Transforming Activity in Both Complementary Strands of *Bacillus subtilis* DNA

Wolfgang Köhnlein

Institut für Strahlenbiologie der Universität Münster

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Transforming Activity, Ultracentrifugation, BrUdR-Sensitization

A *Bacillus subtilis* mutant (strain 168 try⁻ thy⁻) with a thymine-adenine bias in complementary DNA strands was found. After BrU-incorporation into the DNA it was possible to separate physically hybrid DNA-molecules BrU-labeled in different complementary strands using CsCl density gradient centrifugation. These hybrid DNA preparations were used to investigate whether both strands can be informative during transformation. Taking advantage of the selective degradation of BrU-labeled DNA strands by long wavelength UV it was found that the loss of transforming activity was the same for both hybrid DNA preparations. Thus indicating that both complementary DNA strands can be genetically active during transformation. These results are supported by transformation experiments with denatured and reannealed DNA-molecules and heteroduplex DNA-molecules.

Introduction

The question whether both complementary strands of a DNA-molecule can be informative during genetic transformation has been dealt with in several publications [1–3]. This became possible after methods for separating complementary strands have been developed using differential complexing with polyriboguanylic acid [4, 5] or salt gradient elution [6]. In this communication a completely different approach was made to solve the problem of transforming activity in complementary strands exploiting the increased sensitivity of BrU-DNA to ultraviolet light of wavelength 313 nm and using a *B. subtilis* mutant with an adenine-thymine bias in complementary strands. Evidence presented in this paper supports the view that in *B. subtilis* transforming principle either strands can be informative.

Materials and Methods

All biochemicals were obtained from Serva, cesium chloride was purchased from Merck, enzymes like RNase, DNase, pronase, and murimidase were purchased from Worthington Biochem. Corp. Sarkosyl N30 used for bacterial lysis was a gift from Geigy.

A poly autotroph B. subtilis mutant (M 172 ade⁻, his⁻, leu⁻, try⁻, met⁻, str^R) constructed by Don Morrison of Yale University was used as acceptor.

Requests for reprints should be sent to Priv.-Doz. Dr. W. Köhnlein, Institut für Strahlenbiologie der Universität, *D-4400 Münster/Westf.*, Hittorfstr. 17.

Competent bacteria were obtained according to the method of Bott and Wilson [7]. At the maximum of competence bacteria were mixed with glycerol (10% final), frozen in liquid nitrogen, and stored till use. Usually a competence of 0.5 – 1.5% was obtained. The donor DNA was taken from a *B. subtilis* mutant, a derivative from the Marburg strain 168 requiring thymine and tryptophan for growth. For BrU-containing DNA the bacteria were grown under defined conditions as already described [8]. The DNA was extracted and purified by Marmur's procedure [9] using 0.2% Sarkosyl after murimidase treatment to facilitate lysis. For transformation 0.1 μg DNA per 1 ml of competent cells was used.

Preparative CsCl equilibrium centrifugation was used to separate DNA-fractions of different buoyant density due to their various BrU-contents. The gradients were fractionated using a recording flow through system of high resolution with an active volume of 0.004 ml [10].

Analytical centrifugation was performed with a Heraeus-Christ analytical ultracentrifuge (AZ 9100) equipped with UV-optics, photoelectric scanning and multiplexing.

To separate complementary DNA-strands alkali denaturation (0.1 M NaOH) and CsCl equilibrium centrifugation was employed. For renaturation single stranded DNA in SSC at pH 7 was sealed in quartz cuvettes and kept at 70 °C for 5 – 10 hours.

For UV-irradiation a medium pressure mercury lamp (Philips HOQ 700) was used. The UV-light of wavelength shorter than 300 nm was eliminated by interference reflection type filters (Schott & Gen. Mainz UV-R 280) in connection with cut off-filters (Schott & Gen. Mainz WG5, WG6). The lamp was

delivering a fluence of 25.5 erg/mm² sec at the surface of the sample as measured by potassium ferrioxalate actinometry [11].

