Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 10950–10954, November 1992
Biochemistry

Recombination *in vitro* between herpes simplex virus type 1 *a* sequences

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Contributed by I. R. Lehman, August 7, 1992

ABSTRACT **We have partially purified an activity from extracts of cells infected with herpes simplex virus type 1 that mediates recombination between repeated copies of the 317-base-pair *a* sequence of herpes simplex virus type 1. Recombination leads to deletion of a *lacZ* indicator gene situated between two directly repeated copies of the *a* sequence and is scored by transformation of *lacZ*⁻ *Escherichia coli*. The two products of the reaction can be observed directly by restriction enzyme digestion and Southern blot analysis. The recombinase activity is also detectable, but at a lower level, in uninfected cell extracts. The DNA substrate must contain the two *a* sequences arranged in direct orientation to generate the *lacZ* deletion. However, when the *a* sequences are arranged in inverted orientation, an inversion results. A substrate with two homologous sequences of size and G + C content similar to the *a* sequence undergoes recombination at a much lower frequency. The reaction requires a divalent cation (Mg^{2+} or Mn^{2+}) but not ATP or any other nucleoside triphosphate. The simple requirements and specificity for the *a* sequence suggest that the recombination may proceed by a site-specific mechanism.**

The linear 152-kilobase (kb) herpes simplex virus type 1 (HSV-1) genome consists of two components designated U_L and U_S, flanked by the inverted repeated sequences *ab* and *a'b'* for U_L and *a'c'* and *ca* for U_S (1). During infection, the L and S components invert relative to each other by recombination to produce four isomeric forms of the viral DNA (2–4). The *a* sequences appear to be essential for a high level of inversion. Insertion of an *a* sequence into U_L results in additional inversions and deletions (5, 6), and an HSV-1 mutant that lacks the L–S junction, including the *a* sequence, does not undergo inversion (7, 8). Inversion of DNA flanked by two *a* sequences that have been integrated into a stable primate cell line requires HSV-1 infection and amplification dependent upon an HSV-1 origin in cis (9).

The *a* sequence from HSV-1 KOS consists of 83% G + C. It is flanked by two copies of a 20-base-pair (bp) direct repeat (DR1) and has 11 copies of a 12-bp repeat element (DR2) in the center, separating two unique sequence components (Ub and Uc) (10–12). Under superhelical tension, DR2 forms an anomalous structure that has been termed anisomorphic DNA (13, 14). Attempts to identify a specific region within the *a* sequence that is required for the inversion have thus far been inconclusive (15, 16).

HSV-1 DNA undergoes a high frequency of homologous recombination during replication; the *a* sequence could, therefore, be a hot spot for homologous recombination (17, 18). Alternatively, the recombination could proceed by a site-specific mechanism similar to that of the Flp protein of the yeast 2-μm plasmid (19, 20).

We have described (21) an assay that measures recombination between HSV-1 *a* sequences *in vivo*. With this assay, we found that after transfection into primate cells, a plasmid containing two *a* sequences and an HSV-1 origin undergoes a high frequency of recombination upon superinfection with HSV-1. We also observed a low level of recombination in uninfected cells. Using this assay, we have been able to detect an activity that mediates recombination between *a* sequences in fractionated extracts of HSV-1-infected cells.

MATERIALS AND METHODS

Materials. Plasmids pRD104, pRD105, and pRD110 were constructed as described (21). Restriction enzymes were obtained from New England Biolabs and United States Biochemical. DNA polymerase I large fragment, T4 DNA ligase, acetylated bovine serum albumin, proteinase K, and ammonium sulfate were obtained from Bethesda Research Laboratories. Heparin, leupeptin, pepstatin A, phenylmethylsulfonyl fluoride, dithiothreitol, spermidine, and 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-gal) were from Sigma. [α-^{32}P]dCTP (800 Ci/mmol; 1 Ci = 37 GBq) was obtained from Amersham. CV-1 cells were from the American Type Culture Collection. Sterile Hepes, glutamine, and penicillin/streptomycin were from GIBCO. Fetal calf serum was from Irvine Scientific. Heparin was coupled to Sepharose CL-4B (Pharmacia) as described (22). Centricon-30 protein concentrators were from Amicon. A prepacked Superose 12 gel filtration column, molecular weight standards, and poly(dI-dC) were obtained from Pharmacia. Phosphocellulose was obtained from Whatman. A Prime-It labeling kit obtained from Stratagene was used to prepare ^{32}P-labeled linear pRD105. A Geneclean DNA purification kit obtained from Bio 101 was used to isolate DNA from agarose gel slices.

