# Interaction of actin and its 11-amino acid C-terminal peptide as cofactors with the adenovirus proteinase

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**Abstract** **Actin bound to the adenovirus proteinase (AVP) with a lower equilibrium dissociation constant, 4.2 nM, than those exhibited by two viral, nuclear cofactors for AVP, the 11-amino acid peptide pVIc and the viral DNA. The $k_{cat}/K_m$ ratio for substrate hydrolysis by AVP increased 150 000-fold in the presence of actin. The 11-amino acid residue peptide corresponding to the C-terminus of actin, which is highly homologous to pVIc, bound to AVP and stimulated its activity in the presence of DNA. As a cellular cofactor for AVP, AVP(actin) complexes may facilitate the cleavage of cytoskeletal proteins, preparing the infected cell for lysis and release of nascent virions.**


*Key words:* Cysteine proteinase; Viral proteinase; Cell lysis; Equilibrium dissociation constant; Cytoskeleton destruction; Enzyme activation

## 1. Introduction

Recently, actin was shown to be able to act in vitro as a cofactor for the adenovirus proteinase (AVP) in the degradation of cytoskeletal proteins [1]. Studies of infection of HeLa cells by adenovirus [2] or transfection of HeLa cells by AVP [1] reveal AVP localizes with cytokeratin 18, and this is then followed by destruction of the cytokeratin network. For AVP to cleave cytokeratin 18, a cellular cofactor is required, because AVP is synthesized as an inactive proteinase. These and other data implied that a reversible complex between AVP and actin (AVP(actin) complex) may be involved in the cleavage of structural proteins in the cytoplasm that prepares an infected cell for lysis and release of nascent virions [3].

Late in human adenovirus infection, AVP becomes activated inside young virions and cleaves multiple copies of six virion precursor proteins (pTP, pVI, pVII, pVIII, IIIa, and an 11-kDa protein) 3200 times to render virus particles infectious [4]. AVP requires viral cofactors for maximal enzyme activity – pVIc, the 11-amino acid peptide from the C-terminus of virion precursor protein pVI [5,6], and the viral DNA [5]. The cofactors bind to AVP and in doing so dramatically increase the $k_{cat}/K_m$ ratio for substrate hydrolysis [7]. pVIc binds to AVP through the formation of 30 hydrogen bonds and a disulfide bond between Cys10′ of pVIc and Cys104 of AVP [8], as revealed in the crystal structure of AVP bound to pVIc [9,10]. The binding of DNA to AVP is not dependent upon any specific sequence in DNA.

Actin was considered a potential cofactor for AVP, because the C-terminal amino acid sequence of actin (SGPSIVHRKCF) [11,12] is highly homologous to the amino acid sequence of pVIc (GVQSKLRRRCF) [5,6]. Of the last eight amino acid residues of actin, four are identical and three homologous to the last eight amino acid residues of pVIc. Comparison of the last 10 amino acid residues at the C-terminus of the α-, β-, and γ-isomers of actin reveals that these residues are strictly conserved. The penultimate amino acid in actin is Cys374. The penultimate amino acid in pVIc, Cys10′, is a major determinant in the reversible binding of pVIc to AVP [13]. Furthermore, a disulfide bond forms between Cys10′ of pVIc and Cys104 of AVP, both in vitro [9] and in vivo in the virus particle [14]. In vitro, the enzymatic activity of AVP is greatly stimulated by actin [1]. AVP appears to bind to the C-terminus of actin, because AVP can quench the fluorescence of PRODAN covalently bound to Cys374 of actin.

That actin can be a cofactor for AVP may have resolved a conundrum. AVP had been shown in vivo to be responsible for cleavage of cytokeratin 18 in the cytoplasm of adenovirus-infected cells [2]. However, because AVP had also been shown to be relatively inactive in vitro in the absence of cofactors [5,6], the question arose as to whether AVP required a cofactor to cleave cytokeratin 18. AVP could not cleave cytokeratin 18 in vitro. However, in the presence of actin, an AVP(actin) complex could cleave cytokeratin 18. Also, actin itself is a substrate for AVP [1]; in the presence of actin, AVP(actin) complexes cleave actin at two AVP consensus cleavage sites.

