The fibre of bovine adenovirus type 3 is very long but bent

Rob W. H. Ruigrok, Annie Barge, Suresh K. Mittal and Bernard Jacrot

The sequence of the fibre of bovine adenovirus type 3 (BAd3) predicts an extremely long structure due to a large number of 15-residue repeats in the fibre shaft, the tail and head domains being similar in size to the human adenovirus fibres. The length of the fibre was confirmed using negative-strain electron microscopy of BAd3 pentons (fibre plus penton base). The fibre was found to be bent in several discrete places and the bending sites appear to correspond with irregular repeats in the shaft. We suggest that bending of the fibre is needed for the interaction of the penton base with the secondary receptors on the cell surface.

The adenovirus fibre is a long, thin, trimeric structure extending from the 12 fivefold vertices of the icosahedral virion. It consists of an N-terminal tail that is embedded in the penton base, which is part of the capsid. Then comes a shaft consisting of a number of 15 amino acid pseudo-repeats and at the other end of the shaft there is a C-terminal head containing the receptor-binding site (Devaux et al., 1990; Green et al., 1983). The length of the shaft is determined by the number of 15-residue repeats, each repeat contributing about 13 Å to the length (Ruigrok et al., 1990) and is a viral subgroup characteristic (Petterson & Wadell, 1985). Although the 15-residue repeats are not conserved absolutely, they are supposed to consist of alternating stretches of turns and three-residue β-strands (Green et al., 1983). Recent studies have shown that the fibre shaft is subdivided into tail, shaft and head, is shown in Fig. 1. The sequence is remarkable since it predicts 46-5 repeat motifs (Green et al., 1983) in the shaft, which is considerably more than found to date for the HAd fibres, the longest fibre being the HAd2 fibre with 21-5 repeats (Green et al., 1983). This is counting the last repeat (recognized as a full repeat by Green et al., 1983) as only a half repeat since it contains only one of the two β-strands, assuming that the head starts at the conserved TLWT sequence (see legend to Fig. 1). The presence of a half rather than a full repeat would make no difference for the proposed structure of the fibre shaft (Stouten et al., 1992). Most BAd3 repeats are extremely regular and close to the standard repeat: 1 L-x-Hy-x-Hy-x-G(or P)-L-x-Hy-x-x-x-x 15, where x is an unspecified amino acid and Hy means a hydrophobic residue. The residues in positions 2 and 10 are usually hydrophilic. Green et al. (1983) proposed that the first three amino acids would form a short β-strand (Sa), the following x-Hy-x-G(or P) a sharp turn (Ta), then L-x-Hy another β-strand (Sb) and the last four residues a turn (Tb). This proposal was maintained in the new model for the shaft (Stouten et al., 1992). In the HAd2 fibre, in the first half of the shaft the repeats usually have a proline at position 8. In the second half this is usually a glycine. In the BAd3 fibre, glycine is found in 78% of the motifs, nearly always preceded by another glycine at position 6. In the few cases where it is not preceded by another glycine, a serine is found in this position. This suggests that the proper folding of the chain requires either a proline at position 8 or a glycine preceded two residues before by a small amino acid, preferably a glycine.

The shaft is very different in length from that of the...
**Tail**

MKRSVPQDEFLVYPKAKRPNIMPPEFDNGFVENQAE

1 LAM LVEK PL TFDKEGA 24 ASL QGSG PI TYNNSNNGT 1
2 LTL GVGR GI RINPA1LLETND 25 FGL SIGP GM WVDQNR 1
3 LAS AYFP PL ASDEAGN 26 LQV NGPA GL VPQGN 1
4 VTL NMSD GL YTKDND 27 LVF NLAD PL AISDSK 1
5 LAV KVGP GL SLDSNNA 28 ISL SLGP GM TQASNA 1
6 LQV HTGD GL TVTDDNK 29 LTL SLGN GL EFNSQA 1
7 VSL NTQA PL STTSAG 30 VAI KAGR GL RFESSQALE 1
8 LSL LLGP SL HLGEER 31 SSL TVGN GL TLTDTV 1
9 LTV NTGA GL QISNNA 32 IRP NLGD GL EVRDNK 1
10 LAV KVGS GI TVDAQONQ 33 IIV KLGA NL RFENAVTAGTVNPSAPEAP 1
11 LAA SLCD GL ESRDNK 34 PTL TARI PL RASNSH 1
12 TTV KAGP GL TITNQA 35 LQL SLSE GL VVHNNNA 1
13 LTV ATGN GL QNPEGQLQ 36 LAL QLGD GM EVNQHG 1
14 LNI TAGQ GL NFANNS 37 LTL RVGS GL QMRDGI 1
15 LAV ELGS GL HPPGQNOQ 38 LTV TPSGTPI EPRTLAPTQENG 1
16 VSL YPDC GI DIRDNR 39 IGL ALGA GL ELDESA 1
17 VTV PAGP GL RMLNHQ 40 LQV KVGP GM RNPFVEKI 1
18 LAV ASGD GL EVHSDT 41 VTL LLGP GL SFQPNANTRNYD 1
19 LRL KLSH GL TFENGA 42 VTV SVEP PM VFGQROQ 1
20 VRA KLP GP GTDDSGR 43 LTF LVGH GL HIQNSK 1
21 SVV RZGR GL RVANGQ 44 LQL NLQG GL RTDPTVNTQ 1
22 VQI FSGR GT AIGTDSS 45 LEV PLGQ GL EIADESQ 1
23 LTL NIRA PL QFSGPALT 46 VTV KLGD GL QFSQQR 1

