HEAT DENATURATION OF BACILLUS SUBTILIS TRANSFORMING DNA

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Received 17 January 1969

It is well established that when biologically active transforming DNA macromolecules from different strains of bacteria are heat-denatured, a small percentage of the original transforming activity remains [1–5]. The origin of this residual transforming activity has been the subject of considerable interest. The present paper reports the effect of heat denaturation on the DNA from Marburg strain of *Bacillus subtilis*.

DNA, isolated from Marburg strain of *B. subtilis* by the method of Marmur [6], was thermally denaturated by heating for 20 min at several temperatures (90, 93.5 and 100°C) in 0.15 M NaCl containing 0.02 M KH_2PO_4 buffer, pH 6.8, at a concentration of 20 μg/ml and immediately cooled by immersion in an ice-bath. Samples of the native and heat-denatured DNA were examined for biological activity using the competent strain of *B. subtilis*, 3115 (histidine $^-$, tryptophan $^-$, arginine $^-$) prepared as described previously [5]. The results which are shown in fig. 1, indicated that the relative transforming activity of the arginine (89%), histidine (71%) and tryptophan (68%) genes after treatment at 90°C was decreased compared to the original native DNA. Moreover, it was found that by heating DNA to 93.5° (fig. 1), the relative transforming activity of the arginine gene was 57% of the original native DNA, while the activities of the histidine and tryptophan genes were 8 and 11%, respectively. These results indicated: a) a close similarity in properties of the histidine and tryptophan genes (confirming the previous finding that they are genetically linked [5,7]) and b) the arginine gene is less heat-labile than the histidine and tryptophan genes. However, the relativw transforming activities of all three genes were greatly reduced when the temperature was raised to 100°C (fig. 1).

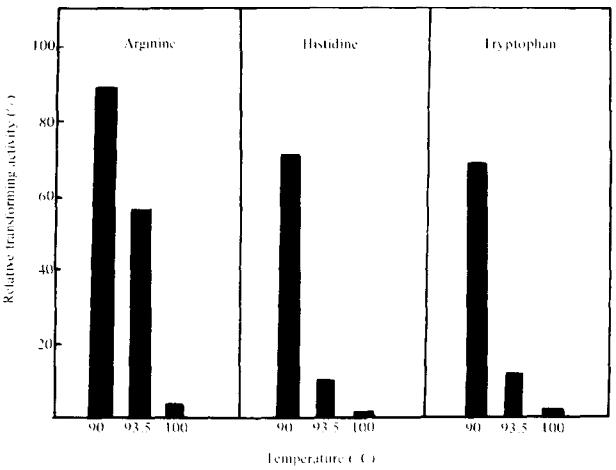

Fig. 1. Heat inactivation of the arginine, histidine and tryptophan genes at different temperatures. *B. subtilis* DNA was heated to different temperatures in a water bath at a concentration of 20 μg/ml for 20 min and immediately cooled. Biological activity was assayed at a DNA concentration of 1 μg/ml using competent strain of *B. subtilis* (histidine $^-$, tryptophan $^-$, arginine $^-$) as described previously [5]. The activity values are expressed as percentage of the original native DNA.

The effect of DNA concentration on the residual transforming activities of histidine, tryptophan and arginine genes were subsequently studied and the results are shown in table 1. It can be seen that the residual transforming activities of the three genes were independent of DNA concentration.

The time course of heat inactivation of *B. subtilis* transforming DNA for the three genes is characterised

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Table 1
Effect of DNA concentration on the residual transforming activity for the histidine, tryptophan and arginine genes.

DNA concentration (μg/ml)	Transforming activity (%)		
	Hist.	Tryp.	Arg.
1	3.5	3.9	5.2
5	3.2	3.8	5.4
10	3.6	3.7	5.6
15	3.4	3.9	5.3
20	3.6	3.7	5.8
25	3.9	3.5	5.9

Samples of *B. subtilis* DNA, at different concentration, were heat denatured at 100°C for 20 min and immediately cooled to 20°C in an ice-bath. The transforming activity was assayed by using competent strain of *B. subtilis* (histidine $^-$, tryptophan $^-$, arginine $^-$) prepared as described previously [5]. Transformation was carried out at a DNA concentration of 1 μg/ml. The activity values are shown as percentage of the original native DNA.