Here we quantitatively characterize the interaction of AVP with actin. The equilibrium dissociation constant, $K_d$, was determined as well as the macroscopic kinetic constants for substrate hydrolysis. Based upon the $K_d$ and the $k_{cat}/K_m$ values, actin was a better cofactor than pVIc. Finally, the peptide with the sequence of the last 11 amino acids of actin was

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*Abbreviations:* 11-Actin-C, the 11-amino acid residue peptide from the C-terminus of actin (SGPSIVHRKCF); AVP, adenovirus proteinase; AVP–pVIc, covalent complex between AVP and pVIc formed by the disulfide bond between Cys104 of AVP and Cys10′ of pVIc; AVP(pVIc), reversible complex between AVP and pVIc; AVP(actin), reversible complex between AVP and actin; AVP(DNA), reversible complex between AVP and DNA; $k_{cat}$, catalytic rate constant for substrate hydrolysis; $K_d$, equilibrium dissociation constant; $K_m$, Michaelis constant; pVIc, 11-amino acid peptide (GVQSLKRRRCF) that originates from the C-terminus of the viral precursor protein pVI; Random-Sequence-Peptide, an 11-amino acid peptide with the same amino acids as in pVIc but in a sequence that was generated randomly (SFRRCGLRQVK)

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shown to stimulate AVP, in the presence of DNA. This directly demonstrated that AVP can bind to the C-terminus of actin.

## 2. Materials and methods

### 2.1. Materials

Bovine thymus G-actin, a gift from Dr. C. Shutt, was purified according to the method of Rozycki et al. [15]. Tris–HCl, EDTA, DNase I, lysozyme, chloramphenicol, isopropyl-β-D-thiogalactopyranoside, and 5,5′-dithio-bis(2-nitrobenzoate) were purchased from Sigma (St. Louis, MO, USA). Octylglucoside was purchased from Roche Molecular Biochemicals (Indianapolis, IN, USA). The viral peptide pVIc (GVQSLKRRRCF), 11-Actin-C (SGPSIVHRKCF), and the Random-Sequence-Peptide (SFRRCGLRQVK) were purchased from Research Genetics (Huntsville, AL, USA). Reduce-Imm Column and buffers were purchased from Pierce (Rockford, IL, USA). The plasmid pT7AD23K8 was from Dr. Carl Anderson. T7 DNA was a gift from Dr. John Dunn. DEAE and S-Sepharose were purchased from TosoHaas (Montgomeryville, PA, USA). The fluorogenic substrate (Leu-Arg-Gly-Gly-NH)$_2$-rhodamine was synthesized and purified as described [5,16].

### 2.2. Determination of protein concentration

Protein concentration was determined by the BCA protein assay from Pierce. For the concentration of AVP, a calculated molar absorbance coefficient at 280 nm of 26 510 $M^{-1}$ $cm^{-1}$ was used [17]. For the concentration of actin, a molar absorbance coefficient at 290 nm of 26 600 $M^{-1}$ $cm^{-1}$ was used [18].

### 2.3. Determination of peptide concentrations

The concentrations of peptides were determined by measuring their thiolate anion concentrations. Prior to an assay, the peptides were reduced by passage through a Reduce-Imm column following the manufacturer's instructions. Assays were performed in 0.516 ml of 50 mM sodium phosphate (pH 8.1) and 1 mM EDTA, containing 350 μM 5,5′-dithio-bis(2-nitrobenzoate) and either pVIc, 11-Actin-C, or Random-Sequence-Peptide. Reactions were incubated at 25°C for 10 min before the absorbance at 412 nm was measured. The concentration of thiolate anion was calculated by subtracting the absorbance in an assay lacking peptide from that measured in its presence, using a molar extinction coefficient for thionitrobenzoate at 412 nm of 14 150 [19].

