Characterization of Streptococcal Bacteriophage c6A

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SUMMARY

Bacteriophage c6A is a lytic phage that infects strains of Streptococcus lactis. Infection of S. lactis C6 under standard conditions yielded 124 ± 8 p.f.u. per infected cell after a latent period of 25 min at 30 °C. The virion of c6A was shown to contain at least 12 polypeptides and a 21.9 kilobase double-stranded, linear DNA genome with complementary 5'-protruding single-stranded termini. The (G + C) content of this DNA was estimated to be 36.7%. A restriction map was constructed which indicates that a number of restriction endonucleases did not digest the DNA and that others cleaved with a much lower frequency than expected.

Lactic streptococci used in the manufacture of cultured milk products are susceptible to bacteriophage infection. Production of lactic acid by infected cultures is often inadequate to promote normal curd formation and an inferior product results. Lytic streptococcal phages with a prolate, polyhedral head and a non-contractile tail are ubiquitous (Keogh & Shimmin, 1974; Nyiendo, 1975; Heap & Jarvis, 1980). Many of these have been shown to carry double-stranded DNA approximately 20 kilobase (kb) long with a (G + C) content of 35 to 40% (Nyiendo, 1975; Jarvis, 1984). Phage c6A, which infects strains of Streptococcus lactis, has been included within this phage group on the basis of its morphology and host range (R. R. Hull, unpublished results). This communication describes the basic molecular characterization of c6A. Both c6A and the host strain S. lactis C6 were provided for this study by Dr R. R. Hull (CSIRO Dairy Research Laboratory, Highton, Victoria, Australia).

Phage c6A was propagated at 30 °C by the delayed infection procedure of Nyiendo et al. (1974) in SL2C broth (20 g Oxoid tryptone, 5 g Oxoid yeast extract, 5 g glucose per litre of 100 mm-Tris–HCl pH 7.2, 10 mm-CaCl₂), collected by polyethylene glycol precipitation (Yamamoto et al., 1970), resuspended in SCT buffer (50 mm-NaCl, 10 mm-CaCl₂, 10 mm-Tris–HCl pH 7.5) and CsCl was dissolved in the phage suspension (0.75 g/ml of suspension). After ultracentrifugation (35000 r.p.m., 48 h in a Beckman 50Ti fixed-angle rotor) the phage band was harvested and stored at 4 °C. When this material was to be used for protein analysis on polyacrylamide gels or as a source of phage DNA, CsCl was removed by extensive dialysis against 1 mm-sodium EDTA pH 8.0.

One-step growth experiments (Fig. 1) showed that infection of S. lactis C6 by c6A resulted in complete lysis of the culture. The release of progeny phage began 25 min after infection. Estimation of burst size is inherently inaccurate in chain-forming streptococci unless the m.o.i. is low enough to eliminate multiple infections of each chain. Under such conditions (m.o.i. = 10⁻⁵), burst size was estimated as 124 ± 8 p.f.u./infected cell. Latent period and burst size may vary with different hosts and under different conditions of host growth and infection (Nicholls & Holloway, 1962; Keogh, 1973). The conditions used in this study were similar to those employed by Nicholls and Holloway where cells were infected in the exponential phase of growth in tryptone–yeast extract broth at 30 °C. Several phages in that study lysed their hosts in 25 to 35 min with burst sizes of 100 to 125 p.f.u./infected cell. Four of these phages have been characterized by electron microscopy (Keogh & Shimmin, 1974) and, like c6A, they are tailed,
Fig. 1. One-step growth of c6A on S. lactis C6. Cells in the exponential phase of growth in medium SLIC (see text) were infected with c6A at 30 °C. O, Turbidity of infected culture (m.o.i. 2 p.f.u./c.f.u.); △, phage titre (m.o.i. 10⁻⁵ p.f.u./c.f.u.).

Fig. 2. Restriction endonuclease cleavage site map of c6A DNA. Fragments are labelled alphabetically in order of their decreasing size. Endonuclease digestions were carried out essentially as described by Maniatis et al. (1982).

prolate, polyhedral phages. Such rapidly replicating, virulent phages are of clear concern in dairy fermentations.

The midpoint melting temperature ($T_m$) of c6A DNA was determined by photometric measurement of the hyperchromic shift at 260 nm in 0.1 x SSC (15 mM-NaCl, 1.5 mM-sodium citrate, pH 7.0). Data were corrected for thermal expansion and the DNA melting curve was constructed. ($G + C$)% was estimated using the formula of Mandel et al. (1970) and Escherichia coli DNA as standard. The ($G + C$) content of c6A DNA was estimated to be 36.7%. This value
Fig. 3. $^{32}$P-labelling and ligation of the single-stranded termini of c6A DNA. (a) Autoradiograph of a 1% agarose gel of an EcoRI digest of c6A DNA end-labelled with $^{32}$P-dATP by DNA polymerase Klenow fragment before (lane 1) and after (lane 2) endonuclease digestion. (b) Ethidium bromide stain of a 1% agarose gel of an EcoRI digest of c6A DNA (lane 1) and c6A DNA ligated with T4 DNA ligase prior to endonuclease digestion (lane 2). λ HindIII was used as a size marker (lane 3). The sizes of the λ HindIII standards are from top to bottom: 23-13, 9-42, 6-56 and 4-36 kb. End labelling and ligation were carried out essentially as described by Maniatis et al. (1982).

