Role of the carboxy terminus of herpes simplex virus type 1 DNA polymerase in its interaction with UL42

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Several recent reports implicate sequences at or near the C terminus of the catalytic subunit (POL) of herpes simplex virus type 1 (HSV-1) DNA polymerase in its interaction with the accessory protein UL42. We have investigated further the involvement of this region by three different approaches: anti-idiotype antibodies, a competition ELISA and inhibition of the interaction by peptides. Antibodies raised in rabbits to peptides corresponding to regions of POL all reacted in Western blots with POL. Surprisingly, the sera raised against C-terminal peptides (amino acids 1221 to 1235 and 1224 to 1235) also reacted with UL42. The UL42 reactivity was shown to be due to the presence of anti-idiotype antibodies, providing direct evidence for complementarity of the structure of the extreme C terminus of POL to a region of UL42. To measure the contribution of the C terminus of POL to UL42 binding we developed a competition ELISA using POL, a truncated polymerase lacking the carboxy-terminal 27 amino acids (POLd1) and UL42. UL42 binding to immobilized POL was inhibited approximately four times more effectively by competition, in solution, with POL than with POLd1, indicating that the C-terminal 27 amino acids of POL are responsible for at least 75% of the binding energy. A peptide corresponding to these 27 amino acids (residues 1209 to 1235) inhibited both the POL-UL42 interaction and the stimulation of POL by UL42 and did so more effectively than peptides corresponding to amino acids just away from the C terminus (residues 1195 to 1223 and 1177 to 1195).

Introduction

The minimal essential requirements for replication of herpes simplex virus type 1 (HSV-1) DNA are an origin of replication and the products of seven viral genes (Challberg, 1991; Weller, 1991). The linear double-stranded 152 kbp genome contains three origins of replication: a single copy of oriL in the long unique region and two copies of oriS in the repeat regions flanking the short unique region. The seven essential products are encoded by genes UL5, UL8 and UL52, UL9, UL29, UL30, UL42 and UL52. The UL5, UL8 and UL52 products form a helicase-primase complex, an ssDNA-binding protein is encoded by gene UL29 and gene UL9 encodes an origin DNA-binding protein. This manuscript is concerned with the products of the two remaining genes, UL30 and UL42.

Gene UL30 encodes the catalytic subunit (POL) of HSV-1 DNA polymerase (Purifoy & Powell, 1977; Chartrand et al., 1980; Coen et al., 1984; Dorsky & Crumpacker, 1988; Marcy et al., 1990). Gene UL42 encodes a dsDNA-binding protein (Parris et al., 1988) which acts as an accessory protein to POL increasing both the rate of incorporation of deoxyribonucleoside triphosphates into activated DNA (Gallo et al., 1989) and processivity on a defined template-primer (Gottlieb et al., 1990; Hernandez & Lehman, 1990). Similar to its HSV-2 counterpart, ICP34.5 (Vaughan et al., 1985), the UL42 protein can physically associate with POL to form a heterodimer (Gallo et al., 1988; Crute et al., 1989; Gottlieb et al., 1990).

The regions on POL and UL42 responsible for their physical and functional interaction have been the subject of several investigations. For UL42, the amino-terminal residues (315 to 338) of the 488 amino acid protein are sufficient for both binding to, and stimulation of, POL (Tenney et al., 1993a; Gao et al., 1993; Digard et al., 1993b; Monahan et al., 1993) and indeed the first 338 amino acids are sufficient for both HSV-1 DNA replication and virus growth (Digard et al., 1993b; Tenney et al., 1993c). The amino-terminal 20 amino acids are not required for either binding of POL or stimulation (Digard et al., 1993b).

The region on POL responsible for its interaction with UL42 was initially localized to the C-terminal 227 amino acids (Digard & Coen, 1990). Subsequent investigations to localize further the essential regions have led to some controversy. On the one hand, Digard et al. (1993a) and Stow (1993) identified the C-terminal 19 and 27 amino acids respectively, residues 1216 to 1235 and 1209 to...
1235, as being crucial for both physical interaction with, and stimulation by, UL42. On the other hand, Tenney et al. (1993b) found the C-terminal 19 amino acids were dispensable for both functions and reported that sequences further upstream (residues 1195 to 1216) stabilized the physical interaction with UL42 but were not required for stimulation of activity. Additionally, Monahan et al. (1993) deduced from coprecipitation experiments employing an antiserum against amino acids 1216 to 1224 of POL that these residues may not be essential for the physical interaction.

