# Mutations in the hydrophobic domain of poliovirus protein 3AB abrogate its permeabilizing activity

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**Abstract** **Poliovirus protein 3AB contains a predicted amphipathic helix that could lead to pore formation in membranes. We have introduced various mutations in the hydrophobic domain of the protein and the membrane-modifying properties of the resulting mutants have been analyzed. Expression of wild type 3AB protein in *E. coli* increases the influx and efflux of different molecules such as nucleosides, lactose analogues and antibiotics. Thus, 3AB expression makes *E. coli* cells two orders of magnitude more sensitive to hygromycin B, a non-permeant inhibitor of translation, and causes a 15–20-fold enhancement in the efflux of uridine. Changes in membrane permeability take place under conditions where no cellular lysis is detected and when other molecules such as β-galactosidase or polyribonucleotides are kept inside the cell. These membrane modifications can be blocked to different extents by amino acid substitutions in the membrane-spanning region of the protein. These results suggest that poliovirus protein 3AB could possess an intrinsic ability to form pores in natural membranes, thus allowing the flux of small hydrophylic molecules through them.**

*Key words:* Poliovirus; Membrane permeability; Pore-forming protein

## 1. Introduction

Cytolytic animal viruses induce profound modifications in the morphology, as well as in the metabolism of infected cells [1]. The molecular basis of these changes, collectively referred to as the cytopathic effect, remains largely undetermined. Poliovirus, a member of the picornavirus group, is highly cytolytic for host cells [2]. Growth of poliovirus in susceptible cells leads to drastic changes in membrane permeability [3–5]. In summary, the progressive membrane damage induces a collapse of the ionic gradients and loss of membrane potential in poliovirus-infected cells [6]. Not only ions, but also other charged molecules traverse the cell membrane from about the third hour after infection [1,7]. The phenomenology of these changes are similar to those observed with membrane-active toxins [8–9], or ionophore compounds [10–11], and may reflect the action of a viral protein with pore-forming activity, the so-called 'viroporins' or proteins with membrane damaging properties [6]. Despite the importance of characterizing the viral products involved in cell lysis and their mode of action, no genetic analyses are available mapping this property, either in picornaviruses, or any other animal virus [1,12].

Human immunodeficiency virus (HIV) is perhaps the virus for which the elucidation of viral proteins toxic for the infected cells is most advanced [13–14]. The mechanisms underlying HIV cytopathicity are thought to be related to the modification of membrane permeability [14–16]. Similarities between some HIV peptides and membrane-active toxins have already been noticed, and some of these HIV peptides possess a lytic activity in both prokaryotic and eukaryotic cells [14,17]. The transmembrane domain of gp41 is involved in virus-induced cytopathogenicity [13,18]. This region of the glycoprotein may form an amphipathic helix that could theoretically aggregate to form a membrane pore [19].

Another viral protein with capacity to modify membrane permeability is influenza M2 polypeptide [20]. Elegant analyses with influenza M2 indicate that this protein has ion-channel activity, although this property has not been linked to the virus lytic potential.

The cloning and individual expression of poliovirus non-structural proteins in *E. coli* led to the identification of two toxic polypeptides, namely 2B and 3A/3AB [21]. Induction of synthesis of any of these polypeptides permeabilized prokaryotic cells, whereas none of the other poliovirus non-structural products presented this effect [21]. Polypeptide 3A, or its precursor 3AB, are known to interact with cellular membranes in infected human cells [22–23]. In addition, 3A possesses a hydrophobic stretch of amino acids (aa 59–80), that may form an amphipathic helix as predicted by computer analysis [5].

The fact that 3A (and 3AB) was highly toxic for *E. coli* cells was interpreted as reflecting the intrinsic lytic potential of this protein [21]. However, we did not discount the possibility that the simple accumulation of a protein with affinity for membranes leads to an unspecific disagregation of the lipid bilayer. In contrast with this idea the possibility existed that 3A was a membrane protein involved in cell lysis and this pore-forming capacity was dependent on its structure [5,21]. Since membrane-active proteins act on all types of cells, irrespective of whether they are eukaryotic or prokaryotic [24], the expression and analysis of their mode of action in *E. coli* cells constitutes a powerful model system for this type of studies. Thus, we have carried out a mutagenesis study on the 3AB gene with the purpose of analyzing its effect on membrane function. Our results indicate that the modification of membrane permeability is an intrinsic characteristic of protein 3AB.

## 2. Materials and methods

### 2.1. Bacterial strains and plasmids

Cloning and expression of the 3AB gene was carried out in BL21(DE3) cells [25]. All 3AB genes were cloned into plasmid pET11B.

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*Abbreviations:* ONPG, *o*-nitrophenyl-β-D-galactopyranoside; IPTG, isopropyl-1-thio-β-D-galactopyranoside.


