EFFECT OF NITROUS ACID ON ABILITY OF DNA TO INHIBIT TRANSFORMATION OF Bacillus subtilis

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Treatment of DNA with nitrous acid modifies its ability to inhibit transformation of Bacillus subtilis. Treatment for 20 min increases the inhibitory power of the DNA, but longer treatment reduces it.

Addition of DNA, isolated from the recipient cells or from other sources, to a transformation mixture of bacteria (transformant DNA + competent recipient cells) leads to the inhibition of transformation [4, 9, 14, 16]. Previous experiments with Bacillus subtilis showed that disturbances of the primary and secondary structure of inhibitory DNA, induced by x-ray irradiation or by exposure to heat or ultrasound, is accompanied by a decrease in its ability to inhibit transformation [6, 8].

The object of this investigation was to study the activity of inhibitory DNA treated with nitrous acid, because this mutagen causes deamination of nitrogenous bases and the formation of intramolecular cross-linkage [12].

EXPERIMENTAL METHOD

Transformant DNA was isolated from cells of strain Bacillus subtilis 23 EMB by the method of Ephrati-Elizur et al. [11], modified by Bresler [1]. Cells of strain B. subtilis 168_2, incapable of synthesizing leucine and tryptophan by themselves (leu^-, try^-), were used as recipients. Both strains were obtained from Professor J. Marmur (USA). Inhibitory DNA was isolated from calf's thymus by Georgiev's method [2]. Phosphorus in the DNA preparations was determined by Spirin's method [5] and protein by Lowry's method [15]. The nitrogen/phosphorus ratio in all preparations did not exceed 1.80, and the protein content, corresponding to this N/P ratio, did not affect the inhibitory activity of the DNA [7]. The viscosity of the samples was determined with a low-gradient capillary viscosimeter, with extrapolation to zero flow gradient. The melting curves of the DNA were recorded on a type SF-4 spectophotometer in thermostatically controlled cells, Transformation experiments were carried out by a modified method of Spizizen and Anagnostopoulos [10]. Treatment of DNA with nitrous acid was carried out by Schramm's method [17]. The inhibitory and transforming activities of DNA were determined by counting the numbers of leu^+, try^+, and $leu^+ + try^+$ transformants.

EXPERIMENTAL RESULTS

To determine the effect of nitrous acid on the ability of DNA to inhibit transformation of B. subtilis, DNA from calf thymus, treated with 2 M $NaNO_2$ solution for 20, 40, and 60 min, was used. The effect of $NaNO_2$ on the transforming activity of DNA (treatment for the same times) was examined in control experiments. To exclude any possible inhibitory action of residues of nitrous acid on transformation, in some experiments, instead of DNA inhibitor, nitrous acid and physiological saline were added to the transformation mixture. In this case no inhibitory effect was observed.

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TABLE 1. Degree of Inhibition of Transformation of B. subtilis in Relation to Time of Addition of Inhibitory DNA, Treated with Nitrous Acid, to Transformation Mixture

Selective marker	Transformation	Inhibition with untreated DNA	Time of add. of mod. DNA rel. to time of add. of transf. DNA (in min)					
			before			after		
			10	20	30	10	20	30
try+	100	42,2	0,2	1	0	0	0	0,01
leu+	100	52,8	1	0	0	0,1	0	0
leu+ +try+	100	41,7	0	0	0	0	0,4	0

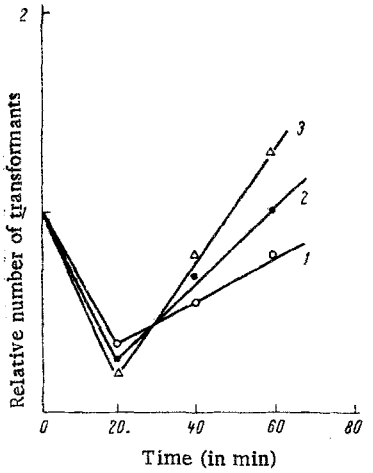

Fig. 1. Kinetics of inactivation of inhibitory DNA: 1) leu+; 2) try+; 3) try+ + leu+.

