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Activity of Deoxyribonucleic Acid Fragments of Defined Size in *Bacillus subtilis* Transformation

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The transforming activity of *Bacillus subtilis* deoxyribonucleic acid (DNA) that had been sheared and purified with respect to size by sucrose gradient sedimentation is given as a function of the DNA molecular weight. It is shown (i) that fragments of median molecular weight 1.2×10^6 have finite activity (10^{-4}), (ii) that the shape of the activity-versus-molecular weight function is qualitatively similar to that observed previously for *Diplococcus pneumoniae*, and (iii) that this shape precludes interpretation in terms of critical size models.

The dependence of the transforming activity of deoxyribonucleic acid (DNA) upon the molecular weight of the DNA has been investigated in detail by Cato and Guild (2) for the pneumococcus system. They reported that measurement of the activity of fractionated, sheared samples of DNA showed (i) that "critical size" models could not fit the data, (ii) that a crossover probability model (4) could fit the data when one assumed that a constant amount of DNA in each donor molecule was excluded from the genetic interaction, and (iii) that the apparent size of this "excluded length" was 450 base pairs. In a later discussion (3), they noted that the data available for the *Bacillus subtilis* transformation system could be fit by the same model with an excluded length (M_0) of 4,500 base pairs and a value for c (recombination switch frequency) of 0.2×10^{-6}.

Since the only data on size dependence of transforming activity for *B. subtilis* were those of Bodmer (1), which were based on populations of randomly produced fragments, we thought it important to determine the activities of more homogeneous samples of DNA. The use of fractionated DNA is particularly important in the region of very low activity. We now report the results of measurements of relative transforming activities of fragments of *B. subtilis* DNA purified with respect to size by sedimentation through sucrose gradients.

MATERIALS AND METHODS

Transformation. Competent cells of strain M152 (*trp-2 hisA1 leu ade*) were prepared as described previously (6). The maximal yield of transformants obtained with saturating DNA concentration was 2×10^6 per ml. Half saturation occurred at about 1 μg/ml for high-molecular-weight DNA. Selection for adenine-independent transformants was accomplished by plating the transformed cells on minimal agar supplemented with 400 μg of Casamino Acids per ml, as described previously (6).

DNA. DNA was isolated from strain 23T⁻ essentially by the method of Marmur (5). The unsheared donor median molecular weight was 52×10^6, and its median single-strand molecular weight was 9.5×10^6. Samples for shearing were dissolved in SSC (0.15 M NaCl plus 0.015 M sodium citrate, pH 7) at a concentration of about 100 μg/ml; shearing was accomplished by stirring with a homogenizer for 20 to 40 min under conditions described by Cato and Guild (2). Fractionation of 1-ml samples of sheared DNA layered on 29-ml linear 5 to 20% sucrose gradients was performed as described previously (2), except that DNA concentration was monitored continuously during collection of fractions. Only fractions near the peak of the distribution (comprising 10–20% of the DNA) were retained from each sheared sample that was fractionated.

Molecular weights. After dialysis to remove sucrose, the median sedimentation coefficient of each fractionated DNA sample was determined by duplicate measurements in a Beckman model E ultracentrifuge by the boundary sedimentation method (A. Cato, Jr., Ph.D. thesis, Duke Univ., Durham, N.C., 1966). The solvent was SSC or (0.1 M NaOH plus 0.9 M NaCl), and the correction factors for solvent viscosity and density were taken to be 1.04 and 1.16, respectively (7). The concentration of DNA in each sample was determined from the step height of the sedimenting boundary.

Activities. Relative transforming activities were determined by incubating DNA samples with competent cells in a series of parallel tubes at 35 C for 45 min and terminating the reaction with deoxyribonuclease I. For samples of low activity, cells from 5 to 10 ml of transformed culture were collected by cen-

trifugation at 5 C, suspended in 1 ml of medium, and spread on 100 ml of selective agar in a 14-cm diameter petri dish. Samples of higher activity were appropriately diluted before plating. DNA concentration in the assays of fractionated samples varied between 0.05 and 0.15 μg/ml; the activies given in Fig. 1 have been corrected to a standard 0.10 μg/ml in each experiment, assuming a linear concentration response. Assays of activities of sheared, but unfractionated, samples were performed in different experiments at DNA concentrations of 0.25 to 5 μg/ml.

