Characterization of Human Papovavirus BK DNA

PETER M. HOWLEY,* MICHAEL F. MULLARKEY, KENNETH K. TAKEMOTO, AND
MALCOLM A. MARTIN

Laboratory of Biology of Viruses and Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20014

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The DNA of the BK virus (BKV) human papovavirus was found to be heterogeneous, consisting of at least four discrete species of DNA. Only the largest of these four species, BKV DNA (i), which has a molecular weight calculated to be 96% that of simian virus 40 (SV40) DNA, was infectious. Homogeneous preparations of BKV DNA were obtained, however, from virions purified after low multiplicity infections of human embryonic kidney cells. BKV DNA (i) was shown to contain a single R·*Eco* RI and four R·*Hind* cleavage sites. The R·*Eco* RI site was localized in the largest R·*Hind* cleavage fragment. Radiolabeled BKV DNA reassociated slightly faster than SV40 DNA; 20 to 30% polynucleotide sequence homology was demonstrated between the genomes of BKV and SV40 when the reaction was monitored by chromatography on hydroxyapatite.

In the last few years, two classes of hemagglutinating papovaviruses have been isolated from humans. One of these was obtained from the brains of patients with progressive multifocal leukoencephalopathy. Most of the isolates of this category of human papovaviruses appear to be related immunologically to the original J. C. prototype virus (17, 15).

The other class of human papovaviruses was first detected in the urine of a renal allograft recipient (B. K.) on immunosuppressive therapy (5). Agents indistinguishable from the original BK virus (BKV) have subsequently been isolated from the urine of four of 74 renal allograft recipients studied (2). Although human pathogenicity remains to be demonstrated, Gardner has shown that antibodies inhibiting hemagglutination of erythrocytes by BKV are ubiquitous in the human population studied (4). Takemoto et al. detected BKV-specific antibodies in 70% of a human sera sample collected between 1949 and 1952, prior to widespread immunization with polio vaccines (25). On the other hand, 2.0 to 3.8% of sera from humans not exposed to polio vaccines contain simian virus 40 (SV40)-specific antibodies (21, 22). This suggests that BKV did not evolve from the SV40 which was a contaminant of some early vaccine preparations.

Although BKV shares some properties with SV40, the two agents can be readily distinguished from one another. SV40 grows to high titer in African green monkey kidney cells; infection of human cells is inefficient, resulting

in low and variable yields of virus. BKV, on the other hand, can be propagated in several different human cell lines and grows poorly in Vero cells (25). Like polyoma and unlike SV40, BKV causes hemagglutination of erythrocytes (5). This reaction can be inhibited by BKV hamster or rabbit antisera but is unaffected by SV40 antisera (25). No cross-reactivity, as measured by complement fixation or immunodiffusion, can be demonstrated between antisera prepared against either SV40 or BKV and the heterologous virus (14).

Hyperimmune serum to BKV can partially neutralize SV40 plaque formation on African green monkey kidney cells (25). In addition, antisera prepared against SV40 V, T, and U antigens cross-react by the indirect fluorescent antibody technique with cells infected with BKV (25; Takemoto, unpublished data). A cross-reaction between capsid antigens has also been detected by immune electron microscopy (18). Whereas the BKV genome has been shown to be in the supercoiled configuration, restriction endonuclease cleavage patterns have led Osborn et al. to suggest that SV40 and BKV DNAs are significantly different (16). This study further characterizes the physical properties of BKV DNA and examines the polynucleotide sequence homology between SV40 and BKV DNAs.

MATERIALS AND METHODS

Cells. BSC-1 cells were grown in Eagle minimal essential medium supplemented with 10% fetal calf

serum. Human embryonic kidney (HEK) cells, purchased from Flow Laboratories, Rockville, Md., were propagated in Eagle minimal essential medium containing $2\times$ vitamins and amino acids and supplemented with 10% fetal bovine serum.

