Some Physiochemical Properties of Yaba Poxvirus Deoxyribonucleic Acid

DAVID S. YOHN AND JOHN F. GALLAGHER

Viral Oncology Section, Roswell Park Memorial Institute, Buffalo, New York 14203

Received for publication 22 October 1968

Yaba tumor poxvirus deoxyribonucleic acid (DNA) has a density of 1.6905 in CsCl and its T_m value in 0.015 M citrate in saline is 82.3. The guanine plus cytosine content estimated from these properties was taken to be $32.5 \pm 0.5\%$, a value 2 to 3% less than for DNA from other poxviruses reported to date.

Yaba poxvirus, recognized as etiologically responsible (1) for an outbreak of subcutaneous tumors in 1956 among rhesus monkeys housed in Yaba, Nigeria (2), has yet to be classified among the subgroups of the poxvirus family (10). It is known to induce antibodies in monkeys to a nucleoprotein common to all poxviruses (21). Morphologically it resembles the vaccinia and myxoma subgroups (5). The present studies were undertaken to determine the composition of Yaba poxvirus deoxyribonucleic acid (DNA); a preliminary report has appeared (Gallagher and Yohn, *Bacteriol. Proc.*, 1967, p. 169). The results indicate that Yaba poxvirus contains 2 to 3% less guanine plus cytosine (GC) than that reported for other poxviruses (9, 13, 14, 19).

MATERIALS AND METHODS

Virus and tumor production. Yaba poxvirus was originally obtained from the laboratory of Sir Christopher Andrewes at Mill Hill, London (1). At Roswell Park the virus has been propagated in rhesus or cynomologous monkeys and more recently in vitro in cercopithecus kidney cells (24). For these studies, virus in its 10th in vivo passage in our laboratory was used as source of inoculum to initiate tumors. Rhesus monkeys were injected with 0.2 ml of a 10% cell-free tumor extract at multiple subcutaneous sites on the abdomen, dorsum, thighs, and shoulders. The inoculum contained $10^{7.7}$ focus-forming units as measured in vitro (26). Within 3 to 4 weeks, approximately 100 g of tumor were removed from each animal and frozen at -70°C until extracted for virus or DNA. Over 2 kg of tumor were used in these studies.

Extraction and partial purification of Yaba poxvirus. The procedure of Eckhart and Yohn (*Bacteriol. Proc.*, 1968, p. 136) was modified in an effort to obtain maximal yields of purified virus. Yaba tumor in 50-g portions was extracted at 10% (w/v) in chilled saline by homogenizing in a Sorvall omnimixer for 3 min at full speed. The homogenate was centrifuged at $700 \times g$ at 4°C for 15 min in an International centrifuge, model PR-2, and the fatty surface layer was removed. An

equal volume of Genetron 113 (fluorocarbon) was added and homogenization was continued for an additional 3 min. The homogenate was centrifuged at 2,000 rev/min for 15 min and the upper aqueous phase was recovered. A volume of fresh saline equal to twice the aqueous phase was added to the sediment and the extraction process was repeated. Aqueous phases were combined, one-fourth volume of fresh, chilled Genetron was added, and the extraction process was repeated. The volume was reduced to 180 ml or less by dialysis against Carbowax. Virus was removed by centrifugation at $64,500 \times g$ for 2 hr at 4°C in an SW25.2 head. The virus was suspended in 10 ml of saline and treated with nucleases, 50 μg of ribonuclease per ml and 25 μg of deoxyribonuclease per ml, for 30 min at 37°C , followed by light trypsinization (0.08%) for 50 min. The virus was sedimented at $73,500 \times g$ in an SW39 head, suspended in 9 ml of citrated saline, and submitted to density gradient centrifugation in sucrose. The virus banded at approximately 65% sucrose after 18 hr of centrifugation at $8,400 \times g$ in an SW25.1 head.

