Molecular cloning and physical mapping of the DNA of bovine adenovirus serotype 4; study of the DNA homology among bovine, human and porcine adenoviruses

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The DNA of bovine adenovirus (BAV) serotype 4, a member of the subgroup 2 BAVs, has been cloned and mapped with 11 restriction enzymes. Southern blot hybridizations probed by a clone containing about 50% of the BAV-4 genome revealed a very strong and extended DNA sequence homology amongst the members of subgroup 2, but no homology was detectable to the subgroup 1 bovine, or any of those human (HAV) and porcine adenovirus serotypes examined. These findings were strengthened by reciprocal hybridizations. When using the cloned hexon gene region of BAV-3 (subgroup 1) or the total genome of HAV-2 as probes, again no homology could be shown to the bovine subgroup 2 serotypes. The extent of DNA homology detectable between the members of bovine subgroup 1, the porcine and the human serotypes was variable, but in general less expressed than that observed within the bovine subgroup 2.

The nine bovine adenovirus (BAV) serotypes isolated so far are divided into two subgroups on the basis of the differences in their biological and serological properties (Bartha, 1969). The representatives of subgroup 1 (BAV-1, 2, 3 and 9) contain a common complement-fixing antigen, thus cross-reacting with other mastadenoviruses in complement fixation or agar gel immunodiffusion tests (Bartha, 1969; Bürki et al., 1979). The apparent lack of this common antigen in the subgroup 2 serotypes (BAV-4 to BAV-8), which do not cross-react with any other examined mastadenoviruses, suggested that these viruses might form a peculiar group within the mastadenovirus genus (Bartha, 1969). DNA restriction enzyme analysis of the reference strains of different BAV serotypes supported the subgrouping (Benkő et al., 1988).

Earlier DNA studies comprising hybridization experiments with two subgroup 1 serotypes, BAV-2 (Belák et al., 1983, 1986) and BAV-3 (Hu et al., 1984a; Kurokawa et al., 1978), revealed a genome size and structure similar to that of human adenovirus (HAV) serotype 2. The only subgroup 2 BAV serotype which has been examined at the DNA level so far is BAV-7. Hu et al. (1984b) demonstrated, that the extent of DNA homology between BAV-7 and BAV-3 is less than that between BAV-7 and HAV-2, or between BAV-3 and HAV-2, thus suggesting that the genetic relationship between two adenoviruses of different host origin might be closer than that of viruses from the same host species.

The aim of the present work was to determine whether other members of the subgroup 1 and 2 BAVs also contain DNA sequences homologous to each other or to human serotypes. The detailed study of the DNA of a subgroup 2 serotype other than BAV-7 was also planned. As a consequence of earlier studies, an almost complete collection of the different BAV and porcine adenovirus (PAV) DNAs was available in our laboratory. The bovine serotype 4 was chosen for more detailed investigations, since this serotype is one of the most frequently isolated in Hungary nowadays.

Molecular cloning of BAV-4 DNA was done in three steps, exploiting the fact that some restriction enzymes (ClaI, SalI and XhoI) had only one cleavage site in the genome. Thus, first an inner part, approximately one half (15 kb) of the total genome was inserted between the ClaI and SalI sites of the pKH47 plasmid (Hayashi, 1980), resulting in a clone of nearly 20 kb in size (pBAV401). Since the BamHI enzyme cleaves the inserted viral DNA fragment almost exactly into two halves, two subclones, containing the ClaI–BamHI (pBAV402) and BamHI–SalI (pBAV403) inserts, were constructed to facilitate the physical mapping. The two ends, consisting of about 6000 and 9000 bp, were cloned by means of synthetic linkers. Because of the likely presence of residues of the terminal protein, a short exonuclease III treatment followed by mung bean

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nuclease digestion was applied, prior to the ligation of SalI and BamHI synthetic linkers. The plasmid containing the supposed left end of the viral genome was constructed by the use of ClaI enzyme and SalI linker (pBAV404), while the other end was inserted into the plasmid by SalI enzyme and BamHI linker (pBAV405). The loss of base pairs at the two ends was estimated by comparing the cloned fragments to the corresponding restriction enzyme pattern of the viral DNA. As no alteration (shifting) in the migration of the DNA fragments of 4200 and 4700 bp could be detected we assumed that no more than 100 bp at each end had been removed. Thus we have cloned three fragments of BAV-4 DNA representing essentially the entire BAV-4 genome.