To shed further light on this situation we have developed an ELISA-based POL–UL42 interaction assay different from the co-immune precipitation techniques used in the earlier studies. The assay utilized purified POL and UL42 proteins as well as POLED1, a truncated version of POL with a deletion of the C-terminal 27 amino acids. The results clearly demonstrate a critical role for these C-terminal 27 amino acids in the interaction with UL42. Further evidence for the involvement of this region was provided by the presence of UL42-reactive anti-idiotypic antibodies in the serum of rabbits immunized with peptides from the extreme C-terminus of POL.

### Methods

Cells and recombinant baculovirus. Spodoptera frugiperda (SF) cells (strain IPLB-SF-21; Kitts et al., 1990) were maintained in TC100 medium (Life Technologies) containing 5% (v/v) fetal calf serum (FCS), penicillin (100 units/ml) and streptomycin (100 µg/ml). The Autographa californica nuclear polyhedrosis virus (AcNPV) recombinants AcUL30, AcPOLd1 and AcUL42, which overexpress POL, POLED1 and UL42 proteins respectively, have all been described (Stow, 1992, 1993). Preparation and titration of virus stocks were carried out as described (Brown & Faulkner, 1977; Matsuura et al., 1987).

**Purification of proteins.** The UL42 protein was purified using only the DNA-cellulose chromatographic step and buffers of the more extensive procedure described by Gottlieb et al. (1990). The protein was extracted from AcUL42-infected SF cells in high salt and dialysed against low-salt buffer before chromatography. POL and POLED1 proteins were purified using the procedure of Gottlieb et al. (1990) except that the cell extract was resuspended in 20 mM-HEPES pH 7.9, 25% glycerol, 600 mM-NaCl, 1.5 mM-MgCl₂, 0.2 mM-EDTA and 1 mM-DTT instead of buffer B. Single-stranded DNA-cellulose (Sigma) was used instead of DNA-agarose. The protease inhibitor bestatin (5 µM) was included in all buffers. Eluted fractions containing purified proteins were identified by SDS–PAGE and Coomassie blue staining, pooled, aliquoted and stored at –70°C.

**Measurement of protein concentration.** Protein concentrations were measured using a Pierce biinchromonic acid protein assay kit with a solution of BSA as a standard. The relative concentrations of POL and POLED1 were also estimated by means of an ELISA. Doubling dilutions of the proteins were coated overnight onto wells of a microtitre plate and bound protein was detected with a 1:100 dilution of a mixture of antisera 22870 and 22871, specific for residues 679 to 690 and 789 to 800 of POL (Table 1). Bound antibodies were detected by incubation with horseradish peroxidase (HRP)-conjugated Protein A (Sigma) diluted 1:1000 and subsequent incubation with the chromogenic enzyme substrate 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) in citrate phosphate buffer (pH 4.0) containing 0.01% hydrogen peroxide. After a 15 to 30 min period of colour development (which was linear with time), the plates were read on a Multiskan plate reader at 405 nm (Titertek; ICN Biomedicals).

**SDS–PAGE and Western blotting.** Purified proteins were analysed on 5 to 12.5% gradients of polyacrylamide using the buffer system of Laemmli (1970). Separated proteins were stained with Coomassie blue or blotted onto nitrocellulose membranes as described (Towbin et al., 1979). Transferred proteins were incubated overnight with antisera and bound antibodies were revealed using HRP-conjugated Protein A and a chromogenic substrate (4 chloro-1-napthol; Bio Rad).

**Measurement of DNA concentration.** The concentration of DNA was measured on a Hoefer minifluorometer (model TK0 100) using the fluorimetric dye bis-benzimidazole following the procedure described by the manufacturer. The DNA standard used was calf thymus DNA (Hoefer).