Further virus purification by density gradient electrophoresis. Yaba virus was further purified by density gradient electrophoresis according to the technique introduced by Brakke (3). A detailed description of the ascending technique has been previously given by Wiarda et al. (20). In our procedure, the lower part of the electrophoresis chamber was filled with 60% (w/v) sucrose (specific gravity, 1.200) made up in 0.05 M barbiturate-acetate-hydrochloride buffer at pH 8.6. A gradient from 50 to 10% sucrose (w/v) prepared in the above buffer was layered in the central tube over the sucrose base. Virus pellets were suspended in approximately 2 ml of 56% sucrose (specific gravity, 1.185) made up in the above buffer and introduced via a capillary tube on the sucrose base. Electrophoresis was for about 20 hr at a constant voltage of 300 v and 20 ma at 7°C . During this interval the virus band ascended 10 to 13 cm; it was collected through the capillary tube and checked for purity by electron microscopy.

Extraction of Yaba poxvirus DNA. Poxvirus DNA extraction techniques of Pfau and McCrea (13), Joklik (9), and Soehner et al. (16) were tested as means to

extract viral DNA. The technique of Joklik was finally selected. Based on the results of others (10, 22, 27), indicating that poxviruses contain 3 to 5% DNA, 50 to 70% of the projected available DNA was consistently obtained. Hyperchromicity during thermal denaturation ranged from 35.0 to 41.0%, indicating double strandedness.

Extraction and purification of Yaba tumor DNA and monkey liver DNA. Normal rhesus monkey liver and Yaba tumor from rhesus monkeys was extracted for DNA as described by Cheng and Sueoka (4). These, and highly polymerized calf thymus DNA (General Biochemicals), were purified by column chromatography on methylated albumin kieselguhr (MAK) columns according to a modification of the technique described by Mandell and Hershey (11). The MAK column was prepared by mixing 8 ml of 1% methylated albumin (18) into 60 g of Celite and packing the mixture into a column (45 by 2 cm). The column was equilibrated with 0.05 M phosphate buffer (pH 6.7) prior to the addition of approximately 10 mg of DNA dissolved in 10 ml of SSC (0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0). The column was flushed with 50 ml of additional buffer and a gradient of 0.1 M NaCl to 1.0 M NaCl made up in 0.05 M sodium phosphate buffer (pH 6.7) was started at a constant flow rate of 20 ml/hr. After 24 hr, the column was washed with 1.5 M NH_4OH to elute the remaining nucleic acid. The various peaks, usually four or five, were dialyzed against several changes of SSC in a cold room. Desired volumes were obtained by dialysis in Carbowax (17) followed by dialysis in citrated saline.

Determination of the T_m values of purified DNA. Thermal transition curves were determined by the procedure outlined by Marmur and Doty (12). All DNA types were dialyzed against 0.015 M citrate in saline at pH 7.0. A 3-ml amount of DNA at a concentration of 20 $\mu\text{g}/\text{ml}$ was placed in a quartz cuvette with a 1-cm light path. A thermister probe was sealed into the fused top of the cuvette. The cuvette was placed in a special chamber of a Cary spectrophotometer, model 14, with two thermal spacers on either side of the cuvette holder. Absorbance at 260 nm was determined at each degree of temperature. The temperature was raised 1 C every 10 min. The GC content was determined from the formula $T_m = 69.3 + 0.41 (\text{GC})$ where T_m is the midpoint of the transition curve and GC is the molar per cent of GC (%GC) in the DNA.

Density equilibrium centrifugation of purified DNA. Buoyant densities were determined in a model E analytical centrifuge with an analytical-D rotor. The techniques of Lfft, Voet, and Vinograd (8) were employed throughout these determinations. Crab DNA, density 1.683 (4), was employed as reference. Additional analysis of Yaba poxvirus DNA, kindly performed by Clyde Goodheart of the A. M. A. Institute for Biomedical Research, Chicago, employed DNA from *Clostridium perfringens* (1.691), *Escherichia coli* (1.710), and *Micrococcus lysodieticus* (1.731) as reference points. Calculations of GC content from buoyant densities were made according to the methods of Rolfe and Meselson (15).

RESULTS

Extraction and purification of Yaba tumor DNA and monkey liver DNA. Generally, four or five peaks were obtained from the eluates of MAK columns. The major peak which eluted at 0.6 to 0.8 M NaCl contained undenatured DNA. Initial peaks contained ribonucleic acid (RNA) and small amounts of DNA. The final peak, obtained by flushing with 1.5 M NH_4OH , contained denatured DNA and occasionally traces of high-molecular-weight DNA and RNA. Highly polymerized calf thymus DNA (General Biochemical), when subjected to MAK chromatography, was found to contain a small amount of RNA. The RNA fraction eluted at 0.1 to 0.2 M NaCl, well separated from the undenatured DNA.

