July 1974

Influence of Molecular Weights of Bacteriophage φ6 Double-Stranded Ribonucleic Acids on Interferon Induction

W.J. Kleinschmidt
The Lilly Research Laboratories. Indianapolis, Indiana

J. L. Van Etten
The Lilly Research Laboratories. Indianapolis, Indiana

Anne M. Vidaver
University of Nebraska - Lincoln, avidaver1@unl.edu

INFECTION AND IMMUNITY, July 1974, p. 284-285

Vol. 10, No. 1
Printed in U.S.A.

Influence of Molecular Weights of Bacteriophage ϕ6 Double-Stranded Ribonucleic Acids on Interferon Induction[1]

W. J. KLEINSCHMIDT, J. L. VAN ETTEN, AND A. K. VIDAVER

The Lilly Research Laboratories, Indianapolis, Indiana 46206 and Department of Plant Pathology, University of Nebraska, Lincoln, Nebraska 68503

Received for publication 27 March 1974

The interferon-inducing capabilities of the three molecular segments of bacteriophage ϕ6 double-stranded ribonucleic acid increased with increasing molecular weight.

The bacteriophage, ϕ6, of *Pseudomonas phaseolicola* (14) possesses three double-stranded ribonucleic acid (dsRNA) segments with molecular weights of 2.3, 3.1, and 5.0 $\times$ 10^6 (11, 13). The melting temperatures and base compositions of the three segments are nearly identical (13).

Colby and Chamberlain (3) reported that associated homopolymers poly rI: rC and the complexed, alternating polymer poly rIrC were equally efficient as inducers of interferon. The sequence of the bases, therefore, appears to be of little consequence in conferring interferon-inducing activity to dsRNA molecules. Phage ϕ6 dsRNA has been shown to be an excellent inducer of interferon (6). Since the base compositions of the three ϕ6 dsRNA segments were similar, these separated natural dsRNAs, arising from the same source, offered a unique opportunity to determine the effect of the remaining variable, molecular weight, on interferon induction. Furthermore, the ϕ6 dsRNA segments are of defined molecular weights, in contrast to the synthetically derived dsRNAs which consist of populations of molecules of varying molecular weights.

The dsRNA was isolated from the phage and the three dsRNA segments were separated as described previously (13). Each of the ϕ6 dsRNAs were injected intraperitoneally into three mice, 13 to 15 g in weight, at 15 μg per mouse; thus, at least twice as many molecules of the small segment were injected as the large segment. At 18 h after injection, the mice were bled and the sera of each group of mice were pooled and assayed for interferon. Interferon was measured by plaque inhibition of vesicular stomatitis virus on monolayers of mouse L-cells. A unit of interferon is defined as the reciprocal of the dilution affording a 50% decrease in plaque counts compared with untreated controls.

Interferon inducing activity increased with increasing molecular weight of the dsRNA (Table 1). The results coincide with those obtained by Niblack and McCreary (10), DeClercq and Merigan (E. DeClercq, Ph.D. thesis, Univ. of Leuven, Belgium, 1971), and Lampson et al. (8) in studies with associated synthetic homopolymers poly rI and poly rC. A correlation of higher antiviral activity to higher molecular weight of the double-stranded complex was generally observed in their studies. Though statistics to test the significance of these results are not available because of the scarcity of the separately isolated ϕ6 dsRNAs, from our assay experience the differences in the interferon produced are considered real and significant. The significance of the results is further emphasized when one takes into account that at least twice as many molecules of the small component as of the large component were injected.

In order for an RNA or DNA (7) to act as an inducer of interferon, two structural characteristics appear to be of primary importance. These are (i) a double-stranded helical structure, and (ii) a capability of conformational transition (5). Double-stranded RNA:deoxyribonucleic acid hybrids, though double-stranded molecules, are inactive as inducers (4), presumably because of their inability to move out of their A conformation (5, 9). The superiority of dsRNAs as inducers may reside in their capability of easily moving out of their A conformation into other transitional states, A' and A'' (1, 5), which is very likely facilitated by their possession of a 2' hydroxy group (2, 3, 12). One might readily expect that, along with the requirement of a conformational change to produce activity, a minimum size of the inducer molecule would be necessary. Lampson et al. (8)

[1] Journal series paper no. 3733, Nebraska Agricultural Experiment Station, Lincoln, Neb.

