ml arildone.

TABLE 3

Heat stability of mutant and wildtype infectivity[a].

Time at 44°C (min)	Proportion of surviving infectivity	
	A^{sens}	A^{res}
0	1.0	1.0
2	0.72	0.79
5	0.22	0.39
15	0.029	0.069
30	0.0048	0.013
60	0.0015	0.0019

[a]Virus stocks were diluted 1:100 in PBS prewarmed to 44°C in a waterbath. Aliquots were removed after various times and cooled immediately in ice. As a control virus was diluted 1:100 in ice-cold PBS and kept for 60 min in ice.
The infectivity of the samples was determined by plaque assay and was expressed relative to the respective control.

Heat stability of mutant and wildtype virus particles

In a previous publication (Schrom et al., 1982) an arildone-dependent mutant of poliovirus has been reported to exhibit increased thermolability in the absence and presence of the drug. We compared the thermal stability of infectivity of our A^{res} mutant to that of the wildtype (Table 3) in the absence of arildone and found no detectable difference. Obviously, the mutation leading to arildone resistance does not influence the thermostability of the virion particle in vitro.

Isoelectric focusing of mutant and wildtype virus particles

Different conformational states of the capsid surface proteins may be reflected by various isoelectric points of the particles (Mandel, 1971, 1976). Purified, radioactively labeled virions of wildtype and arildone-resistant poliovirus 2 were analyzed by isoelectric focusing in the absence and presence of arildone (Fig. 5).

While poliovirus 2 wildtype particles show a biphasic distribution in the pH gradient under our experimental conditions, namely they seem to exist in two conformational states with isoelectric points at pH 6.85 and 5.2, its arildone-resistant mutant bands relatively homogeneously at pH 4.8–4.4. However, if arildone is present in the ampholine solutions, poliovirus wildtype particles, obviously, are stabilized in a conformation with an acidic IEP, pH 4.4. In contrast, arildone-resistant particles do not undergo marked changes in their banding behavior. Thus, the experiments support the assumption, that arildone binds to the wildtype particles, and stabilizes them in a certain conformation in which they, according to the results described above, cannot be uncoated or degraded by alkaline pH. In contrast, the conformation of arildone-resistant particles is not influenced by arildone to the same extent as wildtype virus; possibly, the drug does not significantly bind to these particles.

Analysis of the protein composition of mutant and wildtype poliovirus particles

Analysis of the capsid proteins of the mutant viral particles by SDS-PAGE did

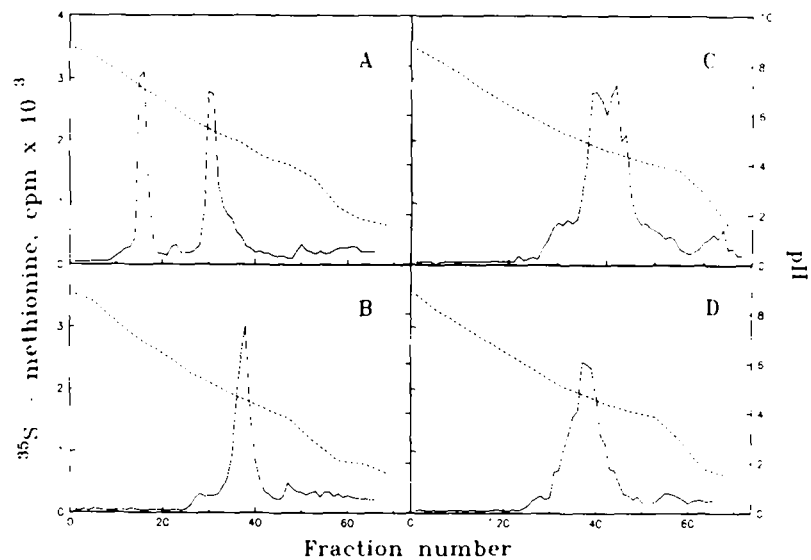

Fig. 5. Isoelectric focusing of poliovirus particles. Purified, [^{35}S]methionine-labeled virus particles were diluted into 1 ml of phosphate-buffered saline, and dialyzed for 1 h against this buffer. A 100 ml sucrose gradient was formed in the LKB 8100-1 column onto the cathode solution (0.25 M NaOH in 60% sucrose) from an ampholyte solution containing 3.6% Ampholine Carrier Ampholytes, pH 3.5–10, in a 50% sucrose solution, and a solution containing 1.2% Ampholytes in 5% sucrose. The anode solution (0.15 M H_3PO_4) was layered on top, and a pH gradient was generated by electrofocusing with a constant voltage of 400 V for 5 h at 10°C. Dry sucrose was added to the dialyzed sample to achieve the buoyant density of the preformed pH gradient at pH 4.5–5.0. The sample was applied by introducing a thin tubing through the upper nipple to the appropriate position where the pH value was 4.5–5.0. Electrofocusing was continued with a constant voltage of 400 V for 20 h at 10°C. The content of the column was collected from the bottom, and the fractions were analyzed for pH and acid-precipitable radioactivity. The values given in the figures represent only the upper two thirds of the column. No radioactivity was ever detected in the lower third. A: A^{sens} virus, without arildone; B: A^{sens} virus, with 1 μg/ml arildone; C: A^{res} virus, without arildone; D: A^{res} virus, with 1 μg/ml arildone; [^{35}S]methionine, cpm × 10^{-3}; ---, pH.