Virus and viral DNA. BSC-1 cells were infected with the small-plaque variant of SV40 at an input multiplicity of approximately 0.1 PFU/cell, and virus was purified 6 to 8 days after infection, as previously described (6). ^{32}P -labeled virus was prepared by carrying out the infection in phosphate-free Eagle minimal essential medium containing carrier-free [^{32}P]orthophosphate (100 $\mu\text{Ci/ml}$) (6). ^3H -labeled SV40 was purified from cells continuously exposed to [^3H]thymidine (10 $\mu\text{Ci/ml}$) beginning at 30 h after infection. Unlabeled and radiolabeled SV40 DNA was prepared from purified virions after an incubation with 1% sodium dodecyl sulfate (50 C) and isopycnic centrifugation in CsCl-ethidium bromide, as previously described (6).

BKV that had been passaged three times in Vero cells was plaque purified in HEK cells. A virus stock was then prepared and used to infect other HEK cells at a multiplicity of approximately 0.1 PFU/cell. Infected cell cultures were usually harvested 8 to 10 days after infection, at which time the hemagglutination titer of the medium was 1:80 or greater. ^{32}P -labeled BKV was prepared as described above for SV40. [^{32}P]orthophosphate (80 $\mu\text{Ci/ml}$) was added to infected HEK cells 24 h after infection; in those studies in which very low (less than 0.01 PFU/cell) input multiplicities of BKV were employed, [^{32}P]orthophosphate was added at the earliest evidence of cytopathic effect (6 to 10 days after infection). BKV DNA was prepared from purified virions as described above or directly from infected cells by differential salt precipitation (8).

Both BKV and SV40 DNAs were mechanically sheared at 50,000 lb/in² in a Ribi cell fractionator (Ivan Sorvall) to a molecular size of 3.1×10^5 prior to DNA-DNA reassociation experiments (6).

DNA-DNA reassociation. ^{32}P -labeled, fragmented, denatured BKV DNA was allowed to reassociate alone or in the presence of an excess of unlabeled SV40 DNA fragments in 0.14 M sodium phosphate buffer at 60 C. Samples were removed at various times and analyzed for single- and double-stranded DNA by hydroxyapatite chromatography as previously described (6).

Restriction endonuclease cleavage of viral DNA.

(i) **R·Eco R1.** Reaction mixtures (0.2 ml) containing 0.1 M Tris-hydrochloride, pH 7.5, 0.005 M MgCl_2 , 10 to 30 ng of supercoiled viral DNA, and 0.1 to 0.5 U of R·Eco R1 (a generous gift of George Fareed) were incubated at 37 C for 30 min (3). Cleavage of viral DNA I was monitored by rate zonal sedimentation through 5 to 30% (wt/vol) neutral sucrose or by electrophoresis in agarose gels.

(ii) **R·Hind.** Preparations of R·Hind were generously supplied by Michael Chen and Daniel Nathans. Mixtures (0.05 ml) consisting of 6.6 mM Tris-hydrochloride, pH 7.5, 6.6 mM MgCl_2 , 50 mM NaCl, 5 to 50 ng of ^{32}P -labeled viral DNA, and sufficient R·Hind to effect complete digestion of substrate DNA were

incubated at 37 C for 16 h. The cleavage products were evaluated by polyacrylamide gel electrophoresis.

Agarose gel electrophoresis. Electrophoresis through 1.4% (wt/vol) agarose slab gels (17 by 12 by 0.3 cm) was carried out at 60 V for 16 h at 20 C in 40 mM Tris-hydrochloride, pH 7.8, 5 mM sodium acetate, and 1 mM EDTA as previously described (7). DNA bands were visualized after the addition of ethidium bromide (0.5 $\mu\text{g/ml}$) to the gel and electrophoresis buffer by the method reported by Sharp et al. (23). DNA was preparatively recovered from agarose gels by homogenization of specific gel fractions with a $2\times$ volume of elution buffer (0.1 $\times$ standard saline-citrate [0.15 M NaCl; 0.015 M sodium citrate], 0.1% sodium dodecyl sulfate, and 1 mM EDTA) in a Teflon tissue homogenizer. The homogenate was shaken on a rotating platform for 16 h at 37 C; agarose was removed by centrifugation at $7,500 \times g$ for 10 min. The agarose was washed with $1\times$ volume of elution buffer, which was then pooled with the original supernatant. Between 60 and 90% of the supercoiled viral DNA could be recovered by this procedure with minimal conversion to nicked circular or fragmented DNA forms. Eluted DNA was further purified on G-100 Sephadex containing Dowex 50 as previously described (26).

