

Influence of Molecular Weights of Bacteriophage $\phi 6$ Double-Stranded Ribonucleic Acids on Interferon Induction¹

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The interferon-inducing capabilities of the three molecular segments of bacteriophage $\phi 6$ double-stranded ribonucleic acid increased with increasing molecular weight.

The bacteriophage, $\phi 6$, of *Pseudomonas phaseolicola* (14) possesses three double-stranded ribonucleic acid (dsRNA) segments with molecular weights of 2.3, 3.1, and 5.0×10^6 (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 rI:rC 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 $\phi 6$ dsRNA has been shown to be an excellent inducer of interferon (6). Since the base compositions of the three $\phi 6$ 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 $\phi 6$ 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 $\phi 6$ 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 $\phi 6$ 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)

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TABLE 1. Interferon-inducing activities of the three dsRNA segments from bacteriophage $\phi 6$

$\phi 6$ dsRNA segment	Average mol wt $\cdot 10^{6a}$	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 $\phi 6$ dsRNA segments with increasing molecular weight is consistent with these considerations.

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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 $\phi 6$ *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 $\phi 6$. *J. Mol. Biol.* **78**:617-625.

12. Steward, D. L., W. C. Herndon, and K. R. Schell. 1972. Influence of 2'-O 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 $\phi 6$. *J. Virol.* **13**:1254-1273.

14. Vidaver, A. K., R. K. Koski, and J. L. Van Etten. 1973. Bacteriophage $\phi 6$: a lipid-containing virus of *Pseudomonas phaseolicola*. *J. Virol.* **11**:799-805.