Buffers. Buffer A contained 20 mM Hepes (pH 7.6), 10% (vol/vol) glycerol, 1 mM dithiothreitol, 1 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, leupeptin (2 μg/ml), pepstatin A (2 μg/ml), and 10 mM sodium bisulfite. The NaCl concentration is indicated in brackets; for example, buffer A[0.1] contains 0.1 M NaCl. Buffer B contained 20 mM Hepes (pH 7.6), 5% glycerol, 1 mM magnesium acetate, 2 mM dithiothreitol, 3 mM spermidine, 0.1 mM EDTA, and bovine serum albumin (100 μg/ml).

Cells and Virus. CV-1 cells were grown at 37°C in Dulbecco's modified Eagle's medium containing 10% (vol/vol) fetal calf serum, supplemented with 2 mM glutamine, penicillin (100 units/ml), and streptomycin (100 mg/ml), and buffered with 10 mM Hepes (pH 7.5). CV-1 cells were grown in roller bottles and harvested when slightly subconfluent. RΔ305, a thymidine kinase-deficient mutant of HSV-1(F) (23), was used to infect roller bottle cultures of CV-1 cells at a multiplicity of infection of 5. HSV-1-infected CV-1 cells were harvested 18 h after infection. Nuclear and cytosolic extracts

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Abbreviations: HSV-1, herpes simplex virus type 1; amp^R, ampicillin resistance; X-gal, 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside.

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were prepared as described (24). Competent *Escherichia coli* cells (DH5α, *recA*$^-$, *lacZ*$^-$) were prepared as described (21, 25) and frozen at −70°C. Transformation efficiency was 1 × 10^7 to 1 × 10^8 colonies per μg of plasmid DNA.

Recombination Assay. The substrate used to measure recombination (21) contains two HSV-1 *a* sequences arranged in direct orientation. Recombination between the two *a* sequences deletes the DNA between them, producing two circular DNA molecules; one containing the *lacZ* gene and the other containing the β-lactamase gene (for ampicillin resistance, amp^R) and the plasmid origin of replication. After incubation with enzyme, the DNA is transformed into *E. coli* (DH5α, *recA*$^-$, *lacZ*$^-$) and plated on LB plates containing ampicillin and X-gal. Colonies that arise from transformation with the parent plasmid stain blue. Because the *lacZ*-containing recombination product lacks the amp^R gene and an origin of replication, it is lost. However, transformation with the other product, containing the amp^R gene and the replication origin, gives rise to white colonies. The recombination frequency is expressed as the percentage of white colonies.

Recombination substrates were incubated with the recombinase fractions in buffer B (25 μl) at 37°C for 15 min. Assays performed with the heparin-Sepharose and phosphocellulose fractions contained 2.5 μg of poly(dI-dC). Reactions were stopped by the addition of 2 μl of 0.5 M EDTA and 1 μl of proteinase K (20 mg/ml), and the incubation was continued for 30 min. Potassium acetate (75 μl of 0.4 M at pH 4.8) containing tRNA (150 μg/ml) was added, and the mixture was extracted with phenol/chloroform. The DNA was precipitated by the addition of 0.2 ml of absolute ethanol. The pellet was washed with 70% ethanol, vacuum-dried, and resuspended in 100 μl of TE (10 mM Tris·HCl, pH 7.5/1 mM EDTA). Typically 1–5 μl was used for the transformation. Cells were plated on LB plates containing ampicillin (100 μg/ml). X-gal was added just prior to spreading the cells.

To analyze the recombination products, white colonies were inoculated into 1-ml cultures of LB containing ampicillin (100 μg/ml) and grown at 37°C overnight. Plasmid DNA was isolated by the alkaline lysis procedure as described (26) and analyzed by restriction enzyme digestion and agarose gel electrophoresis.

Partial Purification of Recombinase Activity. A cytosolic extract of HSV-1-infected CV-1 cells was cleared by centrifugation in a Beckman 45 Ti rotor for 45 min at 40,000 rpm. Protein was precipitated by the addition of ammonium sulfate to 70% saturation. The resulting pellet was resuspended in buffer A to an ionic strength equivalent to 100 mM NaCl and loaded on a 25-ml heparin-Sepharose column equilibrated with buffer A[0.1]. The column was washed with 40 ml of buffer A[0.1] and protein was eluted successively with 40 ml of 0.25 M, 0.5 M, and 1 M NaCl in buffer A. Most of the recombinase activity appeared in the 0.25 M NaCl eluate (Table 1). The 0.25 M eluate (14 ml) was dialyzed against 400 ml of buffer A[0.1] for 4 h and loaded on a 4-ml phosphocellulose column equilibrated in buffer A[0.1]. The column was washed with 4 ml of buffer A[0.1]. The flow-through and wash fractions were combined (11.5 ml) and concentrated to 0.4 ml in a Centricon-30. The concentrated material was applied to a 24-ml Superose 12 gel filtration column, which was eluted at a flow rate of 0.2 ml/min, and 0.5-ml fractions were collected. The major peak of activity appeared in fractions 25–27 (Fig. 1).