**Oligopeptides.** Peptides (Table 1) were synthesized by continuous flow Fmoc chemistry as previously described (Atherton & Sheppard, 1989; McLean et al., 1991). Monomeric peptides were purified by preparative reverse-phase HPLC (Owsianka et al., 1993). The Mr values of monomeric peptides were determined by fast atom bombardment mass spectrometry (M-Scan) and corresponded to the expected weights. The amino acid compositions of branched peptides were determined by amino acid analysis (Cambridge Research Biochemicals) and were in agreement with expectations. Peptides 6 and 19 of UL42 have been described previously (Owsianka et al., 1993).

**Production and titration of antisera.** Monomeric peptides were first coupled via a tyrosine residue to β-galactosidase (Sigma) (Bassiri et al., 1979). Branched peptides, which consist of eight copies of the peptide on a branched lysine core (Tan, 1988) were used directly without coupling to a carrier protein. The methods used and the immunization schedule have been described in detail elsewhere (McLean et al., 1991).

Sera were titrated by ELISA on microtitre wells (Immulon 1, Dynatech) coated with purified POL (0.02 µg/well) or UL42 (0.04 µg/well) diluted in PBS. Plates were blocked with 100 µl of 2% BSA in PBS for 1 h at 37°C. After blocking the plates were washed extensively with PBS containing 0.3% Tween 20 and blotted dry. Sera were initially titrated 10-fold, in PBS containing 2% FCS, and then in serial fourfold dilutions. The diluted sera were incubated in the protein-coated wells and bound antibodies were detected with HRP-conjugated Protein A and chromogenic substrate (ABTS) as described above. The serum titres were determined as the reciprocal of the maximum dilution which yielded an absorbance of 0.5. All results are the mean of duplicate determinations.

**Absorption of sera with POL- or UL42–Sepharose.** Purified POL (100 µg) and UL42 (26 µg) were coupled to 200 µg CNBr-activated Sepharose (Pharmacia) according to the manufacturer's instructions. After coupling the beads were washed, resuspended in 350 µl of 25 mM-HEPES pH 7.5 buffer. The POL–Sepharose and UL42–Sepharose beads were each divided into two equal portions and incubated with 200 µl of either POL- or UL42-specific antisera by gentle end-over-end rotation at 4°C. After 36 h the beads were allowed to settle, and the sera were removed and added to the second portion of the cognate beads. Incubation was continued for a further 24 h as above. The sera were removed and titrated, together with non-absorbed sera, against POL and UL42 proteins.

**POL–UL42 ELISA interaction assay.** Microtitre wells were coated with 0.04 µg of purified POL, blocked with BSA and washed as described above. Fifty µl of purified UL42, at the concentrations indicated in the text, was then added to each well and incubated for 1 h at 37°C. Following further washes, 50 µl of the UL42-specific monoclonal antibody (MAB) Z1F11 (Schenk et al., 1988) diluted 1:400 in PBS containing 2% FCS was reacted for 1 h at 37°C. The wells were
Table 1. Titres against POL and UL42 of sera raised against peptides from different regions of POL

<table>
<thead>
<tr>
<th>Form of immunizing peptide</th>
<th>Serum no.</th>
<th>Amino acid residues in POL</th>
<th>Peptide sequence of immunogen</th>
<th>Serum titre* against POL</th>
<th>Serum titre* against UL42</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monomeric coupled to β-galactosidase</td>
<td>22857</td>
<td>56–67</td>
<td>QKPTGPTQRHTY</td>
<td>640 &lt; 10</td>
<td>640 &lt; 10</td>
</tr>
<tr>
<td>Branched†</td>
<td>22870</td>
<td>679–690</td>
<td>GEREPFEGARETA (Y)</td>
<td>160 &lt; 10</td>
<td>640 2560</td>
</tr>
<tr>
<td>Branched‡</td>
<td>22871</td>
<td>787–800</td>
<td>RSIROSPFPEEA (Y)</td>
<td>40 &lt; 10</td>
<td>40 &lt; 10</td>
</tr>
<tr>
<td>Branched‡</td>
<td>22874</td>
<td>1224–1235</td>
<td>(Y) RRMLHRAFDTLA</td>
<td>640 2560</td>
<td>653 360 &lt; 10</td>
</tr>
<tr>
<td>Branched‡</td>
<td>114</td>
<td>1206–1220</td>
<td>(LRTAGFGAVGAGATA) 8KvA</td>
<td>10 &lt; 10</td>
<td>10 &lt; 10</td>
</tr>
<tr>
<td>Branched‡</td>
<td>115</td>
<td>1206–1220</td>
<td>(LRTAGFGAVGAGATA) 8KvA</td>
<td>&lt; 10 &lt; 10</td>
<td>&lt; 10 &lt; 10</td>
</tr>
<tr>
<td>Branched‡</td>
<td>112</td>
<td>1221–1235</td>
<td>(EETRRMLHRAFDTLA) 8KvA</td>
<td>2560 40960</td>
<td>&lt; 10 &lt; 10</td>
</tr>
<tr>
<td>Branched‡</td>
<td>113</td>
<td>1221–1235</td>
<td>(EETRRMLHRAFDTLA) 8KvA</td>
<td>653 360 &lt; 10</td>
<td>&lt; 10 &lt; 10</td>
</tr>
</tbody>
</table>