Extraction and purification of Yaba poxvirus. Approximately 1 to 3 mg (dry weight) of virus was obtained from 100 g of tumor by the methods described. Purification by density gradient electrophoresis usually yielded two visible bands. One band, which remained at or near the point where the sample was introduced, contained amorphous particulate material with a few entrapped virions. The virus band migrated 10 to 13 cm from the starting point during 20 hr of electrophoresis. This band proved to be highly purified virus devoid of extraneous particulate material when examined by electron microscopy. As much as 10 mg of virus could be purified at one time by density gradient electrophoresis.

Physical properties of Yaba poxvirus DNA. Extraction of Yaba poxvirus DNA by the technique of Joklik (9) yielded 50 to 70% of the available DNA, assuming that 3 to 5% of the virion is DNA as with other poxviruses (10, 22, 27). The DNA obtained was double-stranded; increases in optical density during melting experiments indicated 35 to 41% hyperchromicity (Table 1). Thermal melting curves were performed on a total of nine samples from three separate DNA extractions (Table 1). A typical melting curve is shown in Fig. 1. T_m values in 0.015 M citrate in saline ranged from 81.9 to 82.6 C; the mean T_m value was 82.3 C. This value predicts a GC content of 31.7 mole %.

Controls included determinations of T_m values for DNA from rhesus monkey liver, Yaba tumor (rhesus), and calf thymus (Table 1). Hyperchromicity change was low (25%) in both samples of rhesus liver DNA and in sample B of Yaba tumor DNA. Excluding these samples, the T_m values predicted a mean GC content of 40.5% for Yaba tumor DNA and 40.75% for calf thymus (Table 1). The latter values were in close agreement with the estimations of GC content

TABLE 1. Molar percent guanine plus cytosine content of various DNA's as determined from T_m values

DNA type	Sample and no. of determinations	Maximum hyperchromicity	Mean T_m	Molar %GC
Yaba tumor, poxvirus	A-1	36.0	82.3	31.7
	B-6	41.0	82.3	31.7
	C-2	35.0	82.25	31.6
Rhesus liver	A-1	21.0	85.0	38.5
	B-2	23.0	85.3	38.8
Yaba tumor (rhesus)	A-3	38.0	86.2	41.1
	B-1	19.0	85.4	39.3
	C-2	39.0	85.6	39.8
Calf thymus	A-1	40.8	85.9	40.5
	B-1	33.0	86.1	41.0

TABLE 2. Molar % GC content of various DNA types as determined from buoyant density in $CsCl$

DNA type	Sample and no. of determinations	Mean density	Molar % GC ^a
Yaba tumor poxvirus	A-2	1.690	32.0
	B-1	1.691	33.0
	C-2	1.691	33.0
	D-1 ^b	1.690	32.0
Rhesus liver	A-1	1.699	41.0
Yaba tumor	A-1	1.699	41.0
	C-1	1.699	41.0
Calf thymus	A-1	1.699	41.0

^a Calculated according to the formula of Rolfe and Meselson.

^b This determination was kindly performed by Clyde Goodheart of the American Medical Association's Institute for Biomedical Research in Chicago.

FIG. 1. Thermal denaturation curves for Yaba poxvirus DNA and for calf thymus DNA.

obtained from buoyant density determinations on some of the same DNA samples (Table 2).

Buoyant densities of all samples of mammalian DNA including rhesus liver was 1.699; this value agrees with similar determinations by others. Based on the equation of Rolfe and Meselson (15) where $\%GC = 1.699 - 1.658 \times 10^3$, a

value of 41.0% molar GC is predicted (Table 2). Application of the Rolfe and Meselson equation to the buoyant density values for Yaba poxvirus DNA, 1.690 (Fig. 2) to 1.691, predicts a molar %GC content of 32 to 33%.