TABLE 1. *Interferon-inducing activities of the three dsRNA segments from bacteriophage φ6*

φ6 dsRNA segment	Average mol wt · 10^{6a}	Units of interferon produced[b]
Small	2.29	990
Medium	3.14	1,780
Large	5.00	2,190

[a] Reference 14.
[b] Per 2.5 ml of serum.

have reported that poly rI:rC lost its ability to induce interferon when the average molecular weight was less than 1.2×10^5. Size may not be the only consideration relative to molecular weight. The topological shape of the inducing molecule may also be of importance in the interaction of the inducer with the cellular component. Topological conformation here refers to a tertiary structural property in contrast to helical conformation, a secondary property. The probabilities of topological shapes and therefore the potentiality of interaction of the inducer with the cellular component should increase as the molecular size of the inducer increases, all else being equal. The correlation of increased biological activity of the natural φ6 dsRNA segments with increasing molecular weight is consistent with these considerations.

We thank E. B. Murphy, E. L. Hayes, and R. K. Koski for their excellent technical assistance. This investigation was supported in part by Public Health Service grant AI 10638 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. Arnott, S., W. Fuller, A. Hogston, and I. Prutton. 1968. Molecular conformations and structure transitions of RNA complementary helices and their possible biological significance. Nature (London) **220**:561-564.
2. Black, D. R., F. Eckstein, J. B. Hobbs, H. Sternbach, and T. C. Merigan. 1972. The antiviral activity of certain thiophosphate and 2'-chloro substituted polynucleotide homopolymer duplexes. Virology **48**:537-545.
3. Colby, C., and M. J. Chamberlin. 1969. The specificity of interferon induction in chick embryo cells by helical RNA. Proc. Nat. Acad. Sci. U.S.A. **63**:160-167.
4. Colby, C., B. D. Stollar, and M. I. Simon. 1971. Interferon induction: DNA-RNA hybrid or double-stranded RNA? Nature N. Biol. **229**:172-174.
5. Kleinschmidt, W. J. 1972. Biochemistry of interferon and its inducers. Annu. Rev. Biochem. **41**:517-542.
6. Kleinschmidt, W. J., L. D. Boeck, R. M. Van Frank, and E. B. Murphy. 1973. Interferon production by φ6 *Pseudomonas phaseolicola* phage and its double-stranded RNA. Proc. Soc. Exp. Biol. Med. **144**:304-307.
7. Kleinschmidt, W. J., R. J. Douthart, and E. B. Murphy. 1970. Interferon production by T4 coliphage. Nature (London) **228**:27-30.
8. Lampson, G. P., A. K. Field, A. A. Tytell, M. M. Nemes, and M. R. Hilleman. 1970. Relationship of molecular size of $rI_n:rC_n$ (poly I:C) to induction of interferon and host resistance. Proc. Soc. Exp. Biol. Med. **135**:911-916.
9. Milman, G., R. Langridge, and M. H. Chamberlin. 1967. The structure of a DNA-RNA hybrid. Proc. Nat. Acad. Sci. U.S.A. **57**:1804-1810.
10. Niblack, J. F., and M. B. McCreary. 1971. Relationship of biological activities of poly I·poly C to homopolymer molecular weights. Nature N. Biol. **233**:52-53.
11. Semancik, J. S., A. K. Vidaver, and J. L. Van Etten. 1973. Characterization of a segmented double-helical RNA from bacteriophage φ6. J. Mol. Biol. **78**:617-625.
12. Steward, D. L., W. C. Herndon, and K. R. Schell. 1972. Influence of 2'-0 acetylation on the antiviral activity of polyribonucleotides. Biochim. Biophys. Acta **262**:227-232.
13. Van Etten, J. L., A. K. Vidaver, R. K. Koski, and J. P. Burnett. 1974. Base composition and hybridization studies of the three double-stranded RNA segments of bacteriophage φ6. J. Virol. **13**:1254-1273.
14. Vidaver, A. K., R. K. Koski, and J. L. Van Etten. 1973. Bacteriophage φ6: a lipid-containing virus of *Pseudomonas phaseolicola*. J. Virol. **11**:799-805.