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This plasmid has a *lac* operator downstream of the T7 promoter to control the basal level of expression [26]. After addition of IPTG, cellular RNA and protein synthesis can be blocked by rifampicin without affecting expression of the recombinant gene. Some of the experiments were carried out with bacteria BL21(DE3)pLysS. These bacteria contain a plasmid conferring chloramphenicol resistance and also encoding for T7 lysozyme which is a natural inhibitor of the T7 RNA polymerase.

*2.2. Construction of poliovirus 3AB mutants*

Cloning and expression of the wild type 3AB gene in pET11B (plasmid pT7lac3AB) has been described before [27]. This vector expresses the genuine 3AB poliovirus protein plus an additional methionine at the N-terminus. Amino acid substitutions at positions 62 and 75–76 of the 3AB gene were carried out according to the method of Higuchi et al. [28]. Firstly, two DNA fragments were synthesized by PCR amplification (PCR $A_1$ and $A_2$). These fragments hybridize to each other in a region of 10–12 nt and already contain the desired mutation. These DNA fragments were gel-purified and, after reannealing, were used as template for a second PCR amplification (PCR B). PCR $A_1$ was made by using oligonucleotides 5′-3A [29] and the corresponding $A_1$ oligonucleotide. Oligonucleotide 3′-3B [29] and the corresponding $A_2$ oligonucleotide, were used for PCR$A_2$. PCR $A_1$ and $A_2$ were carried out using as template pT7lac3AB-*Hin*dIII digested DNA. PCR B was carried out with oligonucleotides 5′-3A and 3′-3B. The sequences of oligonucleotides $A_1$ and $A_2$ are:Oligo $A_1$ $Lys^{62}$, ACC GCT TGA AGC TTT GTC ATT GCC C; Oligo $A_2$ $Lys^{62}$, AAT GAC AAA GCT TCA AGC GGT GAC A; Oligo $A_1$ $Glu^{75}/Phe^{76}$ ATG ACA TAG AAT TCT CCA GCC ACT G; Oligo $A_2$ $Glu^{75}/Phe^{76}$, GTG GCT GGA GAA TTC TAT GTC ATG T.

After PCR B, amplified fragments were digested with *Nde*I and *Bcl*I and ligated to pET11B digested with *Nde*I and *Bam*HI. PCR reaction conditions were: 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin (w/v), 10 mM Tris-HCl, pH 8.0, containing 200 μM each dNTP, 1–4 pg/ml of template DNA, 0.5 μM of each primer and 0.05 units/ml of Taq DNA polymerase. Reactions $A_1$ and $A_2$ were done for 20 cycles (1 min at 94°, 1 min at 37° and 1 min at 72°, last cycle 10 min at 72°). PCR B took place for 17 cycles (first 5 cycles as shown above and the last 12 cycles were 1 min at 94°, 45 s at 50° and 1 min at 72°, last cycle 10 min at 72°). Amino acid substitution at position 97 (lysine to arginine) was made by PCR amplification with oligonucleotides 5′–3A and 3′-3B, using pT7-VPg15 as template DNA [30]. Construction of $His^{77}$ and $Phe^{90}$ mutants will be described elsewhere. In all cases, the 3AB genes were completely sequenced by the dideoxy method (Sequenase, U.S. Biochemical Corp.).

*2.3. Permeability changes*

Changes in permeability to hygromycin B, lysozyme, ONPG (*o*-nitrophenyl-β-D-galactopyranoside) and uridine were assayed as described before [21] with the following changes. For measuring ONPG entry, *E. coli* cells were induced with 1 mM IPTG alone. The reaction took place for 15 min at 30°C. Data (absorbance at 420 nm) were normalized to the total β-galactosidase activity found in every 3AB mutant. To estimate the inhibitory effect of hygromycin B, cells were induced in M9 medium with 1 mM IPTG. Thirty min later, rifampicin (150 μg/ml) was added. Hygromycin B (2 mM) was added 35 min after induction. One hour after IPTG addition, cells were labeled with [$^{35}$S]methionine and extracts were submitted to SDS-PAGE as described before [21]. Permeabilization to intracellular T7 lysozyme was evaluated by estimating the cell lysis induced after exit of the bacteriolytic enzyme from the cytoplasm to the periplasmic space. To this end, BL21(DE3) pLysS cells were induced with 1 mM IPTG and rifampicin 150 μg/ml, was added after 30 min of induction. The decrease in absorbance at 660 nm was used to estimate cell lysis.

