THE STABILIZATION OF BACILLUS SUBTILIS TRANSFORMING PRINCIPLE BY SPERMINE

Herbert Tabor

Laboratory of Pharmacology and Toxicology, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland

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The experiments reported in this paper demonstrate the ability of the naturally-occurring polyamine, spermine, $NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$, to increase the stability of a transforming DNA to heat inactivation[1]. The Bacillus subtilis transforming system of Spizizen (1959), as modified by Fox (1960), was used.

DNA was isolated from a tryptophan-independent strain of B. subtilis[2] as described by Fox, and treated as described in Tables 1 and 2. These preparations were then assayed for their ability to transform B. subtilis tryptophan-requiring cells[2] to tryptophan-independent cells.

As indicated in Table 1, approximately 50 per cent of the transforming activity was lost when the DNA was heated in dilute buffer to 70° ("melting-out" temperature[1]). In the presence of 10^{-4} M spermine, a comparable inactivation occurred only at 92°. As indicated in Table 2, some stabilization was observed with as little as 2×10^{-6} M spermine. Some effect was also noted with spermidine, 1,4-diaminobutane, and 1,5-diaminopentane, but considerably higher concentrations were necessary. At this temperature (90°) no effect was noted with $MgCl_2$, $CaCl_2$, or NaCl, although in other experiments, carried out at lower temperatures (70-80°), NaCl did have some stabilizing effect.

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[1] For references to previous studies on the heat-inactivation of DNA see Marmur and Lane (1960) and Doty et al (1960).

[2] Kindly supplied by Dr. M. S. Fox. We also wish to thank Drs. K. Kurahashi and A. Peterkofsky for their advice on the assay procedures for the transforming DNA.

TABLE 1

The effect of 10^{-4} M spermine in protecting transforming DNA against heat inactivation

A DNA preparation (O.D. 260 mμ = 19) from B. subtilis tryptophan$^+$ was diluted 500 fold in 10^{-3} M potassium dimethylglutarate buffer, pH 6.21, containing either (a) no further additions or (b) 10^{-4} M spermine hydrochloride. One ml. aliquots of these dilutions were heated for 30 min. in a 4-liter water-bath at the indicated temperatures, quickly cooled in ice-water, and stored until assayed (24 hrs.) at 0°. Immediately prior to assay sufficient 0.1 M spermine hydrochloride was added* to each tube to give a final concentration of 2×10^{-3} M. These solutions were then assayed for transforming activity by mixing 0.05 ml. aliquots with 0.5 ml. of tryptophan$^-$ recipient cells (5×10^6 cells), incubating the mixture for 30 min. at 37°, and plating 0.1 ml. aliquots on casein-hydrolysate (tryptophan-free) plates. The data below represent the average number of transformants found under these conditions in two separate experiments. In control experiments without the added DNA preparation, no colonies were observed on the casein hydrolysate plate.

Temperature	Transformant colonies observed	
	No spermine during heating*	10^{-4} M Spermine during heating*
0°	40	61
65°	29	73
70°	26	61
75°	6	57
80°	1	66
85°	0	57
90°	1	43
95°	0	3

* We have found that the presence of spermine during the incubation of transforming DNA and recipient cells usually results in a 2 to 4-fold increase in the number of transformants. Consequently, spermine was added to all of the DNA preparations after cooling, so that the final concentration of spermine would be the same in all tubes during the assay procedure. The reason for this effect is not known, and is currently under investigation.

Although the mechanism of this protective action of spermine against heat-denaturation of DNA is not known, the most likely explanation is related to the strong complexing of nucleic acids and polynucleotides by spermine that has been reported by Keister (1958), Razin and Rozansky (1959), Felsenfeld and Huang (1960), and others[3]. The increased stability of the DNA can be explained

[3] For other references on spermine and spermidine, refer to H. Tabor, C. W. Tabor, and S. M. Rosenthal in Ann. Rev. Biochem., Vol. 30 (1961) in press.

TABLE 2

Effect of various concentrations of spermine, spermidine, 1,4-diaminobutane, 1,5-diaminopentane, NaCl, $CaCl_2$, and $MgSO_4$ on heat-stability of transforming DNA

DNA was diluted as described in Table 1, except for the additions indicated below. One ml. aliquots were then either (a) stored at 0° or (b) heated at 90° for 30 min. The tubes were then rapidly cooled in ice-water, and to each tube 20λ of 0.1 M spermine hydrochloride were added (see footnote to Table 1). 0.1 ml. aliquots were then incubated with 0.4 ml. of the recipient cells for 30 min. at 37°. 0.2 ml. aliquots of mixture were then plated on casein-hydrolysate plates. The data below represent the average number of transformants found under these conditions in two separate experiments.

Additions	Transformant colonies observed	
	0° incubation	90° incubation
None	254	0
10^{-4} M Spermine	355	268
10^{-5} M "	371	251
4×10^{-6} M "	329	147
2×10^{-6} M "	387	50
10^{-3} M Spermidine	461	164
10^{-4} M "	266	134
2×10^{-5} M "	321	22
10^{-2} M 1,4-Diaminobutane	330	62
10^{-3} M "	330	19
10^{-4} M "	320	0
10^{-2} M 1,5-Diaminopentane	341	96
10^{-3} M "	339	20
10^{-4} M "	321	0
10^{-2} M $MgSO_4$	358	0
10^{-3} M "	353	0
10^{-2} M $CaCl_2$	330	0
10^{-3} M "	386	0
10^{-1} M NaCl	419	0
10^{-2} M "	347	0
10^{-3} M "	290	0

by the increase in the effective strength of the hydrogen-bonding resulting from the complexing of spermine with the negatively-charged phosphate groups of the DNA, as well as by any polyamine cross-linkages that might be formed.

It is probable that effects similar to those reported in this paper may account for some of the previously reported stabilizing effects of spermine

on protoplasts (Mager, 1959; C. W. Tabor, 1960), bacteriophage T5 (H. Tabor, 1960), and mitochondria (C. W. Tabor, 1960; Herbst and Witherspoon, 1960).

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