Southern Blot Analysis of Recombination Products. After incubation of pRD105 with the Superose 12 fraction in buffer B, the DNA was digested with the indicated restriction endonucleases and subjected to electrophoresis in a 1.0% agarose gel [poly(dI-dC) was omitted from the reaction mixture because it interfered with the analysis]. The DNA was transferred to nitrocellulose in a Stratagene PosiBlot pressure-blot apparatus [the transfer buffer was 20× standard saline citrate (SSC)] and fixed to nitrocellulose in a Stratagene UV Stratalinker model 1800. Bound DNA was probed with linear pRD105, labeled with ^{32}P by the random-primer method (27), and incubated at 60°C for 4 h in 6× Denhardt's solution/5× SSC/0.5% SDS in the presence of denatured sonicated calf thymus DNA (100 μg/ml) (28). The deletion product formed *in vivo* was isolated as described (21). Autoradiography was performed at room temperature.

Table 1. Recombinase activity in heparin-Sepharose eluates of HSV-1-infected and uninfected CV-1 cell extracts

Fraction	Colonies, no. White	Colonies, no. Blue	% white
HSV-1-infected cells			
Flow through	0	3357	<0.03
0.25 M NaCl	46	3039	1.5
0.50 M NaCl	11	2837	0.4
1.0 M NaCl	3	5032	0.06
Uninfected cells			
Flow through	0	3147	<0.03
0.25 M NaCl	11	2766	0.4
0.50 M NaCl	3	2408	0.1
1.0 M NaCl	0	4613	<0.03

One microgram of each fraction was assayed. The concentration of NaCl in each incubation mixture was 40 mM.

RESULTS

Recombinase Activity Is Present in Partially Purified Fractions from HSV-1-Infected and Uninfected CV-1 Cell Extracts. An extract of HSV-1-infected CV-1 cells was chromatographed on heparin-Sepharose, and the eluates were assayed for recombinase activity. As shown in Table 1, most of the recombinase activity appeared in the 0.25 M NaCl eluate (1.5% white colonies), with the 0.5 M NaCl fraction giving 0.4%. Neither the flow-through fraction nor the 1.0 M NaCl eluate showed significant recombinase activity. The frequency of white colonies observed in the absence of protein was 0.003%. The number of blue colonies generated when the heparin-Sepharose fractions were assayed remained constant and corresponded to the number in the control reaction lacking protein, indicating that nuclease activity had been largely suppressed under our assay conditions.

Dra I restriction enzyme analysis of the plasmid DNA from 11 white colonies showed that 6 of them generated a pattern of fragments that was consistent with the predicted amp^R-containing recombination product (Fig. 2 *A* and *C*, lanes 3–7).

When an extract of uninfected CV-1 cells was chromatographed on heparin-Sepharose, recombinase activity was also detected in the 0.25 M and 0.50 M NaCl eluates, but at

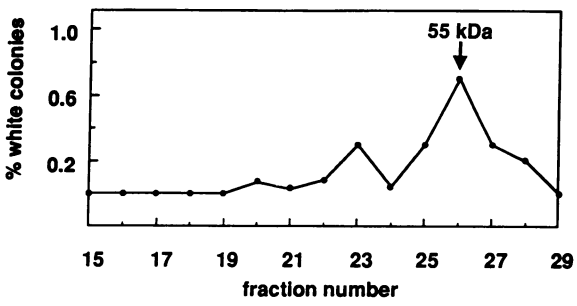

FIG. 1. Superose 12 gel filtration of recombinase activity. The phosphocellulose fraction was filtered through a Superose 12 column and the recombinase activity of the fractions was measured. The peak of activity appeared in fraction 26, which corresponds to 55 kDa.

