Viscoscity Studies on the Sodium Desoxyribonucleates Obtained from Pneumococcus Types III and VI

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In order to gain more information about the molecular characteristics of the sodium desoxyribonucleates isolated from pneumococcus type III and pneumococcus type VI (*1, 2*), a series of viscosity studies was undertaken on each of these desoxyribonucleates (*3*). Since these substances do have the ability to transform under suitable conditions the R strain of pneumococcus into the capsulated type III pneumococcus in one case and the capsulated type VI pneumococcus in the other case, it would seem that more information concerning the molecular properties of these desoxyribonucleates is needed.

The method previously employed to estimate the molecular weight of thymus sodium desoxyribonucleate (*4*) was employed in these experiments to estimate the molecular weights of the desoxyribonucleates from pneumococcus type III and pneumococcus type VI. This method employs the sedimentation constant, the intrinsic viscosity, and the partial specific volume in suitable equations giving the molecular weight.

The desoxyribonucleates studied were those previously isolated (*1, 2*). Because of the small amount of material available, only the ordinary Ostwald laboratory viscometer with a flow time of 80 to 100 sec for water was used. The viscosity studies were carried out at 20° ± 0.1°C. Density determinations were made using the Ostwald pycnometer (*5*). The desoxyribonucleates were dissolved in 0.2*M* NaCl, placed in Visking casings, and put to dialyze against 0.2*M* NaCl at 5°C for 24 hr. Before viscosity determination, the solutions were filtered through a coarse sintered-glass filter to remove large particles. Each solution was diluted serially by weight, giving a series of six different concentrations each. The concentrations of the original solutions were determined as was previously described (*4*). The relative viscosities, η_r, were calculated. The regression lines for ln η_r on c were calculated by the method of least squares. These curves were analyzed statistically and interpreted. The slopes of these lines give the weight intrinsic viscosities. In each case the volume intrinsic viscosity was obtained by multiplying the weight intrinsic viscosity by 100 and dividing by the respective partial specific volume.

Table 1. Viscosity data at 20°C.

Desoxyribonucleate from pneumococcus type III*		Desoxyribonucleate from pneumococcus type VI†	
Concn. (g/100 ml)	$\ln\eta_r$	Concn. (g/100 ml)	$\ln\eta_r$
0.080	1.037	0.085	1.184
0.053	0.703	0.056	0.784
0.034	0.457	0.036	0.519
0.027	0.365	0.028	0.405
0.020	0.270	0.021	0.307
0.011	0.157	0.012	0.174

* $\ln\eta_r = 13.15c$; $\sigma = 0.20$; $r = 0.9992$; volume intrinsic viscosity = 13.15/0.515 × 100 = 2553.4.
† $\ln\eta_r = 14.06c$; $\sigma = 0.18$; $r = 0.9994$; volume intrinsic viscosity = 14.06/0.568 × 100 = 2475.4.

The results of the viscosity determinations are given in Table 1. The standard errors of the slopes, σ, are given and the correlation coefficients, r, for the lines are given. The volume intrinsic viscosities are also listed in the table.

Values for the axial ratio, frictional ratio, molecular weight, and molecular dimensions were calculated for both of the desoxyribonucleates using the equations of Simha, Perrin, and Svedberg (*6*) for prolate ellipsoids of revolution. For sodium desoxyribonucleate from pneumococcus type III using a volume intrinsic viscosity of 2553.4, sedimentation constant of 13.37 (*1*), and partial specific volume of 0.515 (*1*), the following values were obtained: axial ratio, 222.56; molecular weight, 1,200,000; molecular diameter, 1.80 mμ; and molecular length, 399.14 mμ. For sodium desoxyribonucleate from pneumococcus type VI using a volume intrinsic viscosity of 2475.4, sedimentation constant of 17.10 (*2*), and partial specific volume of 0.568 (*2*), the following values were obtained: axial ratio, 219.06; molecular weight, 2,100,000; molecular diameter, 2.26 mμ; and molecular length, 495.08 mμ.

Considering the desoxyribonucleates as flexible chain molecules, one obtains, by making the indicated substitutions in the Mandelkern and Flory equation (*7*), the following values of molecular weight: for desoxyribonucleate from pneumococcus type III, 1,900,000; and for desoxyribonucleate from pneumococcus type VI, 3,400,000.

The weight intrinsic viscosity of the sodium desoxyribonucleate from pneumococcus type VI is significantly higher than that of the sodium desoxyribonucleate obtained from pneumococcus type III. On the other hand, because of the effect of the partial specific volume, the volume intrinsic viscosities are less different. When the necessary physical data are substituted in appropriate equations, the molecular weight of the desoxyribonucleate from pneumococcus type VI is significantly higher than that of the desoxyribonucleate from pneumococcus type III. Both of these desoxyribonucleates have transforming activity, in that they can transform the R strain of pneumococcus into the capsulated pneumococcus type III or the capsulated pneumococcus type VI. Although it is probable that more remains unknown than is known about the transforming process, it is interesting to conjecture that part of this difference in transforming specificities is the result of differences in molecular size. In any event, these molecules are large and show a high degree of asymmetry.