* The titres of the different sera against POL and UL42 were determined by ELISA on wells coated with either POL or UL42 using sera initially diluted 10-fold followed by serial fourfold dilutions.
† Tyrosine residues in parentheses (Y) were added to facilitate coupling of the peptide to a carrier protein.
‡ Branched peptides consisted of eight copies each synthesized onto a branched lysine core (Tam, 1988).

Results

UL42 reactivity of antibodies raised against C-terminal POL peptides

To generate POL-reactive antisera, rabbits were immunized with peptides corresponding to different regions of HSV-1 DNA polymerase (Table 1). In initial experiments, monomeric peptides coupled to β-galactosidase were utilized and the reactivity of the sera was examined by Western blotting. Sera 22857, 22870 and 22871 reacted with POL and, as expected, did not react with UL42. However one serum, 22874, generated against a peptide from the extreme C terminus of POL reacted specifically not only with POL (Fig. 1a, lanes 2 to 5) but also with UL42 (lanes 7 and 8). No reactivity with POL (lane 6) or with UL42 (data not shown) by the preimmune sera was observed. A possible explanation for the UL42-reactive antibodies in serum 22874 was considered to be the presence of anti-idiotype antibodies. Further experiments were performed to examine this possibility.

To exclude the possibility that reactivity with UL42 might arise from a spurious cross-reactivity of anti-β-galactosidase antibodies in serum 22874 (β-galactosidase was used as a carrier for the peptides) and also to localize further the region at the carboxy terminus of POL giving rise to the anti-UL42 antibodies, sera were raised against four branched peptides spanning amino acids 1177 to 1235 of POL (Table 1). The branched peptides consist of eight copies of the peptide synthesized onto a branched lysine core (Tam, 1988) and therefore contain only sequences present in POL by between 10- and 36-fold.

DNA precipitation assay. Activated calf thymus DNA was labelled with 32P, incubated with peptide for 15 min and then centrifuged to pellet any DNA–peptide complexes as described (Owsianska et al., 1993).
was also measured by ELISA using purified proteins. The results are shown in Table 1 and confirmed those found by Western blotting. In particular, sera 22874 and 112 were reactive with both POL and UL42 although serum 113 raised against the same branched peptide as serum 112 reacted only with POL. None of the preimmune sera showed any reactivity with either POL or UL42 (data not shown).

To determine whether the UL42-reactive antibodies in serum 112 could be specifically absorbed by the UL42 protein, the serum was incubated with Sepharose to which either purified POL or UL42 (Fig. 2) had been covalently coupled. The upper panel of Fig. 3 shows that the UL42 reactivity was completely absorbed by the UL42-Sepharose, whereas POL reactivity was reduced by only fourfold. This reduction is most likely nonspecific and due to the Sepharose matrix itself as UL42 reactivity was similarly reduced fourfold by the POL–Sepharose matrix whereas POL reactivity was reduced about 16-fold (lower panel). The remote possibility that the branched peptide corresponding to the C terminus of POL, used to produce serum 112 might mimic UL42, was excluded by the observation that the reactivity of serum 112 with POL but not with UL42 could be blocked by this branched peptide (data not shown).