*2.4. Membrane association of 3AB mutants*

Cells were induced with 1 mM IPTG and rifampicin, 150 μg/ml, was added after 20 min of induction. Recombinant proteins were labeled with [$^{35}$S]methionine (1 μCi/ml) from 35 to 60 min post-induction. Cells were washed twice with 50 mM Tris-HCl, pH 7.6, 100 mM NaCl. Approximately, 20 ml of initial culture were resuspended in 0.5 ml of buffer A (50 mM Tris-HCl, pH 7.6, 100 mM NaCl, 1 mM EDTA, 1 mM DTT, 1 mM PMSF, 5% glycerol). Cells were lysed at 4°C with a sonicator (three bursts at 18 microns) and membrane-bound proteins were sedimented by ultracentrifugation (100,000 × *g* for 30 min). Equivalent amounts of soluble (S phase) and insoluble (P phase) proteins were analyzed by SDS-PAGE. To study the effect of chaotropic agents the initial pellet was resuspended in buffer A plus either 5 M guanidine or 8 M urea and kept at 4°C for 60 min. Soluble protein after 100,000 × *g* centrifugation was measured by estimating the soluble radioactivity.

## 3. Results

*3.1. Characteristics of poliovirus 3AB protein and description of the mutants generated*

Poliovirus polypeptide 3AB is the precursor of the genome-bound VPg protein (3B = VPg) and the 3A protein. The 3A moiety is a basic protein that contains a hydrophobic stretch of amino acids between positions 59–80 (Fig. 1). The Kyte and Doolittle (KD) hydrophobicity plot indicates that this protein domain might interact with membranes. Computer analyses of this 3A domain suggest that it may adopt an amphipathic α-helix conformation. Thus, the protein could be interacting with the lipids of the membrane by one side of this α-helix, whereas the hydrophilic surface may face the cytoplasm or form part of the lumen of a membrane pore. If either 3AB protein, or its cleavage product 3A, had pore-forming activity, this function might be abolished by abrogating the interaction of the polypeptide with cellular membranes. Therefore, we decided to mutagenize this hydrophobic region in order to destabilize the association of 3AB with membranes. Charged amino acids were introduced at positions 62 (isoleucine to lysine), 75–76 (valine to glutamic acid, valine to phenylalanine) and 77 (tyrosine to histidine) of the 3AB gene. As controls, two mutations in the VPg moiety were used. Replacement of tyrosine by phenylalanine at position 90 has been shown to produce non-functional viruses, probably due to the inability of the VPg protein to be nucleotidylated [30]. Amino acid subtitution at position 97 (lysine to arginine) gives rise to a virus with a growth rate indistinguisable from the wild type poliovirus 1 Mahoney strain [30]. All the 3AB mutants depicted in Fig. 1 were cloned in pET11b vectors [31] and expressed in BL21(DE3) *E. coli* cells.

*3.2. Expression of 3AB mutants in* E. coli *cells*

Expression of the 3AB proteins mutated in the hydrophobic region follows a different pattern as compared to wild type one, since they are synthesized at higher levels over longer periods of time (Fig. 2A). Thus, the total amount of $Glu^{75}/Phe^{76}$ and $Lys^{62}$ mutant proteins after 2 h of induction was over 10-fold higher than in wild type bacteria. These results suggest that the simple over-expression and accumulation of 3AB in *E. coli* cells is not necessarily toxic.

The same mutants were also cloned and expressed in BL21 (DE3)pLysS *E. coli* cells. Fig. 2A (lower panel) shows that the expression of wild type 3AB in this system is only observed during the early times of induction, whereas the expression of the mutants in 3A continues even after three hours, further corroborating the idea that these mutant 3AB proteins are not toxic for *E. coli* cells. Amino acid substitutions in 3B do not cause any difference in the level of synthesis of the recombinant proteins. The conclusion from these findings is that a single amino acid substitution in the hydrophobic domain drastically affects the levels of gene expression in this system. The mechanism by which these mutations modulate expression is