not show any differences in band pattern to the parent wildtype strain. Therefore, a more detailed comparison of the individual capsid proteins of mutant and wildtype viral particles was performed. The three largest capsid proteins were subjected to one-dimensional peptide mapping by limited proteolysis in the presence of sodium dodecyl sulfate as described by Cleveland et al. (1977) (Fig. 6).

Several differences were detected between the A^{res} mutant and the wildtype virus after SDS-PAGE of the proteolytic digest of VP1 (see arrows): a) This band migrated faster in the A^{res} than in the A^{sens} VP1 digest; b) this band migrated slower in the A^{res} than in the wildtype digest; c) an additional band was visible in the A^{res} digest; d) a band was missing in the A^{res} VP1 digest. No differences were detected in VP2 or VP3. Thus, the fingerprinting analysis indicates that the mutation leading to A^{res} may be located in the gene coding for VP1.

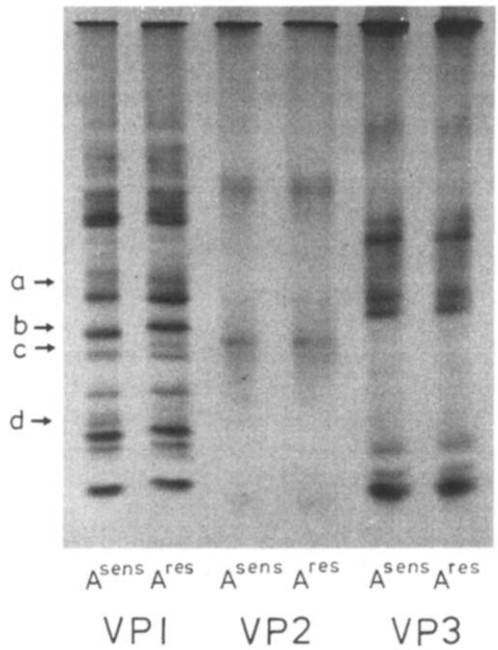

Fig. 6. SDS-polyacrylamide gel autoradiogram of peptides generated by *Staphylococcus aureus* V8 proteolysis of [^{35}S]methionine-labeled poliovirus 2 capsid proteins. [^{35}S]methionine-labeled capsid proteins were separated by SDS-PAGE as before (Rosenwirth and Eggers, 1978a). The visible bands were cut out from the stained gel, were equilibrated for 1 h at room temperature in 0.125 M Tris-HCl, pH 6.8, 0.1% SDS, 1 mM EDTA, and were placed in the slots of an SDS-polyacrylamide slab gel. 5 µl of the equilibration buffer containing 20% glycerol were added to fill the spaces beside the gel pieces, followed by 10 µl *S. aureus* V8 protease (25 µg/ml in equilibration buffer with 10% glycerol). The slots were filled with electrophoresis buffer, and electrophoresis was performed at a current of 20 mA until the tracking dye had almost passed the stacking gel. Then, the current was shut off for 1/2 h to allow proteolytic digestion of the viral proteins in the stacking gel. The cleavage products were separated by subsequent electrophoresis at 25 mA. The separating gel contained 17.5% acrylamide and 0.47% bisacrylamide. The slab gel was stained, dried and analyzed by autoradiography.

Discussion

Arildone has been shown to block uncoating of poliovirus (McSharry et al., 1979) and to stabilize the virion in vitro against alkaline pH- or heat-induced alterations (McSharry et al., 1979; Caliguiri et al., 1980). These results suggested that arildone may interact with the viral protein capsid to stabilize the particle.