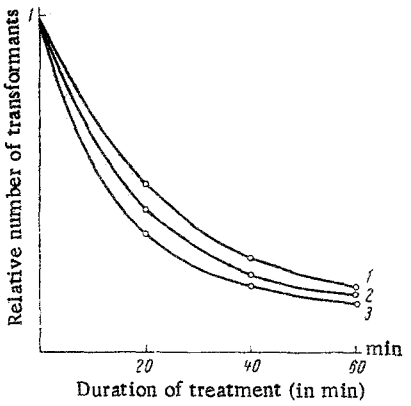

Fig. 2. Kinetics of inactivation of transforming DNA. Legend as in Fig. 1.

The effect of nitrous acid on the ability of thymus DNA to inactivate the transformation of B. subtilis is illustrated in Fig. 1. DNA activity was dependent on the time of nitrous acid treatment, because treatment of this DNA for 20 min caused a further increase in its inhibitory activity. After treatment for 40 min, the number of transformants of all three classes began to increase. After treatment for 60 min, the number of transformants returned to normal, i.e., the ability of the thymus DNA to inhibit transformation was completely suppressed. Changes in the activity of transformant DNA after its treatment with nitrous acid are shown in Fig. 2. With an increase in the duration of treatment (20, 40, and 60 min) its transforming activity diminished and the number of trasnformants of all three classes decreased sharply.

The next step was to investigate the relationship between the inhibitory activity of DNA and damage to its primary and secondary structure. For this purpose the melting temperature of native and treated DNA was determined. Investigation of melting curves of native and modified DNA showed that, during treatment of the DNA with ntrous acid, its melting temperature decreased. In a solution with ionic strength 0.01 it was shifted from 65° for native DNA to 62, 60, and 55° for DNA treated with nitrous acid for 20, 40, and 60 min (respectively). This effect is connected with weakening of the secondary structure due to deamination of guanine by the reaction with nitrous acid and to conversion of the more stable G-C pair into the left stable A-T type of pair [3]. No distubance of the primary DNA structure could be detected by the methods used. An attempt was made to separate the DNA strands by heat in 0.01 M Na^+ solution, but because of the formation of stable covalent cross linkages between the DNA strands [13], it was impossible to separate these strands even at 92-94°.

The results concerning the kinetics of suppression of the inhibitory activity of thymus DNA under the influence of nitrous acid, and also the results of control experiments in which the activity of transformant DNA was suppressed, indicate that the character of inhibition of transformation and inactivation of the try^+, leu^+, and $leu^+ + try^+$ markers is almost identical.

The authors have previously shown [4] that the inhibitory activity of DNA depends on the time of addition of the DNA inhibitor to the transformation mixture, and that the greatest inhibitory activity is observed if inhibitory DNA is added 10 min after transforming DNA. It was therefore decided to study the relationship between the degree of inhibitory activity of DNA isolated from calf thymus and treated with nitrous acid for 20, 40, and 60 min, and the time of its addition to the transformation mixture. On the addition of thymus DNA, treated with nitrous acid 10, 20, and 30 min before and 10, 20, and 30 min after the addition of transforming DNA, to competent cells of B. subtilis, the number of transformants of all three classes was found to be independent of the time of addition of inhibitory DNA to the recipient cells (Table 1).

It is clear from Table 1 that the addition of thymus DNA, treated with nitrous acid, to the transformation mixture at different times was accompanied by practically no inhibition of transformation.

The results of these experiments thus show that treatment of DNA, capable of inhibiting the transformation of B. subtilis, with nitrous acid modifies its activity as an inhibitor of this genetic recombination system. A complex, biphasic process lies at the basis of this modification.

Modification of the inhibitory activity of DNA depends on the duration of treatment, but not on the time of its addition to the transformation mixture.

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