**Shaft**

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**Head**

ITTAPNMVTETLWTGTGSNANVTWVRGTAFLPSKLFLSTTRFSTGLVGLGMDSTNSAFSGQYI

NAGHEQIECFILLDNQGKLLGSLQGTVKNNPASAKAIAFLPSTALYPILENSRSGLPGK

NLVGMQAILGGGCTCITATLNGRSNNYPAGQSIIPVWQEPINTARQPLNHSLTTFYWT

Fig. 1. Amino acid sequence of the BAd3 fibre derived from the corresponding nucleotide sequence (Mittal et al., 1992, 1993). The sequence is divided into a tail, a shaft and a head section according to Green et al. (1983). The amino acids in the tail and head identical to conserved residues in the HAd2 fibres are underlined. The arrangement of the shaft sequence into motifs is in some parts ambiguous. We assume that the head starts at the conserved TLWT sequence and, based on homology with the HAd2 fibre sequences, that the sequence before TLWT (starting with ITT) forms a half repeat.

other adenovirus fibres but the tail and the head are very similar in size to those of the human viruses. Similarities with the HAd fibres are around 15% in the head and 25% in the tail and are of the same extents as those with the canine adenovirus fibre. Despite these homologies we have not been able to define clear phylogenetic relationships between the HAd and BAd3 fibres.

Since the amino acid sequence predicts 46.5 repeats, which would make a very long fibre, we performed negative-stain electron microscopy (EM) to confirm the fibre length. BAd3 (strain WBR1), kindly provided by Dr B. Derbyshire (University of Guelph, Guelph, Ontario, Canada) was grown in MDBK cells. BAd3 plaque purification, working virus stocks and virus titrations were done in MDBK cells. Cells were infected with BAd3 at an m.o.i. of 5 p.f.u. per cell, harvested at 2 to 3 days post-infection and cell-associated virus particles were banded twice on a CsCl gradient (Graham & Prevec, 1991). The purified virus stocks were maintained in PBS (137 mM-NaCl, 2.7 mM-KCl, 8 mM-Na2HPO4, 1.5 mM-K2HPO4, containing 0.01% CaCl2 and 0.01% MgCl2·6H2O) containing 10% glycerol. Samples for
EM were prepared directly out of this solution as described (Ruigrok et al., 1990). Fig. 2 shows a gallery of isolated pentons (fibre plus penton base), which were found in a purified virus preparation. Fig. 2(a) shows pentons in end-on view, the penton base standing up from the carbon support film, often having a pentagonal outline but with the fibre lying flat on the film. Fig. 2(b) shows pentons in side view with the base lying on its side. The dimensions of the pentons in these two orientations are not the same and this has to be taken into account if the fibre length is to be derived from the penton length (Ruigrok et al., 1990; Kidd et al., 1993). We made two independent sets of measurements by two people on two separate EM preparations. The first showed only pentons in end-on view, which measured $795 \pm 24 \text{ Å}$ (60 measurements, the s.d. is that of the population of measurements), from which a fibre length of $768 \text{ Å}$ can be derived (using the comparison of HAd2 penton and fibre lengths; Ruigrok et al., 1990). The second preparation showed pentons in side view, which measured $735 \pm 32 \text{ Å}$ (22 measurements) from the top of the base to the tip of the head, corresponding to $775 \text{ Å}$ for the full length of the fibre. A histogram showing both measurements is given in Fig. 3(a). The length of the base was $106 \pm 8 \text{ Å}$, comparable to the $100 \text{ Å}$ long base of HAd2 and HAd3. The two fibre length estimates agree well and confirm that the BAd3 fibre is indeed much longer than the longest HAd fibres ($373 \text{ Å}$ for the HAd2 fibre). From the average length, $772 \text{ Å}$, we can again derive the length of the shaft by subtracting the length for the N-terminal tail and the height of the head, which are $34$ and $49 \text{ Å}$ respectively for HAd2. This results in a length of $689 \text{ Å}$ for the shaft and thus $14.8 \text{ Å}$ per repeat, which is slightly larger than for HAd2, HAd3 and HAd40 (Ruigrok et al., 1990; Kidd et al., 1993). The use of the HAd2 fibre dimensions for deriving the BAd3 shaft length is justified considering the similar lengths of primary sequence for head and tail. HAd fibres can bend $20 \text{ Å}$ above the penton base at a position that corresponds to the third repeat of the shaft. This is longer than the other repeats and has hydrophilic residues at the conserved hydrophobic positions in the $\beta$-strands (Green et al., 1983; Ruigrok et al., 1990). In Fig. 2, it is striking that most of the BAd3 fibres are bent at one or more places (indicated with arrowheads). Analysis of the pentons (both sets of measurements taken
Fig. 3. (a) Histogram of length measurements on BAd3 pentons (see text) in side view (solid black bars) and in end-on view (hatched bars). Measurements were made from prints with a magnification of 200000 × using an ocular eyepiece giving an additional magnification of 8 × . The magnification was calibrated using negatively stained catalase crystals as described (Ruigrok et al., 1990). The error between different micrographs is estimated to be less than 2% and the measurement error about 1%. The additional spread in the data is probably caused by different arrangements of the molecule on the support film. (b) Schematic representation of the BAd3 penton showing the lengths of the base and the fibre extending from the base, and the position of the kinks measured from the top of the head. The percentages of pentons that possessed kinks in these positions are also indicated.

