INTERACTION OF POLYAMINES WITH TURNIP YELLOW MOSAIC VIRUS RNA

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Johnson and Markham (1962) isolated a polyamine, which they identified as bis(3-amino propyl)amine (BAPA), from turnip yellow mosaic virus (TYMV) grown on Chinese cabbage. This amine is absent in uninfected Chinese cabbage. It is presumed to be synthesized as a consequence of virus infection. Polyamines have been found in bacterial viruses, in ribosomes and in other particulate materials which contain nucleic acids (see, e.g. Colbourn, Witherspoon and Herbst, 1961; Cohen and Lichenstein, 1960).

While their specific biological functions are not known, polyamines and diamines have been shown to interact with DNA and bring about an increased resistance to temperature induced unfolding (Mahler and Mehrotra, 1962; Mandel, 1962; Tabor, 1962). This increase in "melting" temperature has been correlated with adenine-thymine content of the DNA. Effects of polyamines on absorbancy-temperature profiles and on other physical characteristics of high molecular weight RNA have not been reported, although Cantoni (1960) found that S-RNA can be fractionated by precipitation with spermine, and Felsenfeld and Huang (1961) showed by conductometric titration that S-RNA has a strong affinity for spermine.

The present absorbancy, sedimentation and viscosity studies show that BAPA and spermine (which does not occur in TYMV) are remarkably efficient in preventing unfolding of TYMV RNA and in bringing about a compact tertiary structure.

Materials and Methods

RNA was isolated from TYMV by a modified phenol method (Mitra, Enger and Kaesberg, 1963). RNA was then precipitated with alcohol, washed with alcohol, and dialyzed against an appropriate buffer.

Absorbancy-temperature profiles were determined with a Beckman DB Spectrophotometer with thermally regulated cell chambers.

Sedimentation coefficients were determined at low RNA concentrations with a Spinco Model E analytical ultracentrifuge fitted with ultraviolet optics. The position of the sedimenting boundary was determined from densitometer tracings.

Temperature-induced absorbancy increase at 260 mμ was taken as a measure of unfolding of the RNA helical structure (Doty et al., 1959).

BAPA, obtained from the Matheson Company, Inc., was crystallized as the hydrochloride. Spermine-tetrahydrochloride from Mann Research Laboratories was used without further purification. Polyamine solutions were adjusted to a suitable pH with KOH solutions. All other chemicals were of reagent grade.

Results

In Fig. 1 are shown absorbancy-temperature profiles of TYMV RNA (30 μg/ml) in 0.01 M K phosphate (pH 7.0) alone, and in the presence of 10^{-4} M $MgCl_2$, 10^{-5} M BAPA, 10^{-4} M BAPA, and 10^{-4} M spermine. The midpoints, T_m, of the transitions are 30°, 44°, 47°, 56° and 65° C, respectively. Clearly spermine is more

efficient than BAPA in preventing temperature-induced unfolding and BAPA is more efficient than Mg^{++}, even at 10 times lower concentration.

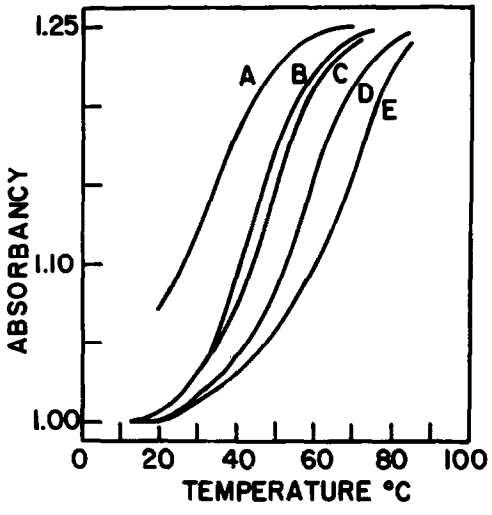

Fig. 1. Absorbancy-temperature profile of RNA in 0.01 M K-phosphate (pH 7.0).

A. Control without any added amine or Mg^{++}. B. 10^{-4} M $MgCl_2$. C. 10^{-5} M BAPA. D. 10^{-4} M BAPA. E. 10^{-4} M spermine.

The possibility of precipitation of the RNA by the polyamines during the melting studies was checked by measuring absorbancy at 320 mμ. In 0.01 M K-phosphate or in higher salt, neither 10^{-4} M spermine nor 10^{-4} M BAPA precipitated RNA when it was present in low concentration (30-50 μg/ml). However, RNA which had been dialyzed against water precipitated both with spermine and BAPA under the above conditions.

The sedimentation rate of RNA (40-45 μg/ml) in 0.01 M K-phosphate (pH 7.0) alone and in the presence of 10^{-4} M $MgCl_2$, 10^{-4} M BAPA and 10^{-4} M spermine are given in Table 1. From the increased sedimentation rates it appears that Mg^{++}, spermine and

TABLE 1

Sedimentation Rate of TYMV RNA in Several Media

	s_{20}
10^{-4} M spermine + 10^{-2} M K phosphate	31
10^{-4} M BAPA + 10^{-2} M K phosphate	24.5
10^{-4} M $MgCl_2$ + 10^{-2} M K phosphate	23.3
10^{-2} M K phosphate	18.8

The sedimentation rates were measured at 20° C. The rate in the presence of 10^{-4} M spermine is uncertain since the sedimenting boundary was quite diffuse.

BAPA induce an RNA structure more compact than it is in 0.01 M K phosphate alone. This effect may not be as great as the increased sedimentation rate indicates because in addition to bringing about a more compact tertiary structure within a single RNA molecule, polyamines could induce intermolecular bonding resulting in aggregation. This evidently occurs in 10^{-4} M spermine in which the sedimentation rate of the RNA is quite large (31 S) and furthermore the sedimenting boundary is diffuse. However, in 10^{-4} M BAPA, the increased sedimentation rate probably can be accounted for by the decrease in viscosity resulting from the more compact RNA configuration and is not due to aggregation. Unfortunately this is difficult to verify precisely. Viscosity

changes are not readily measurable at the low concentrations suitable for the sedimentation analyses. However, the viscosity of TYMV RNA at higher concentration (0.87 mg/ml) in 0.01 M K phosphate was measured as a function of BAPA concentration. It may be seen from Fig. 2 that BAPA reduced the viscosity very markedly, thus implying a more compact structure and consequently an increased sedimentation rate.

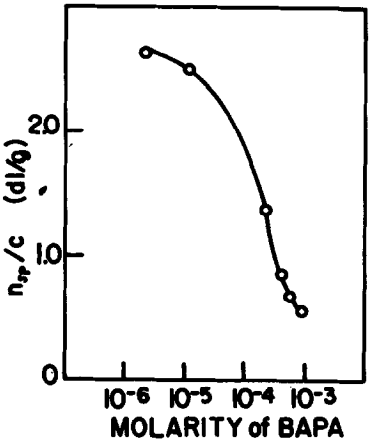

Fig. 2. Dependence of reduced viscosity of RNA (0.87 mg/ml) in 0.01 M K-phosphate (pH 7.0) on concentration of BAPA at 20° C.

Preliminary studies showed that 10^{-4} M BAPA M slowed ribonuclease digestion of TYMV RNA at least 2 to 3-fold.

Discussion

It has been shown that BAPA and spermine interact with TYMV RNA to make it more compact and less susceptible to unfolding and to digestion by ribonuclease. Although the biological role of polyamines is not yet clear, it seems evident from the above results that polyamines may help to bring about and maintain the exceedingly compact RNA structure found in small viruses.

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