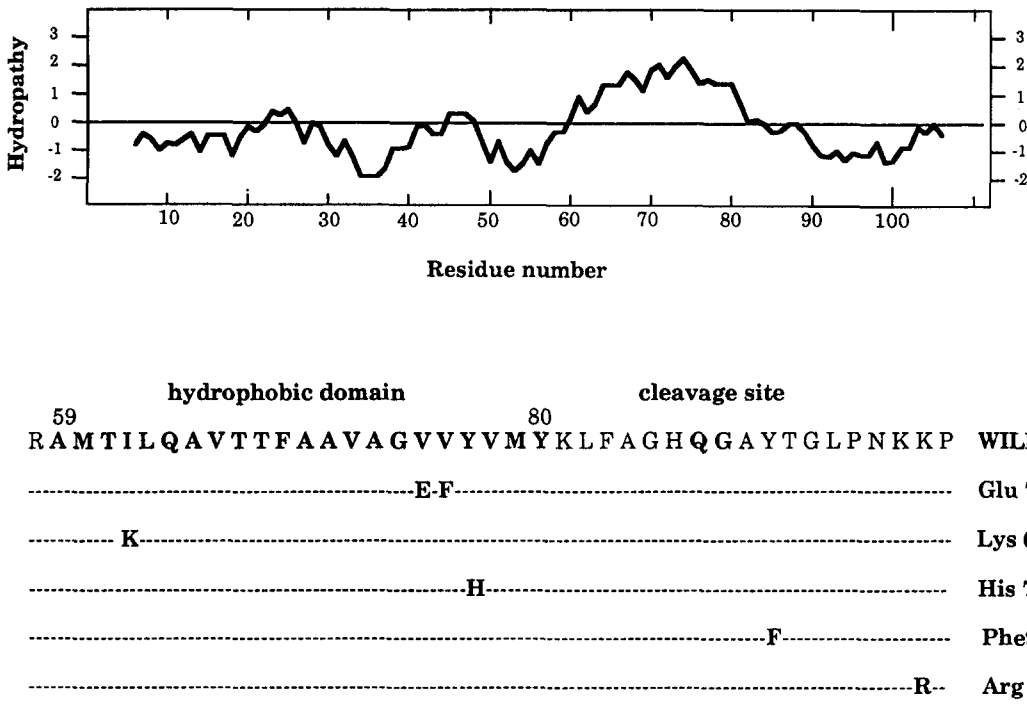


Fig. 1. Schematic representation of the 3AB poliovirus protein. The KD hydrophobicity plot [48] of the 3AB poliovirus protein (poliovirus type 1, Mahoney Strain) is depicted. The wild type amino acid sequence of 3AB around the highly hydrophobic region is represented. The predicted transmembrane domain (hydrophobic region from amino acids 59–80) and the glutamine/glycine cleavage site located between the 3A and 3B proteins are shown with bold characters. Amino acid subtitutions in the mutants used in this work are also depicted.

unknown, but must rely on intrinsic activities of the viral protein that are abolished by modifications of the hydrophobic domain.

### 3.3. *Lytic properties of 3AB mutants*

The expression of poliovirus 3AB in BL21(DE3) pLysS cells induces rapid lysis of *E. coli* as determined by a drastic drop in the absorbance at 660 nm ($A_{660}$) of induced cultures (Fig. 3A). This reduction is not observed with cells that express the different 3A mutants, whereas mutations in 3B has no effect on the lytic activity of the protein. Mutation at position 62 (Ile to Lys) confers some reduction in the lytic potential of 3AB, but changing the two valines at positions 75 and 76 to Glu and Phe respectively, completely suppresses the lytic activity of 3AB.

### 3.4. *Permeabilizing effects of 3AB mutants*

To directly measure membrane permeability changes in cells synthesizing the different 3AB proteins, we first tested the entry of ONPG into cells. Note that experiments shown in Fig. 3B and 3C were done with BL21(DE3) cells. These cells do not contain T7 lysozyme and therefore, no cell-wall degradation and the concomitant lysis is expected after expression of wild type 3AB. When 3AB was synthesized, cells showed a 3-fold increase in the entry of ONPG compared to control cells (Fig. 3B), despite the fact that the *lac* permease can greatly concentrate ONPG in the cytoplasm of *E. coli* [32]. This increase does not appear in either $Glu^{75}/Phe^{76}$ or $His^{77}$ mutants and takes place to an intermediate extent when $Lys^{62}$ mutant protein is expressed.

In the experiment shown in Fig. 3C, cells were loaded with radioactive uridine and the radioactivity released to the medium was measured. An estimation from this experiment shows that uridine leaks out from *E. coli* cells expressing wild type 3AB protein, 15–20 times faster than in either control, or $Glu^{75}/Phe^{76}$ mutant-expressing cells. Mutants $His^{77}$ and $Lys^{62}$ showed a faster leakage of uridine above control bacteria, but still much slower than the efflux measured from wild type bacteria. The released uridine was not TCA-precipitable (Results not shown), suggesting that radioactivity comes from the cellular pool of free-uridine, rather than from the one incorporated in RNA molecules. In addition, the exit of $\beta$-galactosidase activity was measured in parallel with the experiment shown in Fig 3C. Release of $\beta$-galactosidase at zero time was 1% of the total activity, and kept constant until 3 hours of induction (Data not shown). These results suggest that no detectable lysis takes place during the experiment, at least to account for the release of uridine observed (about 20% after 2 hours of induction). Therefore, by the time uridine is rapidly leaking out from cells, membrane integrity is still good enough to keep large macromolecules such as $\beta$-galactosidase or polyribonucleotides inside the cell. This fact suggests that the observed fluxes are taking place through discrete units (e.g. pores), that allow the passage of some molecules, but not others.