Agarose-polyacrylamide gel electrophoresis. Samples (up to 0.05 ml) to be analyzed by composite electrophoresis were incubated for 30 min at 37 C in 1% sodium dodecyl sulfate prior to their application to slab gels (17 by 12 by 0.3 cm) consisting of 3% polyacrylamide and 0.5% agarose. Electrophoresis was carried out for 16 h at 20 C at 50 V in a buffer consisting of 40 mM Tris-hydrochloride, 20 mM sodium acetate, and 1 mM EDTA adjusted to pH 7.2 with acetic acid. Autoradiograms were made from dried gels by direct contact with Kodak RP/R2 X-ray film.

RESULTS

Heterogeneity of BKV DNA. The DNA isolated from purified BK virions co-banded with SV40 DNA I in CsCl-ethidium bromide isopycnic gradients at a density of 1.60 g/cm^3 , confirming a previous report indicating its supercoiled configuration (16). Whereas BKV DNA appeared to cosediment with SV40 DNA I in neutral sucrose, the width of the BKV DNA band suggested that it was not as homogeneous as the SV40 DNA marker. Therefore, ^{32}P -labeled BKV DNA was analyzed by electrophoresis through a 1.4% agarose gel in an attempt to further evaluate its homogeneity (Fig. 1). At least four discrete bands of BKV DNA were resolved after electrophoresis through agarose compared with the single band of SV40 DNA. The four BKV DNA species were present in both the DNA I (Fig. 1, right) and DNA II (Fig. 1, left) regions of the gel. The largest BKV DNA molecules, BKV DNA (i), migrated slightly more rapidly than SV40 DNA I and DNA II,

FIG. 1. Agarose gel electrophoresis of BKV and SV40 DNAs. Approximately 50 ng of ^{32}P -labeled BKV DNA (○) and 200 ng of ^3H -labeled SV40 DNA (●) were subjected to electrophoresis on a 1.4% agarose slab gel (17 by 12 by 0.3 cm) for 16 h at 60 V as described in Materials and Methods. The gel was cut into 1.0-mm slices which were individually dissolved in 0.2 ml of 30% H_2O_2 and assayed for radioactivity.

indicating that BKV DNA (i) was somewhat smaller than SV40 DNA. The more rapidly migrating species of BKV DNA (ii, iii, and iv) have electrophoretic mobilities corresponding to molecules 80 to 90% the physical size of BKV DNA (i). The heterogeneity of this prototype BKV DNA was also suggested by the agarose gel electrophoresis patterns reported by Osborn et al. (16).

Serial high multiplicity passage of papovaviruses has been reported to cause significant rearrangements of viral DNA (28), particularly when nonplaque-purified virus is employed (9). In general, the viral genomes which evolve are smaller than wild-type genomes, are defective in their ability to replicate autonomously, and are often heterogeneous in size. Discrete species of defective supercoiled molecules, however, that are smaller but homogeneous in size have been identified after high multiplicity passage of SV40 (29, 11). The existence of the four species of BKV DNA suggested to us that only BKV DNA (i) was infectious and that the shorter BKV DNAs (ii, iii, and iv) represented defective deletions of the parental genome. Accordingly, ^{32}P -labeled supercoiled BKV DNAs (i to iv) were purified from infected HEK cells by differential salt precipitation (8) and preparatively separated from one another by electrophoresis through a 1.4% agarose gel. Approximately 10^6 HEK cells were separately infected with 3.5×10^{-2} to $8.3 \times 10^{-2} \mu\text{g}$ of each of the four BKV DNAs in the presence of DEAE-dextran (1 mg/ml) (12). A cytopathic