Taken together, these experiments show that the UL42 reactivity is due to UL42-specific antibodies. We conclude that the UL42 reactivity in sera 22874 and 112 is most probably due to the presence of anti-idiotype antibodies in the sera and that this provides direct evidence for complementarity of the structure of the extreme C terminus of POL to a region of UL42.

**POL–UL42 interaction assay**

To investigate the interaction between POL and UL42, an ELISA assay was developed using purified proteins (Fig. 2). In separate experiments, POL, POLd1 and UL42 proteins were found to be stable in the buffers used in the interaction and competition ELISAs (data not shown). In the interaction assay, UL42 binding to POL-coated microtitre wells was detected with the UL42-specific MAb Z1F11. In preliminary experiments the amounts of POL, UL42, Z1F11 and anti-mouse MAb were optimized. Fig. 4 shows the characteristics of the assay and demonstrates that the amount of Z1F11 bound was dependent on both the presence of UL42 and POL. At concentrations of UL42 above 0.2 lag/well the increase in absorbance was due to non-specific binding of UL42 in the absence of POL. In all subsequent experiments wells were coated with 0.04 lag of POL and then incubated with 0.1 µg of UL42. The possibility that the observed interaction was mediated by DNA in the protein preparations could be excluded since the DNA...
content was found to be less than 12.5 ng DNA/µg POL and 1 ng DNA/µg UL42.

**Inhibition of the POL–UL42 interaction by POL and POLd1**

To measure the contribution of the C terminus of POL to UL42 binding a competition ELISA was established whereby binding of UL42 to POL was inhibited by competition in solution with either POL or POLd1, a truncated polymerase lacking the C-terminal 27 amino acids (Stow, 1993). Again POLd1 was produced in insect cells infected with a recombinant baculovirus and the purified protein which was free of contaminating DNA is shown in Fig. 2. The competition assay was performed so that binding of UL42 to immobilized POL took place in the presence of various concentrations of either POL or POLd1. The results of such an experiments are shown in
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Inhibition of UL42 binding to immobilized POL by competition, in solution with POL or POLd1. The POL–UL42 interaction was assayed as usual except that UL42 was added in the presence of either POL (□) or POLd1 (●). The concentration of competing protein was determined using the Pierce colorimetric protein assay.

Fig. 5 in which the relative gradients of the plots demonstrate that soluble POL was approximately four times more effective than was POLd1 in inhibiting binding to the immobilized protein. This indicates that the C-terminal 27 amino acids of POL are responsible for at least 75% of the binding energy.

Three control experiments, important for the validity of the above finding, were performed. Firstly, it was shown that [3H]dTTP was incorporated into a poly (dA)-oligo (dT) template (Tenney et al., 1993) equally well by POL and POLd1 and that incorporation by POL but not POLd1 was stimulated by UL42 (data not shown). This experiment confirmed that the purified proteins behaved as shown previously (Stow, 1993) for the partially purified proteins. Secondly, the proteins BSA and β-galactosidase were used as controls in place of POL and POLd1 and neither inhibited UL42 binding (data not shown). Thirdly, because the result is critically dependent on knowing the relative protein concentration of POL and POLd1, two independent methods were used to obtain this value. The first was a colorimetric protein assay (Pierce) which, although not expected to yield reliable absolute values, should give accurate relative values since 1208 amino acids (of 1235) are common to the two proteins. The second was an immunological assay based on the reactivity of antibodies 22870 and 22871 (Table 1) which are reactive with the central region of the proteins. Serial twofold dilutions of POL and POLd1 were bound to microtitre plates and the amount of the two antibodies bound to each of the proteins was determined by ELISA and plotted. By interpolation within the data, and assuming that the antibodies reacted equally well with the two proteins, the protein dilutions giving the same absorbance (0.6) was found and their relative concentrations established (data not shown). The two methods gave relative concentrations that agreed to within 10%.