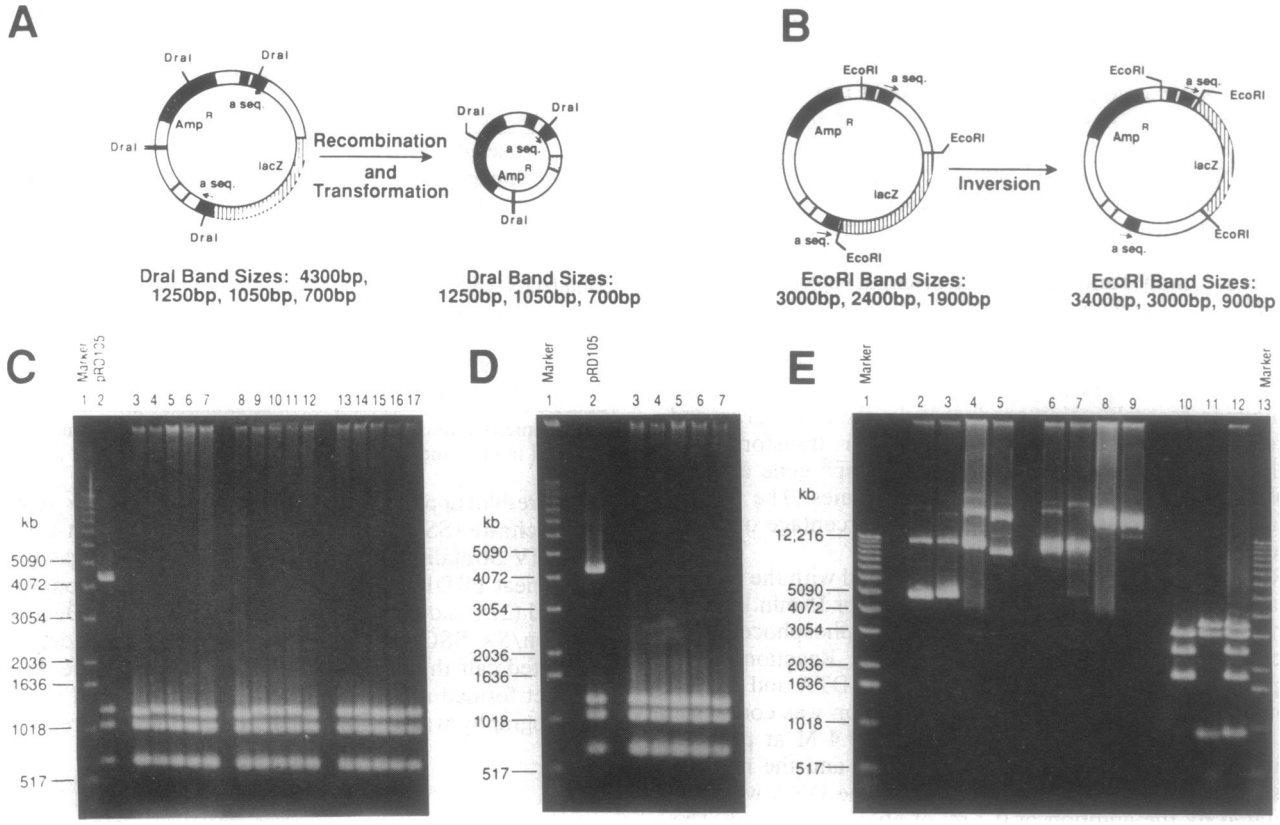

FIG. 2. Analysis of recombination products detected after transformation of *E. coli*. (*A*) *Dra* I sites on pRD105 and the deletion product propagated in *E. coli* are shown with the predicted sizes of the restriction fragments. (*B*) Inversion substrate pRD104 in which the two *a* sequences are arranged in inverted orientation, and the predicted inversion product after recombination. *Eco*RI sites are indicated with the predicted fragment sizes. (*C*) An 0.8% agarose gel analysis of restriction digest of deletion product. Fractions are from HSV-1-infected CV-1 cells. Lanes: 1, 1-kb ladder; 2, *Dra* I digest of the substrate pRD105; 3–7, *Dra* I digest of plasmids obtained after reaction with 0.25 M NaCl heparin-Sepharose fraction; 8–12, *Dra* I digest of plasmids after reaction with phosphocellulose fraction; 13–17, *Dra* I digest of plasmids after reaction with Superose 12 fraction. (*D*) An 0.8% agarose gel analysis of restriction digest of deletion product. Fractions are from uninfected CV-1 cell extract. Lanes: 1, 1-kb ladder; 2, *Dra* I digest of the substrate pRD105; 3–7, *Dra* I digest of plasmids after reaction with 0.25 M NaCl heparin-Sepharose fraction. (*E*) Inversion and intermolecular recombination products. Lanes: 1 and 13, 1-kb ladder; 2–5, supercoiled plasmid DNA; 2, pRD104; 3, inversion product; 4, dimeric intermolecular recombination product; 5, 12.9-kb marker, pVL941/UL9; 6–9, supercoiled DNA treated with topoisomerase I (1 unit, 30 min, 37°C); 6, pRD104; 7, inversion product; 8, dimeric intermolecular recombination product; 9, 12.9-kb marker, pVL941/UL9; 10–12, *Eco*RI digests; 10, pRD104; 11, inversion product; 12, dimeric intermolecular recombination product.