effect was noted in cells infected with BKV DNA (i); the other three BKV DNA preparations (ii to iv) evoked no response under the conditions employed. The virions which appeared after BKV DNA (i) infection were used to infect other HEK cells. The progeny viral genomes obtained from this second infection consisted predominantly of BKV DNA (i), although small amounts of the other BKV DNA species were observed after agarose gel electrophoresis.

A second type of experiment was also performed to assess the infectivity of the various forms of BKV DNA. HEK cells were infected with undiluted BKV stock as well as 10^{-4} and 10^{-6} dilutions of virus and labeled with [^{32}P]orthophosphate as described in Materials and Methods. The progeny viral DNA from the undiluted inoculum was heterogeneous (Fig. 2); at the 10^{-4} dilution, BKV DNA (i) was the predominant newly synthesized viral DNA species, although some BKV DNA (iv) was present. Whereas very small amounts of BKV DNAs (ii, iii, and iv) may have been present in the progeny at the 10^{-6} dilution, only BKV DNA (i) could be detected (Fig. 2). This experiment suggests that BKV DNA (i) can replicate autonomously, whereas the other three BKV DNA species are defective and require the helper function supplied by BKV DNA (i). This study also indicates that defective forms of BKV DNA appear (even at a 10^{-4} dilution) which can accumulate to become the predominant class of BKV DNA (Fig. 1).

FIG. 2. Electropherogram of progeny BKV genomes extracted from cells infected at varying input multiplicities. ³²P-labeled supercoiled DNA was purified from HEK cells infected with undiluted BKV stock or 10⁻⁴ and 10⁻⁶ dilutions by the Hirt procedure (8). Portions were subjected to electrophoresis in agarose gels as described in Materials and Methods. Migration is from top to bottom.

Restriction endonuclease cleavage of BKV DNA. Homogeneous ³²P-labeled supercoiled BKV DNA (i) was prepared from cells infected with a 10⁻⁴ dilution of BKV (Fig. 2). This DNA preparation was cleaved by R·Eco R1 to full-length linear molecules with an *S* value of 14.5 in neutral sucrose, confirming a previous report that a single restriction endonuclease site was present in the BKV genome (16).

Digestion of ³²P-labeled supercoiled BKV DNA (i) with R·Hind enzyme resulted in four

cleavage products (Fig. 3, panel 2). The BKV R·Hind DNA fragments range in size from 0.34 × 10⁶ to 1.5 × 10⁶ daltons (Table 1). Assuming that each of the four cleavage products is present one time in the parental viral genome, the size of BKV DNA is 3.45 × 10⁶ daltons, compared with 3.6 × 10⁶ daltons for SV40, when its molecular weight is calculated in a similar manner. The extra DNA band appearing in the incomplete digestion of BKV DNA (Fig. 3, panel 2) most likely represents the uncleaved

FIG. 3. Electropherogram of restriction endonuclease cleavage products of SV40 and BKV DNAs. (1) *R·Hind* digestion of ^{32}P -labeled SV40 DNA. (2) *R·Hind* digestion of BKV DNA (i) with two concentrations of enzyme. (3) *R·Eco* RI plus *R·Hind* digestion of BKV DNA (i). All samples were subjected to electrophoresis in composite 3% acrylamide, 0.5% agarose slab gels (17 by 12 by 0.3 cm) as described in Materials and Methods.

BKV *R·Hind* fragments C and D, since its size (7.1×10^5 daltons), calculated from its mobility, is very similar to the sum of the sizes of these two cleavage fragments (7.5×10^5 daltons).