Inhibition of the POL–UL42 interaction by POL peptides

In another approach to assess the importance of sequences at or near to the C terminus of POL in its interaction with UL42, peptides corresponding to these regions were tested for their ability to interfere with both the physical and functional interaction (Fig. 6a, b). The functional interaction was measured by the incorpor-
reactive, antibodies in the sera of rabbits immunized with complementarity with a region on UL42. Second, by that the extreme C-terminal 27 amino acid peptide a branched peptide comprising the C-terminal 15 amino acids of POL provides direct evidence for its physical interaction with DNA, their ability to precipitate DNA was tested (Owsianka et al., 1993). The results are shown in Table 2. Of the three peptides tested, peptide 1209–1235 corresponding to the C-terminal 27 amino acids was a potent inhibitor in both assays, peptide 1195–1223 displayed low activity in the interaction assay but appreciable activity in the functional assay and peptide 1177–1195 was inactive in the interaction assay but showed appreciable activity in the functional assay. These data confirm the importance of the C terminus (1195–1235) but do not exclude the possible involvement of upstream sequences in the interaction of POL and UL42.

Interaction of peptides with DNA

To investigate whether the peptides interfered with the functional interaction of POL and UL42 by binding to DNA, their ability to precipitate DNA was tested (Owsianka et al., 1993) using peptide concentrations of 50 μM and 100 μM. The results are shown in Table 2. Peptides 6 and 19 from UL42 were used as controls and, as found previously (Owsianka et al., 1993), peptide 6 did not precipitate DNA whereas peptide 19 did. Neither of the polymerase peptides 1195–1223 and 1209–1235 precipitated DNA at either concentration although peptide 1177–1195 appeared to do so weakly.

Discussion

By three independent methods we have demonstrated the importance of sequences within the extreme C-terminal 27 amino acids of POL in its physical interaction with UL42. First, the presence of anti-idiotypic, UL42-reactive, antibodies in the sera of rabbits immunized with a branched peptide comprising the C-terminal 15 amino acids of POL provides direct evidence for its physical complementarity with a region on UL42. Second, by means of a POL–UL42 interaction ELISA we showed that the extreme C-terminal 27 amino acid peptide (residues 1209 to 1235) blocked the physical interaction between the two proteins. Third, comparison of the abilities of POL and a truncated version, POLd1, to compete for UL42 binding in solution indicated that removal of the C-terminal 27 amino acids of POL reduced its affinity for UL42 by fourfold. Since the deleted sequences correspond exactly with those of a peptide which effectively blocked the physical interaction of POL and UL42 we conclude that these amino acids (1209 to 1235) directly contribute 75% of the binding energy of POL to UL42. The residual 25% of binding energy is presumably contributed by sequences elsewhere in POL. Our results therefore support the findings of Digard et al. (1993a) and Stow (1993). It is possible that the physical interaction observed by Tenney et al. (1993b) between UL42 and a truncated polymerase lacking the C-terminal 19 amino acids may represent weaker binding afforded by residues 1195 to 1216, although this interaction was not detected by Digard et al. (1993a). This possibility is consistent with the slight inhibition of the POL–UL42 physical interaction seen with peptide 1195–1223. The observation (Monahan et al., 1993) that an antiserum against amino acids 1216 to 1224 of POL coprecipitates POL and UL42 does not exclude the possibility that these residues interact with UL42 since they could simultaneously contact both UL42 and the serum antibodies.

The important feature of anti-idiotypic antibodies is that their paratope (combining site) mimics the structure of the original antigen. They have been used extensively to study receptor–ligand interactions and are prepared by first making ligand-specific antibodies which are in turn used to generate anti-idiotypic antibodies capable of binding to the receptor (reviewed by Schick & Kennedy, 1989). More recently anti-idiotypic antibodies have been produced that mimic the structure of the active site of acetylcholinesterase and possess catalytic cholinesterase activity (Izadyar et al., 1993). Antibodies and anti-idiotypic antibodies can be produced concomitantly in the same animal (Cleveland & Erlanger, 1986). The production of anti-idiotypic antibodies by rabbit 112 and not rabbit 113 must reflect different immunological repertoires of these two animals.