a significantly lower level than in the corresponding fractions from infected cells (0.4% and 0.1% white colonies, respectively). *Dra* I restriction enzyme analysis of plasmid DNA isolated from 13 white colonies generated the pattern expected from a deletion between the *a* sequence in 9 of the colonies (Fig. 2*D*, lanes 3–7).

The recombinase activity in both the HSV-1-infected and uninfected cell extracts required only a divalent cation (Mg^{2+} or Mn^{2+}), but not ATP or any other nucleoside triphosphate. Activity was optimal between pH 6.5 and pH 8.0 (data not shown).

The recombinase activity in the 0.25 M NaCl heparin-Sepharose eluate of the HSV-1-infected cell extract was further purified by passage through a phosphocellulose column and gel filtration through a Superose 12 column. As shown in Fig. 1, activity was eluted during gel filtration at a position corresponding to ≈55 kDa.

Plasmid DNA isolated from the white colonies obtained in assays of the phosphocellulose and Superose 12 fractions produced the predicted *Dra* I restriction pattern in 4 of 8 and 10 of 14 of the colonies, respectively (Fig. 2*C*, lanes 8–12 and 13–17).

The Recombinase Activity Promotes Inversion and Intermolecular Recombination. Recombination between two *a* sequences in inverted orientation would be expected to result in the inversion of the DNA that lies between them (Fig. 2*B*). However, the product of the inversion retains the *lacZ* gene and, consequently, would not produce a white colony after transfection of *lacZ*⁻ *E. coli*. To determine whether the recombinase activity will promote inversions, the Superose 12 fraction was incubated with plasmid pRD104 in which the *a* sequences are in the inverted orientation. After transfection of *E. coli*, the plasmid DNA was isolated from 49 colonies without prior selection and subjected to *Eco*RI restriction enzyme analysis. As shown in Fig. 2*E*, lane 11, the restriction pattern of the plasmid DNA isolated from one of the colonies was consistent with an inversion. Plasmid DNA isolated from several other colonies, when treated with *Eco*RI, produced restriction fragments characteristic of both the substrate plasmid (pRD104) and the inversion product (Fig. 2*E*, lane 12). Although this mixture of fragments could result from the coexistence of both the substrate and inversion product within the same bacterial cell, it could also indicate the presence of a dimeric product generated by an asymmetric intermolecular recombination between *a* sequences on two molecules. Electrophoresis of the DNA from one of these colonies without restriction enzyme digestion revealed a single slowly migrating species whose mobility was consistent with a dimeric plasmid (Fig. 2*E*, lane 4). After treatment with topoisomerase I, its migration during agarose gel electrophoresis was identical to that of a relaxed dimer (Fig. 2*E*, lane 8).

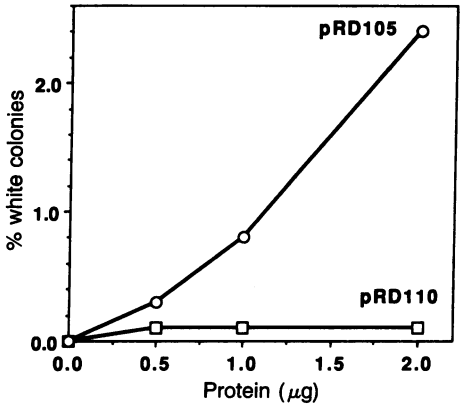

FIG. 3. Sequence specificity of the recombinase reaction. The recombinase reaction was performed using the phosphocellulose fraction with either pRD105 or pRD110 in which the two *a* sequences are replaced by two 370-bp G + C-rich sequences.

Thus, the recombinase activity can generate a deletion, when the two *a* sequences are arranged in direct orientation, and an inversion, when the two *a* sequences are inverted relative to each other. In addition, the recombinase appears to be able to mediate an intermolecular recombination between *a* sequences on different DNA molecules.

The Recombinase Activity Is Specific for *a* Sequences. A plasmid identical to pRD105, but containing two 370-bp G + C-rich sequences (65% G + C) in place of the *a* sequences (pRD110) (21), was incubated with increasing amounts of the phosphocellulose fraction. As shown in Fig. 3, the frequency of white colonies observed with the pRD110 substrate was ≈20-fold lower than that seen with the *a* sequence-containing plasmid. Thus, the recombinase appears to recognize specifically the *a* sequence or a structure imposed by the *a* sequence.