Experimental and Results

Analytical equilibrium centrifugation of BrU-substituted DNA extracted from various thymine auxotrophs of *B. subtilis* gave the wellknown banding pattern of three bands of different intensity representing normal, hybrid, and bifilarly labeled DNA. Usually the hybrid band was found to be broader than the other two bands (Fig. 1 a). With

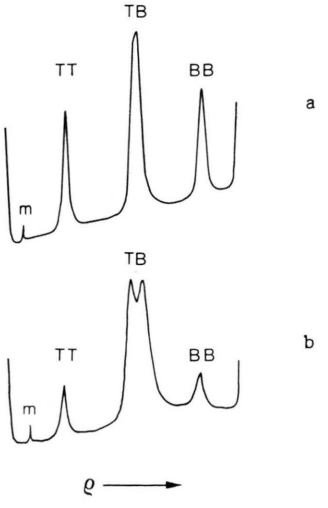

Fig. 1. Scanner tracings of analytical ultracentrifugation experiments with BrU-DNA of *B. subtilis* in CsCl. BrU-DNA with a small (a) and a large (b) adenine-thymine bias in complementary strands. m = meniscus; TT, TB and BB are representing normal, hybrid and bifilarly labeled DNA. Samples were centrifuged to equilibrium at 44,700 rpm; 25 °C; $\varrho_0 = 1.750$ gm/ml.

one thymine and tryptophan requiring mutant (*B. subtilis* 168 thy⁻ try⁻) which originally came from J. L. Farmer's laboratory the hybrid DNA always appeared as a double band of equal intensity and a separation at the maxima corresponding to a $\Delta\varrho$ of 0.010 – 0.012 gm/ml (Fig. 1 b). An explanation for the appearance of two bands at the hybrid position can be given by assuming a different adenine-thymine ratio in complementary strands. The large splitting of the hybrid bands made a preparative separation feasible. Making use of the higher resolution power of an angle head rotor [12] and employing the recording flow through system [10] for evaluating preparative density gradients a DNA distribution as shown in Fig. 2 a was obtained. Corresponding fractions were collected and pooled as indicated in Fig. 2 a. Upon recycling these samples in a CsCl-gradient, it could be shown that DNA

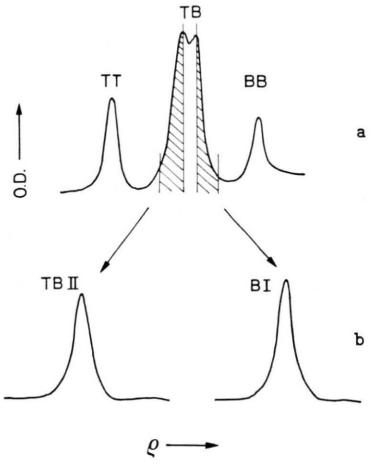

Fig. 2. Banding pattern of BrU-DNA after preparative ultracentrifugation in CsCl (a). DNA-fractions were pooled as indicated, recycled and banded (b). A SSC solution with BrU-substituted DNA was adjusted with CsCl to a density of $\varrho_0 = 1.750$ gm/ml. Aliquots of 5 ml were placed in cellulose nitrate tubes, overlaid with paraffin oil, and centrifuged in a Beckman No. 65 fixed angle rotor for 20 hours at 45,000 rpm and 16 °C. Gradients were fractionated as described in Methods.

BrU-labeled in one strand (I) had been physically separated from DNA BrU-labeled in the complementary strand (II) (Fig. 2 b). Furthermore the hybrid BrU-DNAs were free from bifilarly labeled DNA as shown by analytical ultracentrifugation. Even after "overloading" with hybrid BrU-DNA no UV-absorbing material could be found at the position of bifilarly labeled DNA. The transforming activity was the same within experimental error for both the heavy peak (I) and the light peak (II) hybrid DNA. Usually about 25 000 – 30 000 transformants per 0.1 μg DNA per 1 ml were obtained.