### 2.4. Expression and purification of AVP

The plasmid pT7AD23K8 was transfected into *Escherichia coli* strain BL21(DE3)pLysS. Cells were grown in M9TBYG broth (0.1% $NH_4Cl$, 0.3% $KH_2PO_4$, 0.6% $Na_2PO_4$, 0.4% glucose, 1 mM $MgSO_4$, 0.1% yeast extract, 1% Bacto Tryptone, and 0.5% NaCl) with 50 μg/ml ampicillin and 25 μg/ml chloramphenicol. When the cells reached an $OD_{600}$ of 0.500, transcription of the recombinant gene was induced with 0.6 mM isopropyl-β-D-thiogalactopyranoside. The cells were harvested 16 h later. The cells were centrifuged at 9000×*g* for 10 min at 4°C. The cells in the pellet were resuspended in 20 mM Tris–HCl (pH 7.5), 100 mM NaCl, and 0.1% Triton and stirred for 1 h at 4°C. Then, 1 mM EDTA and 100 μg/ml lysozyme were added, and stirring was continued for an additional hour. $MgCl_2$ to 6 mM was added, and the DNA was digested by the addition of 50 μg/ml DNase I. After stirring for 1 h at 4°C, the cell lysate was shell frozen and thawed three times. Then, the cell lysate was centrifuged at 9000×*g* for 10 min at 4°C.

The supernatant was removed, and its NaCl concentration increased to 200 mM. It was added to 50 ml of DEAE-Sepharose beads equilibrated in 20 mM Tris–HCl (pH 7.5), 200 mM NaCl, and 0.1% Triton X-100. The slurry was repeatedly inverted for 25 min at 4°C, after which it was filtered through a sintered glass funnel. The flow-through was loaded onto a Chelating Sepharose column (Amersham Pharmacia, Piscataway, NJ, USA) charged with $ZnCl_2$. The proteinase eluted from the column in 15 mM EDTA, 20 mM Tris–HCl (pH 7.5), 0.1 M NaCl and 0.1% Triton X-100.

The eluate was diluted to a NaCl concentration of 40 mM, β-mercaptoethanol was added to 5 mM, and the eluate was loaded onto a S-Sepharose column. AVP eluted from the S-Sepharose column in 20 mM Tris–HCl (pH 7.5), 200 mM NaCl, and 1 mM octylglucoside. The protein was dialyzed against nitrogen-saturated buffer consisting of 10 mM Tris (pH 7.5), 5 mM NaCl, 1 mM octylglucoside and 0.1 mM EDTA and stored at −80°C.

### 2.5. Assay for proteinase activity

Standard assays in 1 ml contained 10 mM Tris–HCl (pH 8.0) and 5 mM octylglucoside. Proteinase and cofactor(s) were preincubated for 5 min at 37°C, fluorogenic substrate (Leu-Arg-Gly-Gly-NH)$_2$-rhodamine was added, and the increase in fluorescence as a function of time was measured using an ISS PC-1 fluorometer (Urbana, IL, USA) with an excitation wavelength of 492 nm and emission wavelength of 523 nm, both with a band pass of 8 nm.

### 2.6. Calculation of the equilibrium dissociation constant ($K_d$)

The $K_d$ for the binding of actin to AVP was calculated as follows: increasing concentrations of actin, $[Actin]_i$, were added to a constant amount of AVP, $[AVP]_o$. After 5 min at 37°C, the fluorogenic substrate was added and the increase in fluorescence with time, $F_i$, measured. A plot of $F_i$ versus $[Actin]_i$ yielded a rectangular hyperbola. From this graph, the concentration of actin that bound to AVP, $[Actin]_{bound}$, was obtained:

$$[Actin]_{bound} = [AVP]_o\ (F_i/F_{max})$$

where $F_{max}$ is the maximal rate of substrate hydrolysis, i.e. the rate when AVP is saturated with actin. The concentration of actin not bound to AVP, $[Actin]_{free}$, was determined as follows:

$$[Actin]_{free} = [Actin]_i - [Actin]_{bound}$$

The data were plotted as $[Actin]_{bound}$ versus $[Actin]_{free}$, and from that, the $K_d$ was determined using GRAFIT [20]. The $K_d$ values for the binding of pVIc to AVP and of pVIc or 11-Actin-C to AVP(DNA) complexes were determined in the same manner.

## 3. Results

### 3.1. $K_d$ for the binding of actin to AVP

The equilibrium dissociation constant, $K_d$, for the binding of actin to AVP was measured. This was done by adding increasing concentrations of actin to a constant amount of AVP and measuring the rate of substrate hydrolysis. A plot of the rate of change in fluorescence versus the actin concentration gave a rectangular hyperbola (Fig. 1). The data in Fig. 1 were then converted to concentrations of $[Actin]_{bound}$ and $[Actin]_{free}$ as described in Section 2.6, and, from a plot of