Hygromycin B has been one of the most utilized 'non-permeant inhibitors' [33]. Fig. 4 shows that translation in *E. coli* cells that express 3AB is completely inhibited by this aminoglycoside antibiotic, an effect that is not observed in control cells that do not express any protein [21]. Entry of hygromycin B into cells that express the different 3AB mutants occurs at different extents, depending on the degree of membrane permeabilization caused by the protein. Thus, mutant $Glu^{75}/Phe^{76}$ is almost unable to permeabilize cells to hygromycin B (15% inhi-

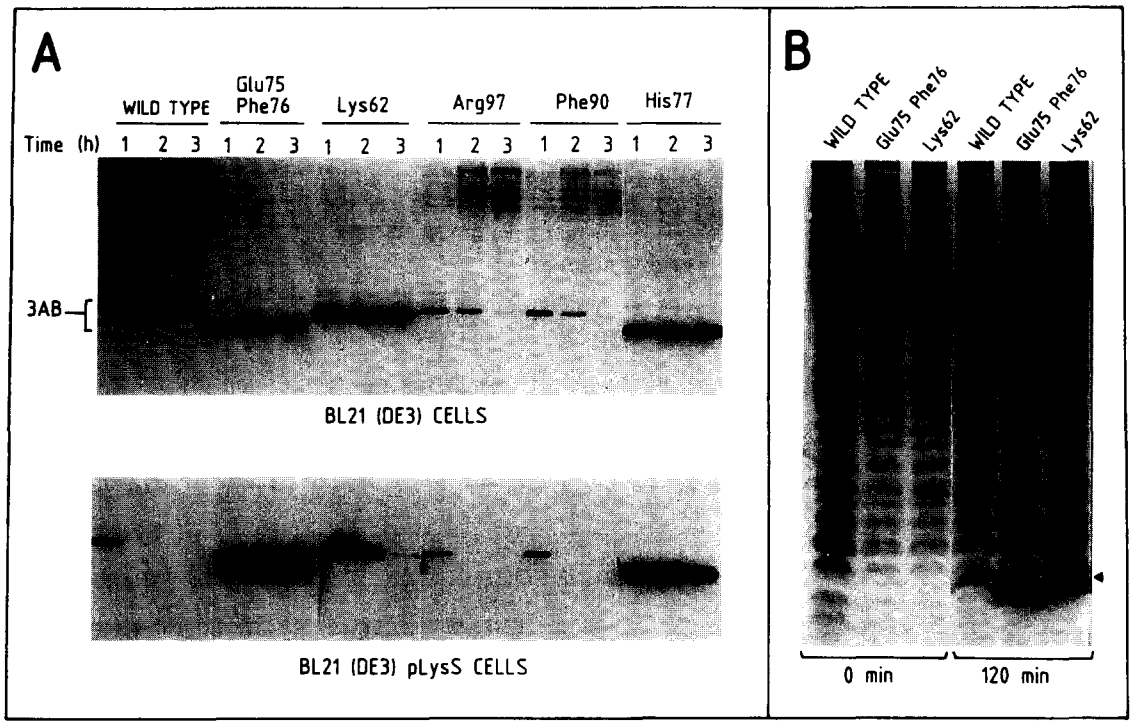


Fig. 2. Expression of wild type and mutant 3AB proteins. Panel A: BL21(DE3) cells (upper panel) or BL21(DE3)pLysS cells (lower panel) containing pT7lac3AB plasmids encoding different 3AB mutants, were induced and labeled with [$^{35}$S]methionine at different times post-induction. Synthesized proteins were analyzed by SDS-PAGE in a 20% acrylamide gel. Gels were then submitted to fluorography. When pLysS cells were used, 150 $\mu$g/ml rifampicin were added after 30 min of induction. Panel B: total protein synthesis was analyzed by coomassie blue staining of polyacrylamide gels. BL21(DE3) cells were induced and lysed before (0 min) or 120 min after IPTG addition. Arrows mark the position of recombinant proteins.

bition; compare lanes 1 to 2, Fig. 4). Even though cells that express the Lys$^{62}$ mutant are more sensitive to hygromycin (80% inhibition; see lanes 7 and 8), a precise estimation indicated that mutant Lys$^{62}$ was still about 100 times more resistant to hygromycin B inhibition, than bacteria expressing wild type protein (Data not shown).