The physical maps of the cleavage sites were determined by double or triple digestion of the cloned and viral DNA with the following restriction enzymes: BamHI, ClaI, EcoRI, HindIII, NdeI, PstI, PvuI, SacI, SalI, SmaI, XbaI and XhoI. The size of the restriction fragments was estimated graphically by relating their migration in 0.5 to 1.8% agarose or 12% polyacrylamide gels (depending on the sizes of fragments of interest) to that of Mr standards used. The orientation of the maps was determined by hybridizing the cloned left end of BAV-7 to the BamHI-cut DNA of BAV-4 and the other subgroup 2 serotypes. The enzyme BamHI cleaves an almost comigrating fragment (of 4 to 4.3 kb in size) from the DNA of all subgroup 2 serotypes examined so far. According to the results of the above mentioned hybridization, these fragments do share homologous sequences with the corresponding fragment, i.e. the left end of the BAV-7 genome, and should therefore be situated at the left end in serotypes 4, 6 and 8 as well. The physical maps of BAV-4 DNA are presented in Fig. 1. Since the group of BAVs to which serotype 4 belongs seems to be very special among other mastadenoviruses, the investigation of the gene organization on the genome of BAV-4 might be of interest. The clones and maps described here will hopefully promote such studies.

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**Fig. 1.** Physical maps of the BAV-4 genome. The location of the restriction enzyme cleavage sites is given in map units. As the genome size of BAV-4 was found to be 30400, one map unit is equal to 304 bp.
Southern blots made from several agarose gels containing the BamHI-, EcoRI-, HindIII- and PstI-cleaved DNA of some HAV, one PAV and eight BAV serotypes were hybridized with the following probes: HAV-2 total genome, pBAV401 and two previously constructed plasmid clones, pBAV301 and pBAV701 (B. Harrach, unpublished results). The pBAV301 contains the EcoRI/C fragment (covering the hexon gene region) of the BAV-3 prototype strain WBR1 (Darbyshire et al., 1965) cloned into the pACYC184 plasmid (Chang & Cohen, 1978), while pBAV701 consists of a HindIII-BamHI fragment from the supposed left end of the BAV-7 prototype strain Fukuroi (Inaba et al., 1968) inserted into the pKH47 plasmid. Both clones were constructed on the basis of the physical maps published by Hu et al. (1984a, b).

The probes made of the HAV-2 total genome (see Fig. 2) and the pBAV301 clone hybridized to all the examined human serotypes, as well as to the PAV and the subgroup 1 BAVs, but none of them gave positive hybridization with the DNA of the subgroup 2 BAVs. On the other hand, the pBAV401 (Fig. 3) and pBAV701 (Fig. 2c) clones hybridized to the subgroup 2 bovine serotypes only, and no DNA homology could be detected with these probes to the subgroup 1 bovine, the porcine or to the human serotypes, even if the autoradiograms were overexposed.

When comparing the BamHI, EcoRI and HindIII physical maps of BAV-4 with those of BAV-7 published by Hu et al. (1984b), virtually no common cleavage sites could be detected for these enzymes. This finding was in accordance with our earlier observation, namely the restriction enzyme analysis of the DNA of BAVs did not reveal comigrating fragments and therefore close genetic relatedness could not have been supposed between the different serotypes even of the same subgroup (Benkö et al., 1988). In this respect, the very strong homology detectable in the genomes of the subgroup 2 serotypes with the probes made from the clones pBAV401 and pBAV701 was somewhat unexpected. The precise localization of the homologous DNA sequences on the genome of the different subgroup 2