On the basis of the foregoing experiments, it can be stated that Yaba poxvirus DNA has a density of $1.6905 \pm .0005$ and has a T_m of 82.3 in SSC. Based on these properties, the DNA is estimated to contain $32.5 \pm 0.5\%$ GC.

DISCUSSION

It is generally accepted that the DNA of vaccinia, rabbitpox, cowpox, and ectromelia viruses contains 36% GC (10, 22). The T_m value of cowpox is 87°C and its density in $CsCl$ is 1.695 (9). The buoyant density of rabbitpox virus DNA is 1.6959 (19). Chemical analyses, except for that of Wyatt and Cohen (23), are consistent with estimates of base composition from physical data (9, 10). These viruses are all members of the vaccinia subgroup (10) and are closely related antigenically.

The DNA of fowlpox virus, a member of the birdpox or avianpox subgroup, differs slightly from the vaccinia subgroup. The T_m in 7.2 M $NaClO_4$ is 39.1°C, which predicts 34.6% GC; by a buoyant density of 1.6945, 35% GC is predicted (19). More recent studies by Randall et al. (14) have confirmed these findings with fowlpox virus DNA.

The present studies revealed that DNA from

BASE COMPOSITION OF YABA VIRUS DNA FROM GRADIENT CENTRIFUGATION
44,700 RPM, 21 HRS., AT 25 °C

FIG. 2. Buoyant density of Yaba poxvirus DNA in CsCl.

Yaba tumor poxvirus has a T_m of 82.3 C in SSC and a buoyant density of 1.6905 in CsCl. These physical properties differ markedly from those of other poxvirus DNA (Table 3); the GC content is approximately 32.5%.

Preliminary chemical analyses of the base composition of Yaba DNA have been less consistent than the physical analyses (Yohn and Gallagher, *unpublished data*). Percent GC in these studies has ranged from 31.9 to 36.9, with an average value of 34.7. Further analyses of base composition by chemical methods are required before it can be stated that the physical and chemical analyses are in total agreement.

It is apparent from the physical analyses that Yaba poxvirus DNA contains less GC than does the DNA from other poxviruses. Such a finding is not surprising in view of the range of base ratios in the DNA in other deoxyriboviruses (6) such as in members of the adenovirus group (7) and of the herpesvirus group (6).

Previous studies on the antigenic relationship of Yaba poxvirus to other members have not established any close relatedness (21, 25). The present studies indicate that its DNA differs from the DNA of members of the vaccinia and avianpox subgroups. Studies of its antigenic relationship to the myxoma-fibroma subgroup are under way; presently, oncogenicity is the only common property.

TABLE 3. Comparison of physicochemical properties of DNA from various poxviruses

Virus	T_m value	Buoyant density	Ratio of base pairs (GC/AT) ^a	% GC	References
Cowpox	87	1.695	1.78	36	9, 10
Rabbit-pox		1.6959		36.5	19
Fowlpox	39.1 ^b	1.6945	1.86	35	14, 19
Yaba	82.3	1.6095	2.0	32.5	

^a AT = adenosine plus thymine.

^b As determined in 7.2 M NaClO₄.

ACKNOWLEDGMENTS

We acknowledge the help and advice given by Ross Hall and Arnold Mittelman during these studies. The technical services of Larry Kline, who performed and analyzed the buoyant density determinations, are greatly appreciated.

This investigation was supported by Public Health Service research grant CA-07998 from the National Cancer Institute.

LITERATURE CITED

- Andrewes, C., A. C. Allison, J. A. Armstrong, G. Bearcroft, J. S. F. Niven, and H. S. Pereira. 1959. A virus disease of monkeys causing large superficial growths. *Acta Unio Intern. Contra Cancrum* 15:760-763.
- Bearcroft, W. C. C., and M. F. Jamieson. 1958. An outbreak of subcutaneous tumors in rhesus monkeys. *Nature* 182:195-196.