Southern Blot Analysis of the Products of Recombination. The assay for recombinase activity between directly repeated *a* sequences relies on transformation of *E. coli* and, therefore, only detects one of the two predicted products of the reaction, the one able to replicate because of its retention of the plasmid origin and the amp^R gene. To observe both products directly, pRD105 was incubated with the Superose 12 fraction and the products digested with *Ssp* I and *Hin*dIII or with *Eco*RI and *Sst* I (Fig. 4). After agarose gel electrophoresis, the DNA was transferred to nitrocellulose and probed with ^{32}P-labeled pRD105. Digestion of the products with *Ssp* I and *Hin*dIII produced a band that migrated to a position corresponding to the large *lacZ*$^-$-containing product (Fig. 4*C*). A similar band appeared after restriction enzyme digestion of the deletion products formed *in vivo* but was absent from the reaction mixture lacking the Superose 12 fraction. When the *lacZ*$^-$-containing fragment was excised from the gel and digested with a variety of restriction enzymes (*Eco*RI, *Bam*HI, *Cla* I, *Pst* I, *Dra* I, *Nhe* I, *Xmn* I, and *Hin*dIII), a restriction map was generated that was consistent with the predicted structure (data not shown). Additionally, more rapidly migrating bands are apparent that are the product of an endonuclease present in the Superose 12 fraction that specifically cleaves within the *a* sequence. This activity is largely suppressed by the addition of poly(dI-dC). However, because poly(dI-dC) interferes with the Southern blot analysis, it was omitted from this reaction. Treatment of the products with the *Eco*RI and *Sst* I restriction enzymes followed by agarose gel electrophoresis produced a band that corresponded to the 3-kb amp^R origin-containing product (Fig. 4*D*). A similar band was formed *in vivo* (21) but was absent in the reaction mixture lacking the Superose 12 fraction. The additional more rapidly migrating bands are again attributable to specific cleavage at a site within the *a* sequence. To confirm the identity of the 3-kb band, a portion of the restriction digest was electrophoresed as before, and