Fig. 4. SDS-polyacrylamide (12%) gel electrophoresis of c6A proteins. Phage in 1mm-sodium EDTA were concentrated by dialysis against Aquacide II (Calbiochem). SDS-PAGE was carried out in a Tris–glycine discontinuous gel system (Laemmli, 1970). Protein bands were visualized by staining with Coomassie Brilliant Blue (Weber et al., 1972). The estimated sizes (kilodalton) of the protein bands are as follows: 169 (a), 85 (b), 64 (c), 51 (d), 35 (e), 31 (f), 26 (g), 23 (h), 22 (i), 21 (j), 20 (k) and 19 (l). Bands c, d, e, and g to l are more apparent at higher gel loadings. Undissociated material is indicated (u).

is within the range previously reported for lactic streptococcal phage DNA (Nyiendo, 1975; Jarvis, 1984) and closely reflects the (G + C) content of the host DNA (Knittel et al., 1965).

DNA was isolated from c6A by SDS-phenol:chloroform (1:5) extraction, and a restriction endonuclease cleavage site map of this DNA was constructed (Fig. 2). The total genome size (21.9 kb) was estimated by averaging results from seven separate gels of EcoRI digests. Cleavage sites were positioned relative to each other by double and triple digests. The DNA was not cleaved by BamHI, BglII, BstNI, HaeIII, PstI, PvuII, Sau3AI, SalI, SmaI or XhoI.

The recognition sequences for two of the enzymes that failed to digest c6A DNA, Sau3AI and HaeIII, should occur approximately 70 and 25 times respectively in c6A DNA [assuming a random sequence for 21.9 kb of 36.7% (G + C) DNA]. Sites for MspI and HhaI digestion, of which only two and three respectively were found, should also occur approximately 25 times. There are two possible explanations for these data: either the appropriate sequences in the DNA are protected from cleavage by the occurrence of modified bases, or the sequences are absent from the genome. These possibilities are currently under investigation. Resistance to cleavage has been observed in other bacteriophage genomes (Kruger & Bickle, 1983) and presumably assists the phage to overcome host cell restriction systems. The presence of restriction modification systems in S. lactis is well established (Boussemaer et al., 1980), although the sequence specificities of the endonucleases are unknown. Isoschizomers of Sau3AI, HaeIII and MspI have been characterized in many Gram-positive bacteria (Kessler et al., 1985).

$^{32}$P-labelling of c6A DNA using the template-dependent 5'→3' polymerase activity of the Klenow fragment of DNA polymerase I resulted in the labelling of only two EcoRI fragments (Fig. 3). These two fragments lie at the ends of the linear DNA (see cleavage site map), indicating that the DNA has 5'-protruding single-stranded ends.
Treatment of c6A DNA with T4 DNA ligase prior to digestion with EcoRI resulted in the disappearance of the two terminal EcoRI fragments and the appearance of a single novel fragment equal in size to the sum of the two terminal fragments (Fig. 3). A faint band, corresponding to this fragment and evidently the result of cohesion between a small proportion of the terminal fragments, was sometimes observed (in addition to the bands shown in lane 1 of Fig. 3b) in ionograms of EcoRI digests (not shown). This band was abolished by heating the digests to 65 °C for 10 min immediately prior to electrophoresis. The specificity of the cohesion (opposite ends of the DNA associate but like ends do not) indicates that the single-stranded protrusions are complementary in base sequence but not palindromic.

The accessibility to DNA polymerase and DNA ligase of c6A DNA isolated by phenol:chloroform extraction indicates that, unlike the small Bacillus phages (Yehle, 1978; Yoshikawa & Ito, 1981), c6A DNA does not have protein molecules covalently attached to its termini. The cohesive ends permit in vitro circularization and concatenation of the DNA; the in vitro significance of this is unknown, but the possibility exists that replication and/or packaging of the DNA involves either a circular or multimeric linear intermediate.

Twelve virion-associated polypeptides in the size range of 19 to 169 kilodalton were detected by SDS-PAGE (Fig. 4). Using the intensity of staining of each band as an index of the amount of protein present, it is clear that some of the bands constitute only a small proportion of the total protein. Although it is possible that these minor bands are not bacteriophage polypeptides but are cell components isolated in association with the phage, different phage preparations reproducibly show the same bands. If each of these polypeptides is encoded by a separate phage gene, approximately 15-4 kb of DNA would be required, leaving about 6-5 kb of DNA to encode any undetected virion proteins and other proteins involved in the phage replicative cycle.

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REFERENCES


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