### 3.5. *Association of 3AB with membranes*

To further understand the loss of pore-forming activity in some of the 3AB mutants, the association of these proteins with cellular membranes was analyzed. Wild type 3AB protein accumulated in the insoluble fraction (Fig. 5A) and no soluble protein was detected in the soluble phase, even after longer exposures of the gel (Results not shown). Nevertheless, a small fraction of the 3A mutant proteins remained soluble after 100,000 × $g$ centrifugation (see lanes 3, 5 and 7, Fig. 5A). VPg mutant proteins behave as wild type protein and were only found in the insoluble fraction. Further evidence supporting the association of wild type 3AB with membranes was obtained by immunogold labeling with anti 3A antibodies of purified membranes from bacteria expressing the 3AB protein (data not shown). Over-expression of recombinant proteins in *E. coli* commonly gives rise to formation of insoluble inclusion bodies that co-purify with cellular membranes. These aggregates can be solubilized by chaotropic reagents [34]. To ascertain whether the insoluble proteins were really membrane-associated proteins, the effect of 5 M guanidine and 8 M urea on the solubility of these proteins was tested. Fig. 5B shows that treatment with 5 M guanidine solubilizes about 80% of the Glu$^{75}$/Phe$^{76}$ mutant protein. Similar results were obtained with the His$^{77}$ mutant and to a lesser extent, with the Lys$^{62}$ mutant. However, less than 20% of the wild type or VPg mutant proteins can be solubilized by the same procedure. Treatment with 8 M urea produces similar results, although this reagent was slightly less effective. These data suggest that those 3AB proteins containing mutations in the hydrophobic domain probably accumulate as inclusion bodies, while wild type protein tightly binds to *E. coli* membranes. Therefore, the loss of pore-forming activity in the 3A mutants could be explained as the inability of these proteins to associate correctly with membranes.

## 4. Discussion

The finding that poliovirus protein 3A (or 3AB) permeabilizes *E. coli* cells [21] opened the possibility of carrying out genetic studies in this system. Our present results show that 3AB is a lytic protein and that changes in its primary sequence that alter its association with membranes lead to a loss of the permeabilizing activity. With regards to the mechanism of 3AB action, we have shown that permeabilty changes in both directions (influx and efflux) to different small molecules (lactose analogues, lysozyme, nucleosides, non-permeant inhibitors) are taking place, at a time when other bigger molecules are effectively excluded by the plasma membrane. This mechanism resembles the action of other membrane-active proteins, including colicins and phage T5 proteins [9]. These findings suggest that 3AB protein may permeabilize membranes by formation of discrete units which permit the flux of some molecules but not others. To date, our attempts to demonstrate the existence of pore-like structures by electron microscopy have failed.