Fig. 2. (a) EcoRI-digested human, porcine and bovine adenovirus DNAs separated in 1% agarose gel. The origin of and the propagation methods for BAV strains, and the virion and DNA purification techniques are as described previously (Benkö et al., 1988). Lane 1, uncut pBAV401; 2, 6, 12 and 17, pBAV401; 3, HAV-2; 4, HAV-9; 5, HAV-4; 7, BAV-4'; 8, BAV-6; 9, BAV-7; 10, BAV-7'; 11, BAV-8; 13, BAV-1; 14, BAV-2; 15, BAV-3; 16, BAV-9; 18, PAV-3. BAV-4' and BAV-7' are field isolates which are genomic variants of the respective BAV reference strains. The porcine adenovirus strain 6618/4, the prototype strain of PAV-3 (Clarke et al., 1967), was propagated on PK-15 (continuous pig kidney) cell line. The Southern blotting and blot washing for sequential hybridization was made on Biodyne A membrane following the recommendations of the manufacturer (Pall Ultrafine Filtration Corporation). (b) Autoradiogram of the above gel after being hybridized with a [32P]dCTP labelled probe made of the HAV-2 total genome by nick translation. Hybridization was carried out according to the procedures described by Maniatis et al. (1982) without formamide, annealing at 57 °C and washing at the same temperature using 2 x SSC. (c) The same blot after being washed and rehybridized with pBAV701. In the lanes containing pBAV401 as the Mr standard, only the vector part of the plasmid became positive since the two clones (pBAV401 and pBAV701) did not contain overlapping regions from the respective virus genomes.
Fig. 3. *Hind*III-digested human, porcine and bovine adenovirus DNAs (a) separated in 1% agarose gel and (b) hybridized with a probe made from pBAV401. This clone contains an insert of a size 15 kb, representing approximately the region between the 30 and 80 map units of the BAV-4 genome. Lanes 1, 6, 11 and 16, pBAV401; 2, HAV-3; 3, HAV-2; 4, HAV-9; 5, HAV-4; 7, BAV-4'; 8, BAV-6; 9, BAV-7; 10, BAV-8; 12, BAV-1; 13, BAV-2; 14, BAV-3; 15, BAV-9; 17, PAV-3.
BAV serotypes was not possible, partly because of the large size of the probes and partly because of the lack of availability of physical maps for types 6 and 8. The extent of the homology was large enough to visualize partially digested fragments which were invisible on the gel in u.v. light (see Fig. 3). In the above mentioned case, the hybridization conditions were less stringent because we aimed at the detection of homologous sequences between the DNAs of HAVs or subgroup 1 BAVs and subgroup 2 BAVs, and this supposed homology was expected to be very weak if any. In spite of the less stringent hybridization conditions the percentage of the mismatching base pairs within the hybridizing sequences (between the DNA of the subgroup 2 serotypes) were estimated to be very low. A serial washing procedure in low salt concentration (0.1 x SSC) at temperatures increased by 5 °C in each step resulted in the complete washing away of the radioactive probe only after 2 h at 85 °C. This finding implies that the BAV serotypes belonging to the subgroup 2 are genetically very closely related, even if their DNA patterns generated by several restriction enzymes did not show much similarity. The DNA homology within the subgroup 2 detectable by the pBAV401 was strong enough to enable this plasmid to be a potential subgroup specific diagnostic tool.

The BAV serotypes 1, 2, 3 and 9 (subgroup 1) and the PAV-3 showed DNA homology to the HAV-2 total genome. According to the results of several hybridization experiments, the order of their genetic relatedness to the HAV-2 was estimated as follows: BAV-9, BAV-3, PAV-3, BAV-2, BAV-1. The cause of the very strong homology found between the HAV-2 and BAV-9 remains to be studied in more detail.

The DNA homology detectable with the clone pBAV301 among the members of the subgroup 1 BAVs was variable, but in general much weaker, than that demonstrated within the subgroup 2 with pBAV401 or pBAV701. Nevertheless, it also seems worth testing the clone pBAV301 as a diagnostic tool for the rapid detection of subgroup 1 BAVs.

In our experiments, we have failed to reproduce the detection of the very weak homology between BAV-7 and BAV-3 or HAV-2 demonstrated by Hu et al. (1984b), although the hybridization conditions applied were similar to those they had described. One possible explanation for this discrepancy might be the difference in the sizes of the probes used. Nevertheless, the positive hybridization results discussed above along with the presented negative results (i.e. no homology found in the DNA of the HAV, PAV or subgroup 1 BAVs to the subgroup 2 BAVs) may be considered as further evidence for the distinctiveness of subgroup 2 BAVs. It is further confirmed that adenovirus strains isolated from the same host species (subgroup 1 and subgroup 2 BAVs) might be genetically more distantly related to each other than are some strains of different host origin (HAV-2, BAV-9 and PAV-3).

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References


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