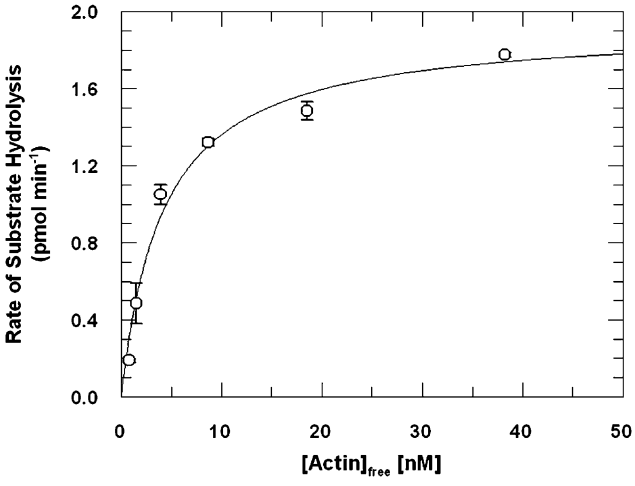


Fig. 1. Binding of actin to AVP, measurement of the $K_d$. The indicated concentrations of actin were added to a constant amount of AVP, 2 nM. After incubation for 5 min at 37°C, (Leu-Arg-Gly-Gly-NH)$_2$-rhodamine was added to 3 μM and the change in fluorescence (counts/s) with time (/s) measured. From these data, a $K_d$ for the binding of actin to AVP was determined, as described in Section 2.

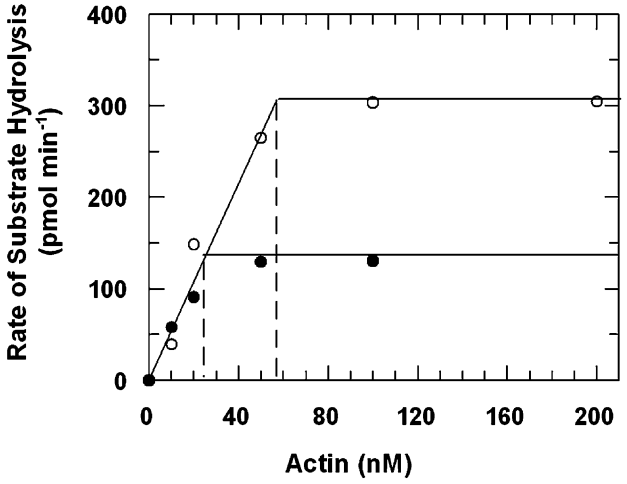


Fig. 2. Stoichiometry of binding of actin to AVP. Reactions containing either 30 (●) or 60 (○) nM AVP were incubated with increasing concentrations of actin. After incubation for 5 min at 37°C, (Leu-Arg-Gly-Gly-NH)$_2$-rhodamine was added to 3 μM and the increase in fluorescence with time measured. The solid lines were drawn with a least squares regression analysis. The dashed lines were drawn from the intersection of the solid lines to the abscissa.

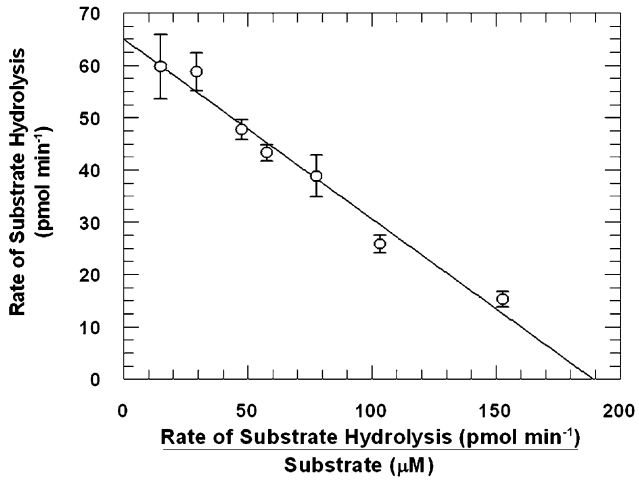


Fig. 3. Macroscopic kinetic constants for the hydrolysis of substrate by actin(AVP) complexes. Reactions containing 10 nM AVP and 100 nM actin were incubated for 5 min at 37°C. (Leu-Arg-Gly-Gly-NH)$_2$-rhodamine, ranging in concentration from 0.1 to 4 μM, was added and the increase in fluorescence with time measured. The data are presented in an Eadie–Hofstee plot.