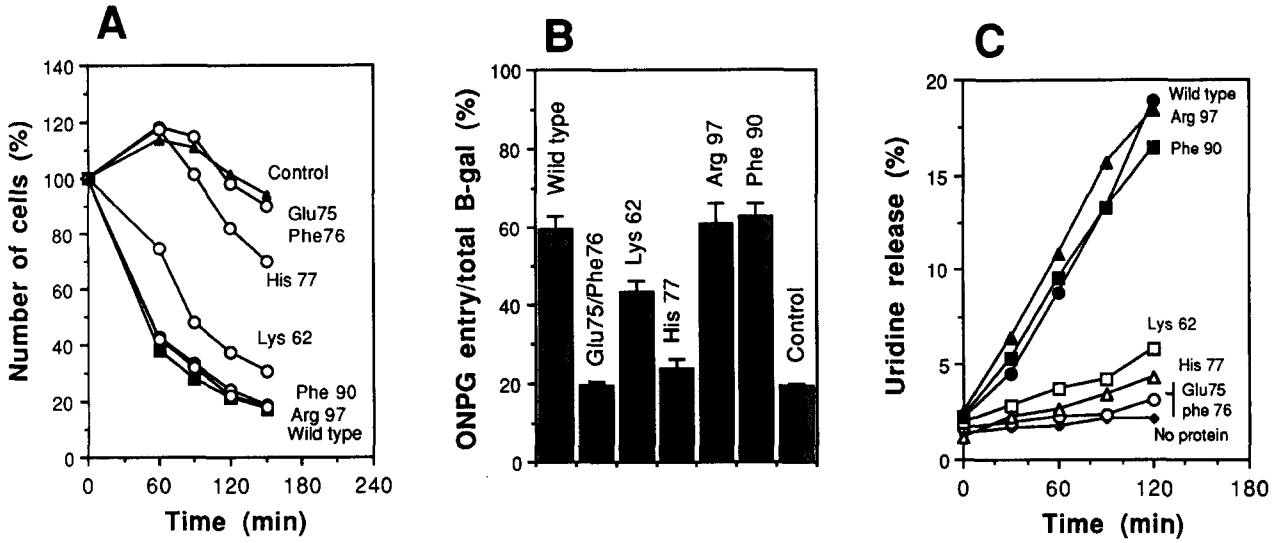


Fig. 3. Modification of membrane permeability to lysozyme, ONPG and [$^3$H]uridine. Panel A: BL21(DE3)-pLysS cells expressing the different 3AB mutants were induced with 1 mM IPTG at zero time. Thirty min later, rifampicin (150 $\mu$g/ml) was added to the cultures. The number of cells in the cultures were estimated by measuring $A_{660}$. Control cells contain the parental plasmid pET11B and express no recombinant protein. Panel B: BL21(DE3) cells were induced and 90 min after IPTG addition, the entry of ONPG was measured as described in section 2. ONPG entry is shown as the percentage of the total $\beta$-galactosidase activity. The mean value ± S.E.M from five independent experiments is shown. Panel C: BL21(DE3) cells were preloaded for 2 hours with 2 $\mu$Ci/ml [$^3$H]uridine. Excess uridine was removed by washing with M9 medium at 4°C. At zero time, 1 mM IPTG was added. Uridine release was estimated by measuring the radioactivity remaining in the medium after centrifugation to remove cells. 100% represents the radioactivity accumulated in cells before addition of IPTG. Cells containing pET11B plasmid without insert are indicated as 'no protein'. Leakage of $\beta$-galactosidase during the experiment was always lower than one percent of the total activity.

Nevertheless, this failure should not be strange. Such structures should probably have dimensions close of below the resolution limits of electron microscopy. In fact, similar attempts to identify pore-like structures formed by proteins that permeabilize the membranes of phage-infected bacteria have also been unsuccessful [36]. Otherwise, electrophysiological measurements with 3AB protein inserted into liposomes will be required to further confirm these results.

Although we have shown that poliovirus protein 3AB is endowed with permeabilizing activity in *E. coli* cells, it is still far from proven that this protein is responsible for the cytolytic effects of poliovirus. Regarding this possibility, Morace et al. [37] have shown that cytopathic strains of hepatitis A virus, another picornavirus, have acquired mutations in the 3A gene, suggesting a potential role for this protein in the generation of the cytopathic effect induced by picornaviruses. To find out

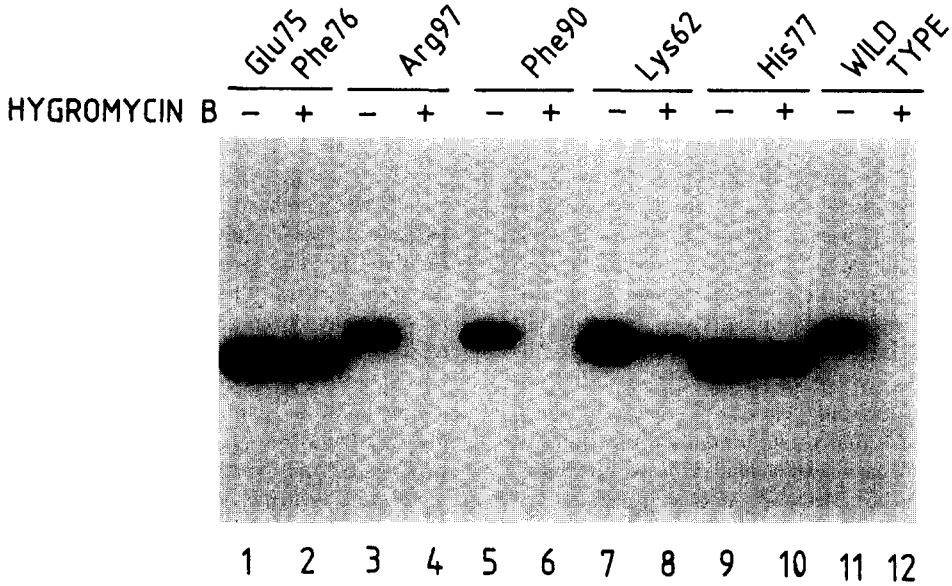


Fig. 4. Effect of hygromycin B on translation of 3AB proteins. BL21(DE3) cells containing each 3AB expressing plasmid were induced with 1 mM IPTG. Rifampicin was added 30 min later to block cellular protein synthesis. After 35 min of induction, either growth medium (−) or 2 mM hygromycin B (+) was added. At 60 min post-induction proteins were labeled with [$^{35}$S]methionine for 10 min and analyzed by SDS-PAGE. The gel was submitted to fluorography and exposed to X-ray films.