With long wavelength UV it is possible to inactivate the transforming activity of BrU-DNA by producing single strand breaks almost exclusively in the BrU-containing strands [13, 14]. Thus we have the possibility to inactivate different complementary DNA strands in the hybrid BrU-DNA samples I and II respectively. In Fig. 3 the transforming activity is plotted *versus* UV-fluence for hybrid BrU-DNA I and II.

As can be seen heavy and light peak hybrid BrU-DNA is inactivated in the same mode by long wave-

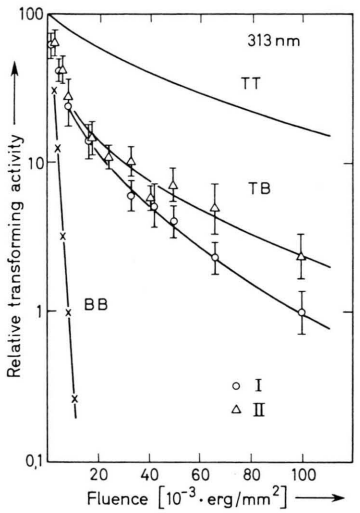

Fig. 3. Relative transforming activity after UV-irradiation of BrU-containing hybrid DNA I (–○–) and II (–△–) compared with the inactivation of TT-DNA and bifilarly labeled BB-DNA. Transformation assays were made for (his) prototrophy. Similar relations were obtained when assaying for methionine or adenine prototrophy. The absolute values of transformants per 0.1 μg DNA per 1 ml for the unirradiated samples of TT-, BB-, hybrid I- and II-DNA were about 40,000; 23,000; 25,000; 30,000 respectively. The error bars indicate the mean deviation of at least 8 different irradiation experiments.

length UV. These results are consistent with the idea that genetic information can be transferred by either complementary strand in transforming principle of *B. subtilis*. If only one DNA strand is informative we should have obtained for one hybrid BrU-DNA an inactivation curve as for normal (TT) DNA, whereas the other hybrid BrU-DNA would have been inactivated like bifilarly labeled BrU-DNA (Fig. 3).

The assumption made in interpreting the results of the UV-irradiation experiments can be confirmed by an independent method: Heavy and light hybrid BrU-DNA was alkali denatured in separate vials. The BrU-labeled strands and thymine-containing strands were separated by density gradient centrifugation. After reannealing single stranded DNA molecules which originally formed double stranded DNA molecules up to 20% of the original transforming activity was obtained. In an attempt to form heteroduplex molecules by mixing BrU-labeled DNA strands of the heavy hybrid with thymine-containing strands of the light hybrid no or less than 1% transforming activity was reclaimed as expected thus supporting the idea that hybrid BrU-DNA I and II are BrU-labeled in complementary strands.

Discussion

For a thymine tryptophan auxotroph of *B. subtilis* two distinct DNA peaks are obtained at the position of hybrid DNA after BrU-incorporation and CsCl density gradient centrifugation thus indicating a bias in base composition of complementary strands. Upon recycling two hybrid DNA fractions are physically separated, which are BrU-labeled in different complementary strands. Making use of the selective degradation of BrU-substituted DNA by long wavelength UV irradiation it was possible with a very simple system to obtain information about transforming principle on the molecular level. Thus complicated handling of the DNA – necessary to obtain similar information in other systems – could be avoided. The finding that both complementary DNA strands can be informative during genetic transformation is in good agreement with the results of Chilton [2] and Goodgal *et al.* [15] and do not support the idea of an informative and not informative DNA strand [3, 16]. The small difference in the UV-inactivation curves between hybrid I and II BrU-DNA cannot be taken as a strand selection bias in native DNA transformation but is certainly due to the higher BrU-substitution in hybrid I DNA. The results obtained with denatured and reannealed DNA support the conclusions drawn from the UV-inactivation curves.

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