[Actin]$_{bound}$ versus [Actin]$_{free}$ (data not shown), an apparent $K_d$ of 4.2 ± 0.8 nM was obtained.

### 3.2. Stoichiometry of binding of actin to AVP

The method used to calculate the $K_d$ assumed the stoichiometry of binding of actin to AVP was 1:1. This 1:1 ratio was verified by measuring the stoichiometry of binding under tight binding conditions, e.g. at AVP concentrations 15–30 times higher than the apparent $K_d$. AVP was incubated with increasing concentrations of actin and the rate of substrate hydrolysis measured. As the actin concentration was increased, below the concentration of actin binding sites on AVP, no free actin was present and the rate of substrate hydrolysis was directly proportional to the concentration of actin. When the actin concentration exceeded that of the actin binding sites on AVP, no further increase in the rate of substrate hydrolysis was observed. The data were characterized by two straight lines whose intersection reflected the minimal concentration of actin required to saturate AVP (Fig. 2). Extrapolation of the intersection points to the abscissa indicated that 25 nM actin saturated 30 nM AVP and that 57 nM actin saturated 60 nM AVP. Therefore, the stoichiometry of binding was 1:1, and the $K_d$, as opposed to apparent $K_d$, was 4.2 nM (Table 1).

### 3.3. Comparison of the cofactor activity of actin to that of viral cofactors

How does the cofactor activity of actin compare to that of pVIc? The macroscopic kinetic constants, $K_m$ and $k_{cat}$, for the hydrolysis of (Leu-Arg-Gly-Gly-NH)$_2$-rhodamine by AVP in the presence of different cofactors were measured (Table 1). With AVP alone, in the absence of cofactors, the $K_m$ is 95 μM and the $k_{cat}$ is 0.002 $s^{-1}$ [7]. With pVIc and AVP, the $K_m$ was 9.9 μM and the $k_{cat}$ was 0.27 $s^{-1}$. The data with actin and AVP are shown in the form of an Eadie–Hofstee plot (Fig. 3). The $K_m$ was 0.34 μM and the $k_{cat}$ was 0.22 $s^{-1}$. Actin was a better cofactor than pVIc, based upon the specificity constant, $k_{cat}/K_m$, 645 000 $M^{-1}$ $s^{-1}$ for actin and 27 400 $M^{-1}$ $s^{-1}$ for pVIc.

### 3.4. Stimulation of AVP activity by a peptide containing the C-terminal 11-amino acids of actin, in the presence of DNA

Since the C-terminus of actin is homologous to pVIc, the question arose as to whether the peptide containing the last 11 amino acids at the C-terminus of actin (SGPSIVHRKCF), abbreviated 11-Actin-C, would stimulate AVP activity. Increasing concentrations of 11-Actin-C were added to a constant amount of AVP and the rate of substrate hydrolysis measured. Minimal stimulation of enzyme activity was observed (Fig. 4). As a control, a similar experiment was done

Table 1
Equilibrium dissociation constants and macroscopic kinetic constants

| | $K_d$ (μM) | $K_m$ (μM) | $k_{cat}$ ($s^{-1}$) | $k_{cat}/K_m$ ($s^{-1}$ $M^{-1}$) |
|---|---|---|---|---|
| AVP[a] | | 94.8 ± 7.0 | 0.0023 | 4.3 |
| Ligand binding to AVP | | | | |
| pVIc | 3.46 ± 0.10 | 9.88 ± 0.025 | 0.271 | 27 400 |
| Actin | 0.00418 ± 0.00082 | 0.344 ± 0.025 | 0.222 | 645 000 |
| 11-Actin-C | could not measure; little or no stimulation of AVP | | | |
| Ligand binding to AVP(T7 DNA) | | | | |
| pVIc | 0.049 ± 0.005 | 0.152 ± 0.023 | 2.46 | 16 200 000 |
| 11-Actin-C | 9.52 ± 0.89 | 0.225 ± 0.052 | 2.04 | 9 070 000 |
| Random-Sequence-Peptide[b] | no stimulation of AVP activity observed | | | |

[a]Data obtained from [7].
[b]An 11-amino acid peptide with the same amino acids as in pVIc but in a sequence that was generated randomly.