3. Brakke, M. K. 1955. Zone electrophoresis of dyes, proteins, and viruses in density-gradient columns of sucrose solutions. *Arch. Biochem. Biophys.* **55**:175-190.
4. Cheng, T., and N. Sueoka. 1964. Polymer similar to poly-deoxyadenylate-thymidylate in various tissues of a marine crab. *Science* **143**:1442-1443.
5. deHarven, E., and D. S. Yohn. 1966. The fine structure of Yaba monkey tumor poxvirus. *Cancer Res.* **26**:995-1008.
6. Fenner, F. 1968. The biology of animal viruses. Vol. I. Molecular and cellular biology, p. 85-99. Academic Press, Inc., New York.
7. Green, M., and M. Pina. 1964. Biochemical studies on adenovirus multiplication. VI. Properties of highly purified tumorigenic human adenoviruses and their DNA's. *Proc. Natl. Acad. Sci. U.S.* **51**:1251-1258.
8. Ifft, J. B., D. H. Voet, and J. Vinograd. 1961. The determination of density distributions and density gradients in binary solutions at equilibrium in the ultracentrifuge. *J. Phys. Chem.* **65**:1138-1145.
9. Joklik, W. K. 1962. Some properties of poxvirus DNA. *J. Mol. Biol.* **5**:265-274.
10. Joklik, W. K. 1966. The poxviruses. *Bacteriol. Rev.* **30**:33-66.
11. Mandell, J. D., and A. D. Hershey. 1960. A fractionating column for analysis of nucleic acids. *Analyt. Biochem.* **11**:66-77.
12. Marmur, J., and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J. Mol. Biol.* **5**:109-118.
13. Pfau, C. J., and J. F. McCrea. 1962. Release of deoxyribonucleic acid from vaccinia virus by mercapto-ethanol and pronase. *Nature* **194**:894-895.
14. Randall, C. C., L. G. Gafford, R. L. Soehner, and J. M. Hyde. 1966. Physicochemical properties of fowlpox virus deoxyribonucleic acid and its anomalous infectious behavior. *J. Bacteriol.* **91**:95-100.
15. Rolfe, R., and M. Meselson. 1959. The relative homogeneity of microbial DNA. *Proc. Natl. Acad. Sci. U.S.* **45**:1039-1043.
16. Soehner, R. L., G. A. Gentry, and C. C. Randall. 1965. Some physicochemical characteristics of equine abortion virus nucleic acid. *Virology* **26**:394-405.
17. Solter, A. 1961. Procedure for rapid concentration of phage lysate. *Virology* **13**:267-268.
18. Sueoka, N., and T. Cheng. 1962. Fractionation of nucleic acids with the methylated albumin column. *J. Mol. Biol.* **4**:161-172.
19. Szybalski, W., R. L. Erickson, G. A. Gentry, L. G. Gafford, and C. C. Randall. 1963. Unusual properties of fowlpox DNA. *Virology* **19**:586-589.
20. Wiarda, K. A., A. J. Hyman, and A. A. H. Bassenaer. 1960. The application of density-gradient electrophoresis to the separation of serum proteins. *Clin. Chim. Acta* **5**:289-295.
21. Woodroffe, G. M., and F. Fenner. 1962. Serological relationships within the poxvirus group: an antigen common to all members of the group. *Virology* **16**:334-341.
22. Woodson, B. 1968. Recent progress in poxvirus research. *Bacteriol. Rev.* **32**:127-137.
23. Wyatt, G. R., and S. S. Cohen. 1953. The bases of the nucleic acids of some bacterial and animal viruses: the occurrence of 5-hydroxymethyl cytosine. *Biochem. J.* **55**:774-782.
24. Yohn, D. S., J. T. Grace, Jr., and V. A. Haendiges. 1964. A quantitative cell culture assay for Yaba tumour virus. *Nature* **202**:881-883.
25. Yohn, D. S., V. A. Haendiges, and E. deHarven. 1966. Yaba tumor poxvirus synthesis in vitro. III. Growth kinetics. *J. Bacteriol.* **91**:1986-1991.
26. Yohn, D. S., F. R. Marmol, V. A. Haendiges, and J. T. Grace, Jr. 1966. Yaba tumor poxvirus synthesis in vitro. II. Adsorption, inactivation, and assay studies. *J. Bacteriol.* **91**:1953-1958.
27. Zwartouw, H. T. 1964. The chemical composition of vaccinia virus. *J. Gen. Microbiol.* **34**:115-123.