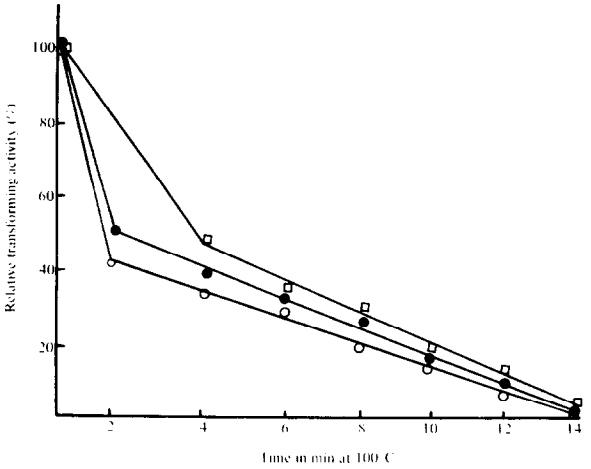

Fig. 2. Heat inactivation of the arginine, histidine and tryptophan genes at 100°C. DNA was thermally denatured at a concentration of 20 μg/ml in 0.15 M NaCl containing 0.02 M potassium phosphate buffer, pH 6.8, for different intervals of time and immediately cooled in an ice-bath. Transforming activity was carried out at a DNA concentration of 1 μg/ml using competent strain of *B. subtilis* (histidine $^-$, tryptophan $^-$, arginine $^-$) prepared as described previously [5]. The activity values are shown as percentage of the original native DNA. ●—● histidine gene; ○—○ tryptophane gene; □—□ arginine gene.

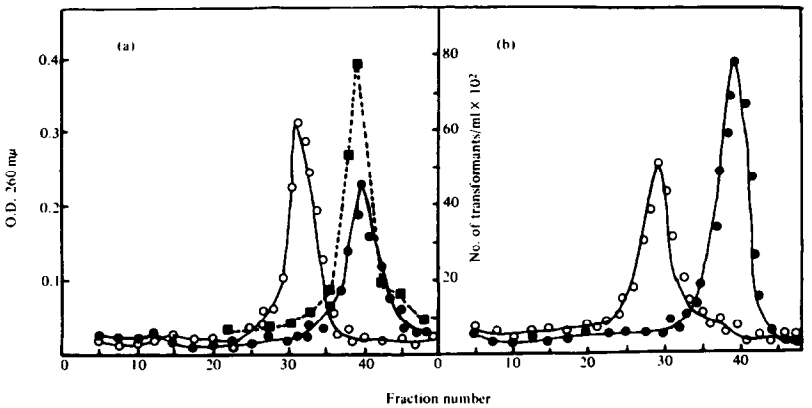

Fig. 3. (a) Buoyant density distribution and transforming activity of heat denatured DNA fractions eluted from methylated albumin coated kieselguhr [5]. Solid CsCl was added to fractions containing 100 μg DNA to give a refractive index of 1.400 and centrifuged at 35,000 rpm for 66 hr in the SW 39 rotor of the spinco model L centrifuge and 60 μl fractions were collected. One drop was used to measure the refractive index and the rest was diluted to 0.3 ml with 0.15 M NaCl containing 0.02 M KH_2PO_4, pH 6.8, and the extinction at 260 mμ was measured. Transforming activity of the fractions was carried out using competent strain of *B. subtilis* as described previously [5]. ○—○ denatured DNA (ρ = 1.720 g/cm^{-3}); ●—● native-like DNA (ρ = 1.705 g/cm^{-3}); ■—■ transforming activity. (b) Buoyant density distribution of native and denatured DNA as a control. ○—○ denatured DNA (ρ = 1.721 g/cm^{-3}); ●—● native DNA (ρ = 1.703 g/cm^{-3}).

by a very sharp drop in the biological activity in the first few minutes followed by a slower decrease (fig.2). The initial rapid decline in the biological activity may correspond to the time required for complete unwinding of the double helix, while the second more gradual drop may indicate a special reversibly denaturable fraction of DNA which loses its transforming activity only with increasing time of heating.

In order to examine the observation that the biological activity of *B. subtilis* DNA which remains after heat denaturation for a long period of time may indicate a type of DNA with a native-like structure [5], the following experiment was carried out. Marburg DNA was heat denatured at 100°C and fractionated on methylated albumin coated kieselguhr as described previously [5]. Five fractions eluting at 0.8 M, 0.85 M, 0.9 M, 0.95 M and 1.0 M NaCl were obtained, two of which exhibited residual biological activity for histidine, tryptophan and arginine genes [5] (0.8 M and 0.85 M NaCl). 20 μg of each of the five fractions were mixed and fractionated by preparative CsCl density gradient centrifugation and the fractions obtained were assayed for absorbance at 260 mμ and for biological activity as described above. Two peaks were observed (fig. 3a) with densities of 1.705 g/cm^{-3} and 1.720 g/cm^3 corresponding to native-like and fully denatured DNA molecules, respectively. The residual transforming activity for the three genes was found only in the native-like DNA fraction.

The result presented above indicated the presence of a DNA fraction which is independent of DNA concentration and had a native-like configuration after heat treatment confirming the previous observation [5]. A similar finding of a native-like pnymococcal DNA fraction has been reported by Rodger, Beckman and Hotchkiss [4]. The native-like fraction which remains after heat denaturation could arise from a heat resistant segment of the DNA in which the two strands never separate [8] (due to the presence of "cross-link"). Alternatively, since a high temperature of 110°C is necessary to completely melt dG.dC polymers [2] and the critical inactivation temperature of certain genes depends on the distribution of GC sequences [9] the residual transforming activity could represent a DNA segment in which the strands do not separate due to their specific GC content.

I would like to thank Professor G.R.Barker for many helpful discussions.

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