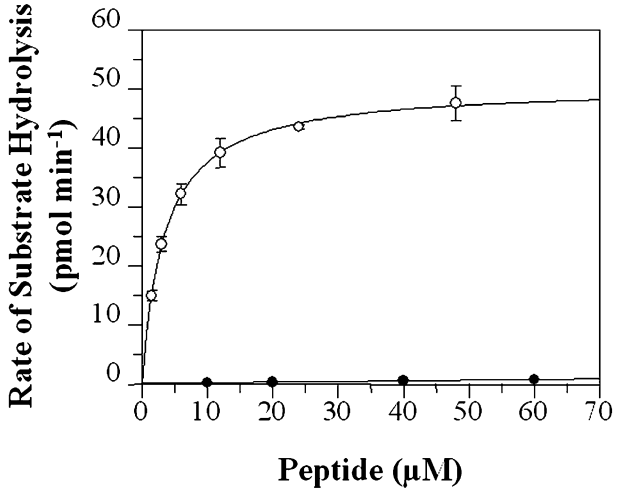


Fig. 4. Stimulation of AVP activity by 11-Actin-C and by pVIc. To 10 nM AVP were added increasing concentrations of 11-Actin-C (●) or pVIc (○). After 5 min at 37°C, (Leu-Arg-Gly-Gly-NH)$_2$-rhodamine was added to 5 μM and the increase in fluorescence with time measured.

but with pVIc. When the rate of substrate hydrolysis was plotted versus the concentration of pVIc, a rectangular hyperbola was observed (Fig. 4); conversion of these data to $[pVIc]_{bound}$ versus $[pVIc]_{free}$, as described in Section 2.6, yielded a $K_d$ of pVIc for AVP of 3.5 μM (Table 1).

Since DNA is known to increase the affinity of pVIc for AVP [8], the above experiment to determine whether 11-Actin-C would stimulate AVP activity was repeated, but in the presence of T7 DNA. Increasing concentrations of 11-Actin-C were added to a constant amount of AVP and T7 DNA, and the rate of substrate hydrolysis was measured. Under these conditions, AVP activity was greatly stimulated by 11-Actin-C (Fig. 5). And the degree of stimulation was proportional to the 11-Actin-C concentration.

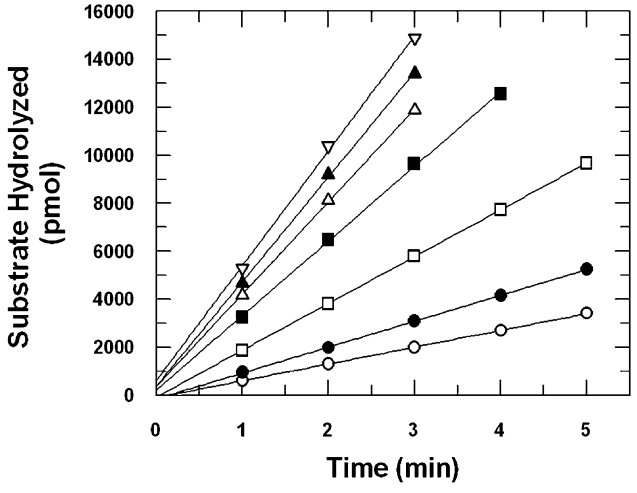


Fig. 5. Stimulation of AVP activity by 11-Actin-C, in the presence of T7 DNA. Increasing concentrations of 11-Actin-C were added to 60 nM AVP and 0.286 μg/ml T7 DNA, in 10 mM Tris (pH 8.0) and 2 mM octylglucoside. After 5 min at 37°C, (Leu-Arg-Gly-Gly-NH)$_2$-rhodamine was added to 5 μM and the increase in fluorescence with time measured. The 11-Actin-C concentrations were 1.25 (○), 2.5 (●), 5 (□), 12.5 (■), 25 (△), 50 (▲), and 100 μM (▽).

### 3.5. $K_d$ for the binding to AVP of a peptide containing the C-terminal 11 amino acids of actin

The $K_d$ for the binding of 11-Actin-C to AVP in the presence of T7 DNA was determined by the same method used to measure the $K_d$ for the binding of actin to AVP. The data from Fig. 5 were converted into $[11\text{-Actin-C}]_{bound}$ and $[11\text{-Actin-C}]_{free}$ (Fig. 6A), and that graph yielded a $K_d$ of 9.5 μM (Table 1). For comparison, the $K_d$ for the binding of pVIc to AVP in the presence of T7 DNA was 49 nM (Fig. 6B and Table 1).