together, Fig. 3 b) shows that 56% of the fibres have a kink at 249 Å, 20% at 526 Å and 18% at 685 Å below the tip of the head. In total, 24% of the fibres were straight, 58% showed one bend, 14% two bends and 4% three bends.

The kink found in most of the pentons may correspond to the position of repeats 33 and 34. Repeat 33 is clearly the most irregular repeat of all, with a hydrophobic residue at position 2, neither a proline nor a glycine at position 8 but an asparagine and a very long sequence for Tb. Repeat 34 is unusual in having prolines at position 7 and 8. The two other kinks were seen much less frequently and seem to correspond to less important irregularities. Considering its position, the kink closest to the penton base should correspond to repeats 3 and 4. Repeats 2 and 3 of the BAd3 fibre are irregular; repeat 2 is longer than the others and repeat 3 has serines at hydrophobic positions 3 and 11. In fact, the two repeats could be lined up in several different ways and this ambiguity could be the cause of the bend. Interestingly, repeat 3 also has two prolines at positions 7 and 8, like repeat 34. The third kink, found in 20% of the pentons, could correspond to the position of repeats 14 and 15. These two repeats seem normal apart from two consecutive proline residues in turn Tb of repeat 15. In general we may conclude that the shaft of the BAd3 fibre consists of a series of very regular repeats and that those repeats where the kinks were found are amongst the most irregular.

The shaft length per repeat of the BAd3 fibre, 14.8 Å, is larger than the average value for HAds of 13.8 Å (HAd2, shaft length of 290 Å for 21.5 repeats gives 13.5 Å; HAd5, 289/21.5 gives 13.4 Å; HAd3 77/5.5 gives 14 Å; HAd40, long fibre 285/20.5 gives 13.9 Å and short fibre 165/11.5 gives 14.3 Å; Ruigrok et al., 1990; Kidd et al., 1993). This is not due to systematic errors in measuring the lengths of the kinked fibres, since the straight pentons had the same average length as the kinked pentons. One possible explanation would be that the length per repeat at the three kink positions is larger than the other, regular repeats, i.e. 44 repeats of 13.8 Å and three repeats of 27 Å. However, the presence of three very long repeats in the straight pentons could be expected to lead to constrictions in the shaft or other irregularities, which were not observed. The slightly longer length could also be due to the very high percentage of glycine-containing repeats and the specific differences in folding between a glycine and a proline turn. It should also be noted that we originally derived a repeat length of 13.2 Å for the HAd2 fibre, for those repeats which were present in the HAd2 fibre but not in the HAd3 fibre, by comparing HAd2 and HAd3 fibre lengths. The HAd2 and HAd3 fibres have repeats 1 to 3 and 20 to 21.5 in common. If the length per repeat for the common repeats is then calculated, a value of 14 Å is derived. This suggests that the shaft length per repeat could depend on the structure of each individual repeat. The answers will come when the structure of a fibre can be elucidated at the atomic level.
As mentioned above, the fibres of the HAd5s are straight, apart from the possibility of a bend at the third repeat. This aberrant repeat is conserved in most known fibre sequences, suggesting some biological function. Recently, it was shown that primary receptor attachment through the fibre is not sufficient for viral infection. There has to be a second interaction of an RGD sequence in the penton base with host cell integrins for internalization (Wickham et al., 1993). If the fibre on the virus were rigid, interaction of the base with the cell surface would be difficult. We suggest that the conserved area of possible bending in the fibre just above the top of the penton base allows this penton base–second receptor interaction.

The only other fibre known to bend in places other than close to the base is the long fibre of the avian adenovirus FAV1 (or CELO virus), which is also much longer than the human virus fibres (Laver et al., 1971; Gelderblom & Maichle-Lauppe, 1982). After the initial primary receptor attachment with several fibre heads at the same time, the secondary penton base–integrin interaction can only take place if the fibre heads with their bound receptors slide aside. This may well be possible for short fibres but for the FAV1 fibres of about 500 Å in length and also for the BAd3 fibres of 775 Å it may be difficult because of the displacement or avoidance of other structures on the cell surface. The bending of the fibre shaft will certainly facilitate the approach of the virus to the cell surface while remaining attached to the primary receptors.

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References