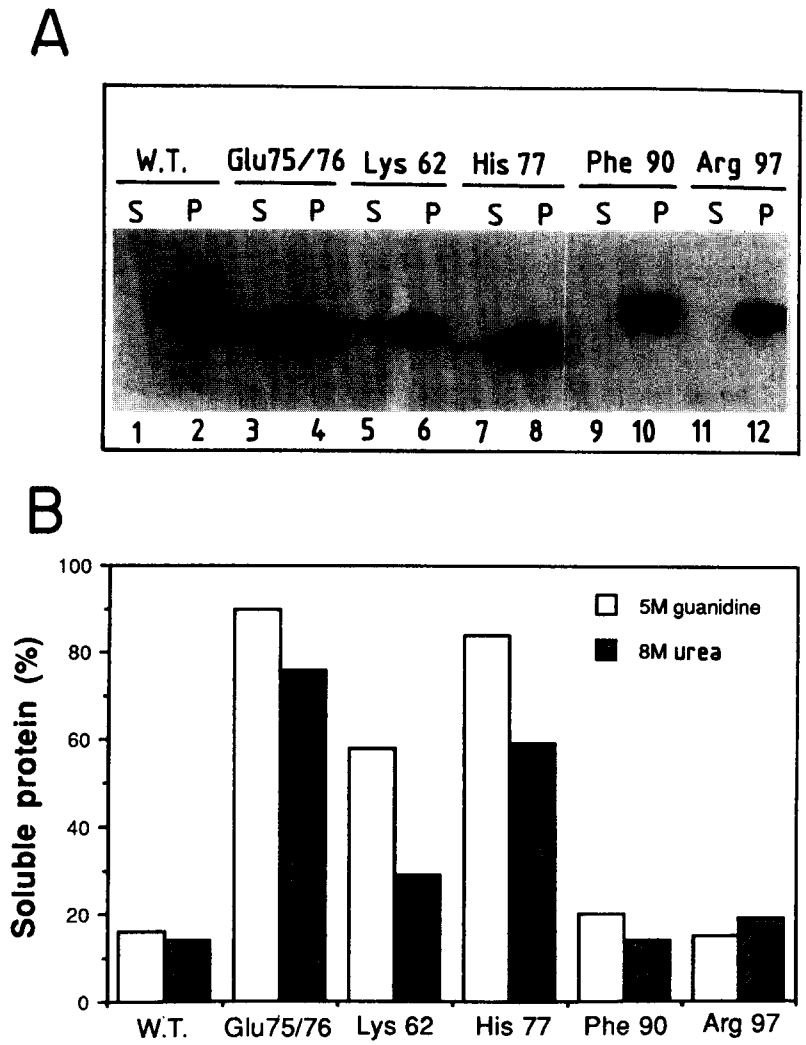


Fig. 5. Membrane-association of 3AB recombinant proteins. Panel A: BL21(DE3) cells containing pT7lac3AB plasmids to express either wild type or mutant 3AB proteins were induced, labeled with [$^{35}$S]methionine and lysed as described in section 2. Soluble (S) and insoluble (P) protein were separated by centrifugation at 100,000 × *g*. Recombinant proteins were analyzed by SDS-PAGE. The gel was submitted to fluorography and exposed to X-ray films. Panel B: effect of chaotropic agents on the solubility of 3AB proteins. Insoluble protein after centrifugation at 100,000 × *g* was resuspended in buffer A plus either 5 M guanidine or 8 M urea. After 60 min at 4°C, soluble protein was estimated by measuring the percentage of radioactivity still soluble after another 100,000 × *g* centrifugation.

whether 3AB protein is involved in the membrane permeability changes that take place in infected cells, studies with mammalian cells will be necessary. However, most of the poliovirus 3A mutants thus far generated are not viable [38], probably due to the fact that 3AB may play different roles during poliovirus infection. Recently, poliovirus protein 3AB was found to enhance the activity of the viral 3D polymerase in cell-free systems [39] and this property was involved in the replication of the viral genomes [40]. 3A-containing polypeptides have been localized in the membranous vesicles that are induced after infection and where active genome replication takes place [23]. Nevertheless, these proteins have never been detected on the cell surface. The possibility remains that 3AB protein might exert cytopathic effects despite being localized in cytoplasmic vesicles. Thus, 3A/3AB proteins may form pores in these structures and thereby increase the cytoplasmic calcium concetration. Such an increase has been implicated in the cytotoxic effects induced by rotavirus [41].

Other viral proteins with the capacity to modify membrane permeability have already been identified. Proteins encoded by bacteriophages endowed with such activity have long been known [36]. The ion-channel activity of influenza virus M2 protein has already been indicated. A number of animal virus proteins have been reported to be involved in cell lysis and release of virions. Among these are the 6K protein of Semliki Forest virus [42], the M protein of vesicular stomatitis virus [43], the Vpu protein of human immunodeficiency virus type 1 [44] and the 11.6K protein of human adenovirus [45]. All these proteins contain between 50–100 amino acids and are integral membrane proteins. We believe that these proteins could also form pores as 3AB protein does. The expression of pore-forming proteins might be a general mechanism used by animal viruses to kill infected cells and release virions.

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