### 3.6. Comparison of the cofactor activity of 11-Actin-C to that of pVIc, in the presence of DNA

How does the cofactor activity of 11-Actin-C compare to that of pVIc with AVP in the presence of DNA? The macroscopic kinetic constants for the hydrolysis of (Leu-Arg-Gly-Gly-NH)$_2$-rhodamine by these two forms of AVP were measured. With the AVP(T7 DNA) complex saturated with pVIc, the $K_m$ was 0.152 μM and the $k_{cat}$ was 2.46 $s^{-1}$ (Table 1). With the AVP(T7 DNA) complex saturated with 11-Actin-C, the $K_m$ was 0.225 μM and the $k_{cat}$ was 2.04 $s^{-1}$ (Table 1).

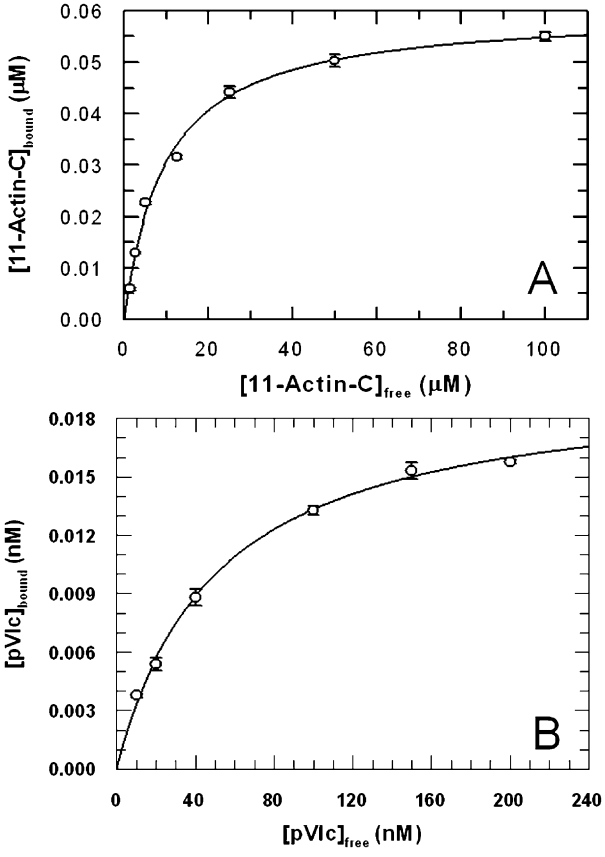


Fig. 6. Binding of 11-Actin-C (A) or pVIc (B) to AVP, in the presence of T7 DNA. A: Increasing concentrations of 11-Actin-C were added to 60 nM AVP and 0.286 μg/ml T7 DNA. After 5 min at 37°C, 5 μM (Leu-Arg-Gly-Gly-NH)$_2$-rhodamine was added and the rate of substrate hydrolysis measured. The concentrations of $[11\text{-Actin-C}]_{bound}$ and $[11\text{-Actin-C}]_{free}$ were determined as described in Section 2. B: Increasing concentrations of pVIc were added to 20 nM AVP and 0.286 μg/ml T7 DNA. After 5 min at 37°C, (Leu-Arg-Gly-Gly-NH)$_2$-rhodamine was added to 5 μM and the rate of substrate hydrolysis measured. The concentrations of $[pVIc]_{bound}$ and $[pVIc]_{free}$ were determined as described in Section 2.

Thus, both 11-Actin-C and pVIc stimulated AVP(T7 DNA) complexes to the same extent; the macroscopic kinetic constants were similar.

### 3.7. Control experiments with an 11-amino acid peptide containing a different sequence than that of pVIc

As a control, experiments similar to those described with pVIc and 11-Actin-C were done but with a peptide that had the same amino acid composition as in pVIc but in a different sequence, SFRRCGLRQVK, abbreviated Random-Sequence-Peptide. No stimulation of AVP activity was observed with the Random-Sequence-Peptide (data not shown), either in the absence or in the presence of T7 DNA (Table 1).