To localize the *R·Eco* RI cleavage site in BKV DNA (i), ^{32}P -labeled viral DNA was successively cleaved with *R·Eco* RI and *R·Hind* restriction endonucleases, and the reaction products were analyzed by electrophoresis in composite polyacrylamide-agarose gels (Fig. 3, panel 3). The two new cleavage products which appeared (A_1 and A_2) were 9.2×10^5 and

6.0×10^5 daltons in size, respectively, as calculated from their electrophoretic mobilities (Table 1). The sum of their molecular weights (1.52×10^6) corresponds closely to that calculated for fragment A (1.50×10^6). The amount of *R·Hind* fragment A remaining after digestion with both restriction endonucleases has been greatly reduced. This result indicates that the *R·Eco* RI site is located within *R·Hind* fragment A.

Polynucleotide sequence homology between BKV and SV40 DNAs. In view of the cross-reactivity detected by indirect immuno-

TABLE 1. *Molecular weights of BKV restriction endonuclease DNA fragments*

BKV DNA fragment	Relative electrophoretic mobility ^a	Mol wt ^b
R·Hind A	0.540	1.50×10^6
R·Eco RI, R·Hind A ₁	0.849	0.92×10^6
R·Eco RI, R·Hind A ₂	1.302	0.60×10^6
R·Hind B	0.651	1.20×10^6
R·Hind C	1.784	0.41×10^6
R·Hind D	2.047	0.34×10^6
R·Hind (CD) ^c	1.140	0.71×10^6

^a The ratio of the electrophoretic mobility of the BKV DNA fragment to the electrophoretic mobility of SV40 R·Hind DNA fragment A in a composite 3% acrylamide, 0.5% agarose gel.

^b Molecular weights calculated from electrophoretic mobilities calibrated with SV40 R·Hind DNA fragments and adenovirus 2 R·Eco RI DNA fragments. Intact molecular weights of 3.6×10^6 for SV40 DNA and 23×10^6 for adenovirus 2 DNA were used in making determinations (19).

^c Fragment representing a R·Hind partial digestion product of BKV DNA migrating between fragments B and C (Fig. 3, panel 2).

fluorescence, we decided to examine the polynucleotide sequence homology between these two agents. When ³²P-labeled, fragmented, unfractionated BKV DNA was allowed to reassociate in 0.14 M phosphate buffer, its $C_0t_{1/2}$ was 1.7×10^{-3} ; under the same conditions, SV40 DNA reanneals with a $C_0t_{1/2}$ of 2.0×10^{-3} .

Nucleotide sequence homology between SV40 and BKV DNAs was assessed by observing the effect of unlabeled SV40 DNA on the kinetics of reassociation of ³²P-labeled BKV DNA. Since it quickly became apparent that only a portion of the polynucleotide sequences were held in common, it was necessary to analyze the data in such a way that a quantitative relationship could be established. The time required for half-renaturation ($t_{1/2}$) of the radiolabeled BKV DNA was calculated by modifying the equation for a second-order reaction described by Wetmur and Davidson (27). Thus: $1/f_{ss} = t/t_{1/2} + 1$, where f_{ss} is the fraction of the radiolabeled DNA remaining single stranded, t is the period of time the DNA has been allowed to reassociate, and $t_{1/2}$ is the time required for 50% of the DNA to reanneal. The $t_{1/2}$ value, calculated as the average of six different determinations during the reassociation of ³²P-labeled BKV DNA, was 17.1 h. The curve of $1/f_{ss}$ plotted versus $t/t_{1/2}$ for this reassociation is a straight line (Fig. 4, open circles), indicating a second-order reaction. The reassociation of ³²P-labeled BKV DNA, in the presence of an excess of unlabeled SV40 DNA