Differences in molecular weight are observed when one assumes that the desoxyribonucleates are a prolate

ellipsoid of revolution or a flexible chain molecule. More work probably will have to be done before an ideal model can be designed. It is felt that the differences in observed viscosity values for the two desoxyribonucleates are real. The viscosity values are not considered to be absolute, because insufficient material was available for an investigation of the effect of shear gradient.

References and Notes

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1. V. L. Koenig, L. Larkins, and J. D. Perrings, *Arch. Biochem. Biophys.*, in press.
2. ———, *ibid.* **39**, 355 (1952).
3. Work done under auspices of the U.S. Atomic Energy Commission.
4. V. L. Koenig and J. D. Perrings, *J. Colloid Sci.* **8**, 452 (1953).
5. ———, *Arch. Biochem. Biophys.* **38**, 105 (1952).
6. M. A. Lauffer, *J. Am. Chem. Soc.* **66**, 1188 (1944).
7. L. Mandelkern and P. J. Flory, *J. Chem. Phys.* **20**, 212 (1952).

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Reversible Photoreaction Controlling Expansion of Etiolated Bean-Leaf Disks

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Several developmental responses of higher plants are known to be controlled by the same or a quite similar reversible photoreaction. Thus lettuce seed germination (*1*), cuticle coloration of tomato (*2*), photoperiodic induction (*3*), and auxin-induced growth of the oat coleoptile (*4*) are all dependent on reactions either promoted or inhibited by red light (6500 A). These red-light-induced reactions, whether promotive or inhibitory, are all reversed by subsequent exposure to far-red irradiation (7350 A). The expansion of etiolated leaves is also known to be light-dependent, with red light being maximally effective in promoting the expansion (*5*). The experimental results reported here (*6*) are the first demonstration that this red-light-promoted expansion is reversed by subsequent exposure to far-red irradiation.

Seed of Ferry-Morse dwarf stringless greenpod beans that had been sterilized in 15-percent Purex were thoroughly washed in distilled water, soaked for 3 hr, and then grown in a sand-vermiculite mixture kept in a darkroom maintained at 26° ±1°C. Disks, 5 mm in diameter, were prepared from the unexpanded simple leaves of these dark-grown beans according to the method of Miller (*7*). The leaf sections were then placed in petri dishes on filter-paper disks treated with 5 ml of solution at *p*H 5.6 containing 3 percent D-glucose, by weight, and 0.08*M* KNO_3. All manipulations prior to final measurement were performed under a dim green safelight (*4*). Durations of the red and far-red irradiations are indicated in Tables 1 and 2. All measurements were made after 48 hr with the aid of a binocular microscope equipped with an ocular micrometer.

The data of Table 1 show that red light (*4*) promotes expansion of etiolated bean-leaf disks and that this promotion is reversed by subsequent exposure to far-red irradiation (*4*). A comparison of treatments 5 and 6 of Table 1 shows that red light given continuously is more effective than a single short exposure. This is in agreement with Miller's finding (*8*) that an additional exposure to red light given on the second day of incubation causes a marked increase in expansion over that elicited by a single exposure.

The data of Table 2 summarize the results of a series of experiments in which red light alone, red light followed by far-red, or no irradiation were given to separate lots of leaf sections. These results are in agreement with those presented in Table 1. Downs

Table 1. Promotion of expansion of etiolated bean-leaf disks by red light and reversal of the red-light effect by far-red light. Results expressed as millimeters increase in diameter of 5-mm disks after 48-hr growth in darkness following exposure.

Treatment	Duration of treatment (hr)	Increase in diameter
1) Red (fluorescent source and filter)	0.5	1.7 ± 0.04*
2) Same as No. 1, followed by far red	1	1.1 ± 0.02
3) Red (Mazda and filter)	0.5	1.6 ± 0.03
4) Same as No. 3, followed by far red	1	1.0 ± 0.03
5) Red (photographic safelight, 60 w)	0.5	1.5 ± 0.04
6) Same as No. 5, given continuously	48	2.0 ± 0.02
7) Far-red control	1	0.9 ± 0.02
8) Dark control	48	1.0 ± 0.02

* Standard error.

Table 2. Suppression of the red-light-promoted bean-leaf disk expansion by far-red exposure. Results expressed as millimeters increase in diameter of 5-mm disks after 48 hr.

Experiment no. and conditions	Expansion in		
	Red	Red followed by far red	Dark
L-3 17.5-hr irradiation	2.6		1.2
L-8 20 min red, 40 min far red	1.53 1.62	1.19 1.08	1.01 1.04
L-10 30 min red, 45 min far red	1.86	1.13	1.05
Average of L-8 and L-10	1.67	1.13	1.03