## 4. Discussion

These experiments were designed to characterize quantitatively the interaction between actin and AVP. Actin bound to AVP with a low $K_d$. Secondly, AVP(actin) complexes were shown to hydrolyze a specific fluorogenic substrate with a $k_{cat}/K_m$ ratio higher than that exhibited by AVP(pVIc) complexes. Lastly, the 11-amino acid peptide with the sequence of the C-terminus of actin was shown in the presence of DNA to bind to AVP and to stimulate its activity, directly demonstrating that the C-terminus of actin could be involved in the binding of actin to AVP.

Previously, the binding of actin to AVP was measured with actin labeled at its penultimate amino acid, Cys374, with PRODAN [1]. The quenching of the fluorescence of PRODAN indicated AVP binds at the C-terminus of actin; by varying the AVP concentration a binding isotherm was obtained from which a $K_d$ of 1.7 μM was calculated. But, the labeling of actin with PRODAN can affect the binding of ligand. For example, profilin binds to the C-terminus of actin but profilin cannot bind to PRODAN-labeled actin [21]. For this reason, the $K_d$ for the binding of actin to AVP was determined using unlabeled actin, and the $K_d$ for unlabeled actin turned out to be more than 400-fold lower than the $K_d$ for PRODAN-labeled actin.

There were several reasons to think a peptide with the last 11 amino acids of actin could stimulate the activity of AVP. One reason is the high homology between the last 11 amino acids of actin and pVIc. Two, alanine scanning mutagenesis of pVIc has revealed that Cys10′ and Phe11′ of pVIc are important in the binding of pVIc to AVP and in the stimulation of proteinase activity [8]; the last two amino acids in actin are Cys374 and Phe375. The 11-amino acid residue peptide identical to the C-terminus of actin, 11-Actin-C, showed little stimulation of AVP activity (Fig. 4). However, in the presence of DNA, AVP activity was greatly stimulated (Fig. 5). 11-Actin-C was shown to bind to AVP(T7 DNA) complexes with a $K_d$ of 9.5 μM. The macroscopic kinetic constants exhibited by AVP(T7 DNA) complexes in the presence of 11-Actin-C were the same as those exhibited by AVP(T7 DNA) in the presence of the viral cofactor pVIc.

Why was DNA required in order for 11-Actin-C to bind to AVP? Quite possibly more than the 11 C-terminal amino acids of actin are required for optimal binding of actin to AVP. DNA has been shown to suppress mutations in pVIc that alter the ability of mutant peptides to bind to AVP [8]. For example, the Cys10′Ala mutant of pVIc has a $K_d$ much greater than 440 μM for binding to AVP [13]. The $K_d$ for wild-type pVIc binding to AVP was 3.5 μM. However, in the presence of 12-mer single-stranded DNA, the $K_d$ for the Cys10′Ala mutant drops more than 60-fold, to 7 μM [13]. The $K_d$ for wild-type pVIc in the presence of DNA was 49 nM.

The homology between the last eight amino acids in actin and the last eight amino acids of pVIc led to the prediction that actin could act as a cellular cofactor for AVP. How similar is actin to pVIc, as a cofactor? The $K_d$ of actin for AVP is 4.2 nM; that of pVIc to AVP is 3.5 μM. Once actin is bound to AVP, the $K_m$ for substrate hydrolysis is 0.34 μM, and the $k_{cat}$ is 0.22 $s^{-1}$; with AVP–pVIc, the $K_m$ is 9.9 μM and the $k_{cat}$ is 0.27 $s^{-1}$. Thus, the $K_d$ for actin binding to AVP is 833-fold lower than that for pVIc binding to AVP, and the $k_{cat}/K_m$ is 24-fold higher. From the point of view of $K_d$ and $k_{cat}/K_m$, actin is a better cofactor than pVIc.

The $K_d$ of 4.2 nM of actin for AVP implies, given the much higher concentration of actin in the cytoplasm, that AVP will bind to actin even in the presence of the multitude of actin binding proteins present in the cytoplasm. Actin has already been shown to be able to act in vitro as a cofactor in the cleavage of cytokeratin 18 which contains two AVP consensus cleavage sequences [1]. Furthermore, actin was shown to be a cofactor and a substrate for its own destruction as incubation of actin with AVP results in the cleavage of actin at two AVP consensus cleavage sequences. Other cytoskeletal proteins also contain AVP consensus cleavage sequences, including tubulin and vimentin. Cleavage of cytoskeletal proteins has been reported to weaken the mechanical structure of the cell [2], and AVP activated by actin may play a significant role in cell lysis and release of nascent virions.

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