which is only partially homologous to the labeled BKV DNA, is a more complex reaction. The rate of reassociation of that portion of the BKV DNA which is homologous to a portion of the SV40 genome will be accelerated, whereas the kinetics of reassociation of the remaining BKV genome sharing no homology with SV40 will be unaffected. We have analyzed such biphasic reactions using the equations developed by Sharp et al. (24). For ³²P-labeled unfractionated BKV DNA reassociating in the presence of a 100-fold molar excess of unlabeled SV40 DNA, theoretical curves for various degrees of homology can be generated from the relationship:

$$\frac{1}{f_{ss}} = \frac{1}{\frac{x_1}{101 \frac{t}{t_{1/2}} + 1} + \frac{x_2}{\frac{t}{t_{1/2}} + 1}}$$

where x_1 is the fraction of BKV DNA with homology to SV40 DNA and x_2 is the fraction of BKV DNA with no homology to SV40 DNA. The results of this experiment as well as the theoretical curve for 20% polynucleotide sequence homology are shown in Fig. 4. The experimental data (Fig. 4, closed circles) correspond closely to the theoretical curve generated for 20% homology.

Since unfractionated BKV DNA consists of at least four separable DNA species (Fig. 1), we repeated the experiment shown in Fig. 4 with each of the individual radiolabeled BKV DNA components separated by preparative agarose gel electrophoresis. The results of this study (Fig. 5) indicate that 20 to 30% of each of the BKV DNA species is homologous with SV40 DNA. The data for the infectious BKV DNA (i) approach the theoretical curve for 20% homology.

DISCUSSION

The results of these studies show that the supercoiled DNA isolated from purified BKV is heterogeneous and consists of at least four separable species. A previous report also suggests that viral DNA prepared from prototype BKV is not homogeneous (16). Whereas the heterogeneity of BKV DNA could serve a necessary role during the lytic interaction between BKV and human cells, it seems more likely that it reflects its initial isolation or subsequent passage history. In this regard, serial undiluted passage of another papovavirus (SV40) results in deletions, substitutions, and insertions of host cell

FIG. 4. Reassociation of radiolabeled BKV DNA in the presence of an excess of unlabeled SV40 DNA. ^{32}P -labeled BKV DNA (10^5 counts/min per μg) was allowed to reassociate at a concentration of 1.18×10^{-2} $\mu\text{g}/\text{ml}$ in the presence (●) or absence (○) of a 100-fold excess of unlabeled, fragmented SV40 DNA in 0.14 M sodium phosphate buffer at 60 C. The percent DNA in duplex molecules was assayed by hydroxyapatite chromatography as described in Materials and Methods. The $t_{1/2}$ for the reassociation of ^{32}P -labeled BKV DNA alone (17.1 h) was the average of six separate determinations. The dashed line is the theoretical curve for the reassociation of BKV DNA alone; the solid line represents the theoretical curve for the renaturation of labeled BKV DNA in the presence of a 100-fold excess of SV40 DNA, assuming 20% polynucleotide sequence homology.

DNA, leading to the accumulation of defective viral genomes (1, 9, 10, 20, 28). The rearrangements that occur result in viral DNA molecules that are shorter than parental genomes (28) and that fall in the size range we observed for the BKV DNAs (ii, iii, and iv). Whereas there is no evidence that the BKV isolate was ever passaged at very high virus-to-cell ratios, efforts were not made to propagate this agent at low input multiplicities. It is worth noting that BKV was initially passaged in Vero cells rather than human cells, a step that may favor the emergence of defective viral genomes. Subsequent studies have indicated that BKV infection of monkey cells is inefficient (25).