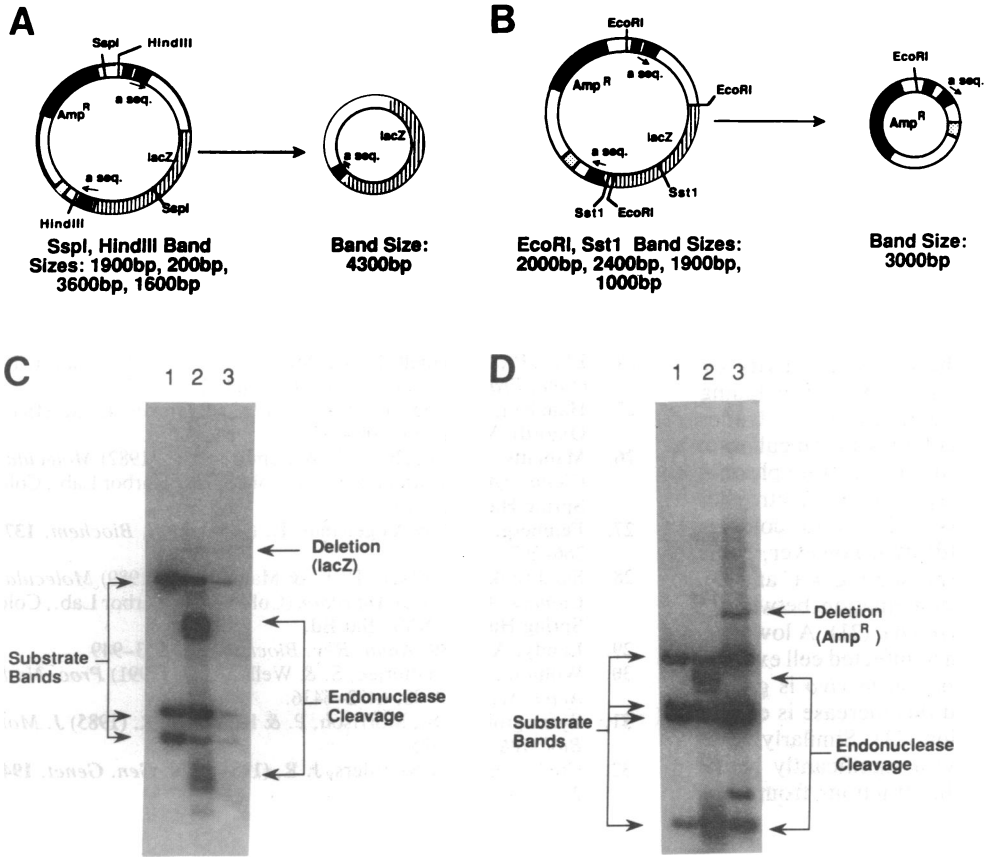

FIG. 4. Southern blot analysis of recombinase products. (*A*) *Ssp* I and *Hin*dIII sites on pRD105 and the *lacZ* deletion product are shown with the predicted restriction fragments. (*B*) *Eco*RI and *Sst* I sites on pRD105 and the amp^R deletion product are shown with the predicted restriction fragments. (*C*) pRD105 was incubated with 500 ng of the Superose 12 fraction for 10 min at 37°C in buffer B containing 40 mM NaCl. The DNA was digested with *Ssp* I and *Hin*dIII, transferred to nitrocellulose, and probed with labeled pRD105. Lanes: 1, no protein; 2, Superose 12 fraction; 3, deletion product generated *in vivo*. Recombination product and substrate bands are indicated by arrows. (*D*) Same reaction as in *C* except that the DNA was digested with *Eco*RI and *Sst* I. Lanes: 1, no protein; 2, Superose 12 fraction; 3, deletion product generated *in vivo*. The sites of endonuclease cleavage within the *a* sequences are indicated.

the DNA was isolated from the 3-kb region of the gel. After ligation and transformation of *lacZ*$^-$ *E. coli*, plasmids were recovered from the white colonies that had a restriction pattern corresponding to the amp^R origin-containing product of the recombination reaction (data not shown).

DISCUSSION

We have partially purified a recombinase activity from extracts of HSV-1-infected mammalian cells that promotes recombination between two copies of the HSV-1 *a* sequence. A similar activity is present in extracts of uninfected cells, but at a significantly lower level. When the *a* sequences are arranged in direct orientation, a deletion is produced; when the *a* sequences are in the inverted orientation, an inversion results in a manner similar to the inversion-segment deletion process characterized by using recombinant HSV-1 (5, 6). Thus, flipping the orientation of one of the two *a* sequences changes the reaction from deletion to inversion, indicating that the crossover point of the recombination occurs within the *a* sequences. Substitution of the *a* sequences by a G + C-rich sequence of approximately the same size resulted in a 20-fold decrease in recombinase activity. A specific sequence or structure within the *a* sequence must, therefore, be required for efficient recombination.

The recombinase activity requires Mg^{2+} (or Mn^{2+}); neither ATP nor other nucleoside triphosphates are needed. The apparent sequence specificity and lack of an external energy requirement are consistent with several well-studied site-specific recombinational systems (19, 20, 29).

Recombinase activity was measured by determining the proportion of white colonies formed after transformation of the plasmid substrate and products of the reaction into *E. coli*. Several nucleases, including one similar to that reported by Wohlrab *et al.* (30) that specifically cleaves within the *a* sequences, are present in our protein fractions (R.C.B. and I.R.L., unpublished observations). However, cleavage of the substrate plasmid at the *a* sequences by this nuclease activity cannot entirely account for the recombination that we observe. The efficiency of transformation of *E. coli* by linear DNA is less than 1×10^{-3} that of circular DNA (31, 32). Moreover, intramolecular ligation of the termini formed by nuclease action to generate a circular product prior to transformation requires ATP, which is not provided in the reaction mixture. The formation of inversion products is even more difficult to explain by a nuclease activity. Inversion would require cleavage within both *a* sequences, followed by ligation of the two half molecules in the inverted orientation. More decisively, however, both products of the deletion as well as the inversion product can be observed directly without resorting to transformation.

We have been able to suppress the nuclease activity by maintaining the Mg^{2+} concentration at 1 mM and including spermidine and poly(dI-dC) in the reaction mixture. Under these conditions, the number of blue colonies after incubation remains unchanged (see Table 1), and the substrate plasmid remains largely intact as judged by agarose gel electrophoresis (data not shown). Nevertheless, 1–2% white colonies are formed. In the absence of poly(dI-dC), however, some cleavage of the *a* sequence is apparent (see Fig. 4 *C* and *D*).