In principle, it should be possible to identify the region(s) on UL42 with which the C terminus of POL interacts by epitope-mapping the anti-idiotypic, UL42-reactive antibodies. We have attempted to do this by utilizing a set of 96 pentadecapeptides spanning the whole of the UL42 protein (Owsianka et al., 1993). However, no single peptides were unambiguously recognized by sera 22874 or 112 (data not shown). This finding suggests to us that no single contiguous region on UL42 is responsible for binding the C terminus of POL but rather the site is formed by two or more non-contiguous

<table>
<thead>
<tr>
<th>Table 2. Precipitation of DNA by peptides</th>
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<tbody>
<tr>
<td>Peptide</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>None</td>
</tr>
<tr>
<td>Residues 23–28 of UL42 (peptide 6†)</td>
</tr>
<tr>
<td>Residues 89–102 of UL42 (peptide 19†)</td>
</tr>
<tr>
<td>Residues 117–1195 of POL</td>
</tr>
<tr>
<td>Residues 1195–1223 of POL</td>
</tr>
<tr>
<td>Residues 1209–1235 of POL</td>
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* The radioactivity remaining in solution in the absence of peptide was 1339 c.p.m. per 10 μl.
† Peptides previously described by Owsianka et al. (1993).
regions. This conclusion is supported by the finding that none of the UL42 pentadecapeptides was able to disrupt specifically the POL–UL42 functional interaction (Owsianka et al., 1993) or physical interaction (unpublished data). Interestingly, analysis of insertion and deletion mutants of UL42 have also implicated non-contiguous regions within the protein as being important for its interaction with POL (Monahan et al., 1993; Digard et al., 1993b).

Our results on the binding of POL and UL42 are reminiscent of the interaction between the large and small subunits of ribonucleotide reductase enzymes having the general form $\alpha_4\beta_2$ which is mediated by the C terminus of the small subunit (R2) complexing with the large subunit (R1) (reviewed by Marsden, 1992; Conner et al., 1993b). In both cases the C terminus of one subunit, POL or R2 (Clement et al., 1991, 1992), contribute most or all of the binding energy, whilst the interacting sequences of the other subunit, UL42 or R1, appear to be discontinuous (Conner et al., 1993a).

The concentration of peptide required to inhibit the POL–UL42 physical interaction by 50% was approximately 25 $\mu$M (Fig. 6a), a value comparable to that observed for the HSV-1 R1–R2 interaction by the R2 C-terminal nonapeptide YAGAVVNDL (Dutia et al., 1986; Cohen et al., 1986; Conner et al., 1992). However in contrast to the inhibition of the R1–R2 interaction it was not possible to inhibit completely the POL–UL42 physical interaction. It is not clear whether this reflects our finding that other sequences within POL may contribute weakly to the POL–UL42 interaction. Inhibition by the C-terminal 27 amino acid POL peptide does not appear to be due to a non-specific ‘stickiness’ since at concentrations as high as 1 mM, it did not inhibit the R1–R2 interaction, in contrast to the marked inhibition by peptide YAGAVVNDL (data not shown).

The concentration of C-terminal peptide required to inhibit the POL–UL42 functional interaction by 50% was very similar (approximately 25 $\mu$M; Fig. 6b). Peptide 1177–1195 inhibited the functional interaction, most probably by binding to the template DNA (Table 2) rather than by any specific mechanism. A surprising finding was that the peptide comprising residues 1195 to 1223 inhibited the POL–UL42 functional interaction (IC$_{50}$ 120 $\mu$M) but had a much smaller effect on the physical interaction. This inhibition of the functional interaction was not due to the peptide binding to DNA (Table 2) and is probably a consequence of the peptide inhibiting POL activity in the absence of UL42 (data not shown).

Amongst the reasons for studying HSV protein–protein interactions is the possibility that they may be targeted as an antiviral strategy (reviewed by Marsden, 1992). Mimetic compounds have been developed (Bio-Mega patent, 1990) that inhibit HSV replication in tissue culture (M. Luizzi and R. Deziel, Bio-Mega, personal communication). It remains to be determined whether mimetic compounds based on the peptides described in our paper or on other compounds that block the POL–UL42 interaction, will have any effect in vivo.

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We thank Drs Paul Digard and Don Coen, who have independently shown that C-terminal POL peptides can disrupt the interaction between POL and UL42 and have obtained evidence suggesting an $\alpha$ helical structure for the C-terminal POL peptide, for communicating their results to us prior to publication.

References


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