In this communication we describe the isolation of an arildone-resistant mutant which is not or at best partially stabilized against alkaline degradation by the drug. Cell-mediated uncoating of this mutant takes place in the presence of arildone as is shown directly and quantitatively by using neutral red-sensitized virus (Eggers, 1977). The isoelectric point (IEP) of wild-type virus particles is clearly influenced by arildone: in the absence of the drug wildtype particles show a biphasic distribution in the pH gradient whereas in the presence of arildone the particles are sta-

bilized in one conformation, namely the one with an acidic IEP. The arildone-resistant virus particles, however, do not considerably change their banding behavior and show a similar distribution in the pH gradient with or without arildone, which is different from that of wildtype. These results are compatible with the assumption that arildone does not bind or does bind in an altered way to our resistant variant. The behavior of wildtype particles in isoelectric focusing indicates that a major conformational change is induced by arildone in at least those particles with an IEP around neutrality. The particles, which are stabilized in the acidic conformation – according to the results described above – cannot be uncoated or degraded by alkaline pH. Arildone-resistant particles exhibiting a similar IEP as the arildone-stabilized wildtype particles, on the other hand, are being uncoated. Consequently, it may not be the conformation with an acidic IEP per se, but the bound arildone, which inhibits uncoating; the stabilization of the wildtype particles in this conformation can possibly be considered as an additional effect of arildone. However, the similarity in the IEPs of arildone-stabilized wildtype particles and arildone-resistant particles by no means proves that the conformations are identical.

No difference was detected between the A^{res} and A^{sens} strains in neutralization kinetics and adsorption behaviour. The A^{res} virus particles were also indistinguishable from wildtype in thermostability. Thus, the mutation leading to arildone resistance and to changes in capsid protein, did not alter the antigenic determinants recognized by neutralizing antibodies as measured with our polyclonal antiserum in a one-way neutralization test. Nor did it affect the structures interacting with the cellular receptor. Nor did it cause an increased heat-lability of the capsid.

By fingerprinting analysis we obtained evidence that the arildone-resistant poliovirus contained mutational changes in VP1. Thus, it may be assumed that arildone interacts with epitopes on the capsid formed mainly by sequences of VP1. Recently, compounds being structurally related to arildone were described as inhibitors of rhinoviruses and related picornaviruses (Diana et al., 1985; Otto et al., 1985). Two of these compounds, WIN 51711 and WIN 52084, have been bound to crystals of human rhinovirus type 14 and their interaction with the viral particle was analyzed at atomic resolution (Smith et al., 1986). The compounds consist of a 3-methylisoxazole group which inserts itself into an hydrophobic interior of the VP1 β-barrel, a connecting seven membered aliphatic chain, and a 4-oxazolinylphenoxy group that covers the entrance to an ion channel in the floor of the 'canyon' (Rossmann et al., 1985). These compounds have the same mode of action as arildone, i.e. they inhibit uncoating (Fox et al., 1986), and since the three dimensional structure of poliovirus (Hogle et al., 1985) was found to be very similar to that of human rhinovirus 14 (Rossmann et al., 1985), it is reasonable to hypothesize that arildone may also interact with analogous regions of the VP1 β-barrel of poliovirus. Our finding that an arildone-resistant mutant differs from its parent virus in VP1 thus is consistent with the hypothesis that uncoating in some way may be initiated by conformational changes in VP1.

Mutants being resistant to or dependent on arildone have been isolated independently by Schrom et al. (1982). However, the experimental results of these investigators indicated that arildone still interacts with the variant virus particles; no

difference in capsid proteins as compared to wildtype could be detected. Treatment of poliovirus by heat or alkali results in an irreversible alteration of the virion (Breindl, 1971). It has been suggested that these alterations represent an analogue of the uncoating process in vivo (Joklik and Darnell, 1961; Holland, 1962; Fenwick and Cooper, 1962; Mandel, 1965; Eggers, 1977). The existence of two different types of arildone-resistant mutants, those which are stabilized by arildone to the same extent as wildtype (Schrom et al., 1982) and the one we describe here, which is only marginally stabilized by the drug, demonstrates that uncoating in vivo and degradation in vitro must not involve the same mechanisms. Obviously, interaction of the drug with the resistant virus particle can occur, and this can stabilize the virion, but this interaction per se is not sufficient to inhibit uncoating. The fact that Schrom et al. (1982) did not find any differences in capsid protein does not exclude the existence of more subtle mutational changes in their mutants; our result, on the contrary, demonstrates that an altered VP1 is associated with arildone resistance.

We have reported previously that the antiviral drug rhodanine also interacts with the viral capsid and thus prevents echovirus type 12 uncoating in vivo and modification of the virion in vitro (Eggers, 1977; Rosenwirth and Eggers, 1979). Though rhodanine and arildone are chemically unrelated they inhibit picornaviruses by the same mechanism, namely by preventing uncoating. It remains to be elucidated if rhodanine, too, interacts with VP1. To approach this question we have isolated rhodanine-resistant and -dependent mutants and are presently characterizing them.

In conclusion, the isolation of an arildone-resistant mutant which is not stabilized against alkaline degradation by the drug, shows an altered behavior in isoelectric focusing and contains a mutational change in VP1, strongly suggests that arildone interacts directly with the capsid protein, probably with VP1, of arildone-sensitive wildtype virus and thus stabilizes the particle against cell-mediated uncoating and in vitro degradation.

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