A low but significant level of recombination between *a* sequences occurs in uninfected cells *in vivo* (21). A low level of recombinase activity is also seen in uninfected cell extracts *in vitro*. The frequency of recombination *in vivo* is greatly increased after HSV-1 infection, and this increase is closely associated with viral DNA replication (21). Similarly, fractionated infected cell extracts show a significantly higher recombinase activity than comparable fractions from uninfected cells. However, in the latter case, we cannot yet assess the impact of DNA replication on recombination. Nonetheless, the deletion and inversion products generated in the two systems are very similar.

The significance and normal function of the recombinase activity in uninfected primate cells remain intriguing questions, as does the role of HSV-1 infection in increasing its level.

This research was supported by Grants AI26538 to I.R.L. and AI20211 to E.S.M. R.E.D. is a Predoctoral Fellow of the National Science Foundation.

1. Roizman, B. & Sears, A. (1990) in *Virology*, eds. Fields, B. & Knipe, D. M. (Raven, New York), Vol. 2, pp. 1795–1843.
2. Sheldrick, P. & Berthelot, N. (1975) *Cold Spring Harbor Symp. Quant. Biol.* **39**, 667–678.
3. Hayward, G. S., Jacob, F. J., Wadsworth, S. C. & Roizman, B. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 4243–4247.
4. Delius, H. & Clements, J. B. (1976) *J. Gen. Virol.* **33**, 125–133.
5. Mocarski, E. S., Post, L. E. & Roizman, B. (1980) *Cell* **22**, 243–255.
6. Smiley, J. R., Fong, B. S. & Leung, W. L. (1981) *Virology* **113**, 345–362.
7. Poffenberger, K. L., Tabares, E. & Roizman, B. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 2690–2694.
8. Jenkins, F. J. & Roizman, B. (1986) *J. Virol.* **59**, 494–499.
9. Mocarski, E. S. & Roizman, B. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 5626–5630.
10. Davison, A. & Wilkie, N. (1981) *J. Gen. Virol.* **55**, 315–331.
11. Mocarski, E. S. & Roizman, B. (1982) *Proc. Natl. Acad. Sci. USA* **78**, 7047–7051.
12. Varmuza, S. L. & Smiley, J. R. (1985) *Cell* **41**, 793–802.
13. Wohlrab, F., McLean, M. J. & Wells, R. D. (1987) *J. Biol. Chem.* **262**, 6407–6416.
14. Wohlrab, F. & Wells, R. D. (1989) *J. Biol. Chem.* **264**, 8207–8213.
15. Smiley, J. R., Duncan, J. & Howes, M. (1990) *J. Virol.* **64**, 5036–5050.
16. Chou, J. & Roizman, B. (1985) *Cell* **41**, 803–811.
17. Weber, P. C., Challberg, M. D., Nelson, N. J., Levine, M. & Glorioso, J. L. (1988) *Cell* **54**, 369–381.
18. Weber, P. C., Levine, M. & Glorioso, J. C. (1990) *J. Virol.* **64**, 300–306.
19. Sadowski, P. D., Beatty, L. F., Clary, D. & Ollerhead, S. (1987) in *DNA Replication and Recombination*, eds. McMacken, R. & Kelly, T. J. (Liss, New York), pp. 691–701.
20. Cox, M. M. (1989) in *Mobile DNA*, eds. Berg, D. E. & Howe, M. M. (Am. Soc. Microbiol., Washington), pp. 661–670.
21. Dutch, R. E., Bruckner, R. C., Mocarski, E. S. & Lehman, I. R. (1992) *J. Virol.* **66**, 277–285.
22. Arndt-Jovin, D. J., Jovin, T. M., Bahr, W., Frischauf, A. M. & Marquardt, M. (1975) *Eur. J. Biochem.* **54**, 411–418.
23. Post, L. E., Mackem, S. & Roizman, B. (1981) *Cell* **24**, 556–565.
24. Elias, P., O'Donnell, M. E., Mocarski, E. S. & Lehman, I. R. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 6322–6326.
25. Hanahan, D. (1985) in *DNA Cloning*, ed. Glover, D. M. (IRL, Oxford), Vol. 1, pp. 109–135.
26. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
27. Feinberg, A. P. & Vogelstein, B. (1984) *Anal. Biochem.* **137**, 266–267.
28. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Practical Approach* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), 2nd Ed.
29. Landy, A. (1989) *Annu. Rev. Biochem.* **58**, 913–949.
30. Wohlrab, F., Chatterjee, S. & Wells, R. D. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 6432–6436.
31. Symington, L. S., Morrison, P. & Kolodner, R. (1985) *J. Mol. Biol.* **186**, 515–525.
32. Conley, E. C. & Saunders, J. R. (1984) *Mol. Gen. Genet.* **194**, 211–218.