It must be concluded, however, that shortened, defective forms of BKV DNA evolve despite our attempts to infect cells at low input multiplicities. Of the four DNA species examined, only BKV DNA (i) was infectious under the conditions employed. This result was confirmed when the progeny of serially diluted BKV were evaluated. When the BKV stock was diluted 10^{-4} and used to infect HEK cells, the predominant DNA species present was BKV

FIG. 5. Reassociation of fractionated, radiolabeled BKV DNA in the presence of an excess of unlabeled SV40 DNA. ^{32}P -labeled, fractionated BKV DNAs (i [○], ii [Δ], iii [□], and iv [◇]) (10^5 counts/min per μg) were allowed to reassociate alone (2.9×10^{-2} $\mu\text{g}/\text{ml}$) or in the presence of unlabeled SV40 DNA (5.8 $\mu\text{g}/\text{ml}$) in 0.14 M sodium phosphate buffer at 60 C. The percent ^{32}P -labeled DNA reassociated was assayed by hydroxyapatite chromatography. The $t_{1/2}$ for the reassociation of each of the four ^{32}P -labeled BKV DNA fractions alone was the average of six separate determinations: BKV DNA (i), 10.1 h; BKV DNA (ii), 7.6 h; BKV DNA (iii), 5.8 h; and BKV DNA (iv), 6.8 h. The theoretical curves shown in the figure represent, from bottom to top: no homology, and 10, 20, and 30% polynucleotide sequence homology to SV40 DNA.

DNA (i); at a 10^{-6} dilution, only BKV DNA (i) was detected (Fig. 2). Our interpretation of these findings is that the BKV stock contains high concentrations of defective viral genomes. As the inoculum is diluted, the probability of a single cell being infected with both infectious and defective virions is reduced. Thus, lower relative amounts of "helper" virus are present at the 10^{-6} dilution of the virus stock, resulting in the appearance of only nondefective BKV DNA (i).

Recent studies of two other papovavirus isolates that are serologically indistinguishable from BKV suggest that genome heterogeneity is not an intrinsic property of this agent. Both isolates were obtained from the urine of patients with Wiscott-Aldrich syndrome (Takemoto et al., J. Nat. Cancer Inst., in press), and their viral DNAs comigrated with BKV DNA (i) in agarose gels (Howley et al., manuscript in preparation). No additional short DNA mole-

cles were visualized. Cleavage of the viral DNA from one of these isolates with R-*Hind* resulted in four fragments which comigrated with the four bands we observed with BKV DNA (i) (Fig. 3). In addition, polynucleotide sequence homology studies between the nucleic acids of SV40 and each of these two isolates, both of which have been passaged exclusively in human cells, confirm the 20% homology detected with the DNA of the original BKV isolate (Fig. 4) (Howley et al., manuscript in preparation).

Despite the dissimilarity between the R-*Hind* cleavage patterns of SV40 and BKV DNAs, the two viral genomes share approximately 20% of their nucleotide sequences when the hybridization reaction is analyzed by hydroxyapatite chromatography. The observed homology was somewhat greater for the purified, shortened, defective BKV (ii, iii, and iv) genomes. The higher values could reflect the selective deletion of sequences unique to BKV DNA. Alternatively, the sequences homologous to SV40 DNA may be reiterated during the generation of the defective viral genomes.

In a somewhat similar experiment carried out in our laboratory, the plus strand of ³²P-labeled SV40 DNA was reannealed to an excess of ³H-labeled BKV DNA, and the extent of reaction was determined by resistance to the single-strand specific nuclease, S1. Approximately 11% homology was observed with either unfractionated BKV DNA or purified BKV DNA (i) (Khoury et al., Proc. Nat. Acad. Sci. U.S.A., in press).

Takemoto has demonstrated a cross-reaction between the T antigens of BKV and SV40 (25) as well as a cross-reaction with SV40 U antigen (K. Takemoto, unpublished data). The nucleotide sequences specifying T and U antigens have been assigned to the early region on the physical map of SV40 DNA (13). Such immunological cross-reactivity suggests that homology between BKV and SV40 DNA involves sequences located in the early region of the SV40 genome. The partial neutralization of SV40 infectivity by hyperimmune BKV serum, and the cross-reaction between viral capsid antigens measured by the fluorescent antibody test and immune electron microscopy, suggest possible nucleotide sequence homology between the late region of SV40 DNA and the BKV genome (5, 18, 25). Experiments delineating the specific areas of homology are in progress in this laboratory.

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