RESULTS

The relative transforming activities of sheared samples of DNA after purification by sedimentation through sucrose gradients are given in Fig. 1. The activities of some unfractionated samples are also given since, as seen in the figure, relative activities do not change significantly with fractionation for the sheared samples of molecular weights 2×10^6 to 9×10^6, confirming the observation of Cato and Guild (2).

The relative transforming activities given in Fig. 1 are the combined results of seven separate assays. For the samples of fractionated DNA, the results of each separate assay were normalized to the activity of the sample of median molecular weight 3×10^6. For the samples assayed without fractionation, the results of four assays (at DNA concentrations of 0.25–5 μg/ml) were fully consistent in the molecular weight range of 1.5 to 10^6 to 9×10^6. The relative activity of the unsheared DNA control (molecular weight of 52×10^6, native; 9.5×10^6 single strand) was determined in most assays, but, unlike the sheared samples of lower molecular weight, the relative activity varied with the concentration of DNA and other variables, over a fourfold range. This behavior is not unexpected, since Green (E. W. Green, M. A. thesis, Duke Univ., Durham, N.C., 1967) found a size dependence of the apparent K_m for pneumococcus. The relative activities for samples of DNA smaller than nine million daltons from different assays can be combined only because their relative activities are observed here to be independent of concentration. Full activity in the figure is arbitrarily set at the average activity of the unsheared DNA seen in these assays. All activities for assays of fractionated samples were normalized to yield coherence with the activities of samples of unfractionated DNA in the region 2×10^6 to 4×10^6 daltons. This final normalization procedure was justified by the observations that the relative activities of samples in the region 2×10^6 to 4×10^6 daltons were reproducible in the seven assays and that the resulting ranges of relative activities of the control sample were the same for both sets of assays.

It is seen from the data of Fig. 1 that activity is very dependent on size below 6×10^6 daltons, but that the residual activity at 3×10^6 daltons is a substantial 1% of maximum. Activity is finite, but small (about 10^{-4}) at 1.2×10^6 to 1.6×10^6 daltons. The activity of a purified sample of molecular weight $0.8 \times 10^6 \pm 0.1 \times 10^6$ was not clearly above background reversion levels at an activity of 10^{-5}, but the possibility of a real activity as high as 4×10^{-5} was not excluded by the data.

DISCUSSION

Guild et al. (3) showed that Bodmer's data obtained with unfractionated samples of randomly nicked *B. subtilis* DNA fitted the same formal model they had applied to their results with pneumococcus, with values for the model's parameters of $M_0 = 3 \times 10^6$ and $c = 0.2 \times 10^{-6}$. If the size dependence of transforming activity were in fact given by that model, then DNA of molecular weight 3.0×10^6 or less would have *no* transforming activity. To test that suggestion as well as to establish the size dependence of transformation in *B. subtilis*, we took advantage of the observations of Cato on sucrose gradient fractionation. Specifically, he found (2; A. Cato, Jr., Ph.D. thesis, Duke Univ., Durham, N.C., 1966) that the DNA taken from the peak fraction of a sucrose gradient exhibited the specific transforming activity found to be characteristic of its median molecular weight on further successive fractionations, whereas the activities exhibited by other fractions were unreliable, showing significant contamination from neighboring fractions. Furthermore, this result held even for preparations with specific activities of 0.01%, in a size region where activity is a very strong function of molecular weight. Thus, a single sedimentation purification is sufficient to achieve the real specific activity of DNA near the median molecular weight of a sample of sheared DNA.

The results given here, of measurements of activities of fractionated sheared DNA samples, show that fragments of molecular weight 1.2×10^6 have finite, but small, activity and that, if there is a size absolutely incapable of producing a transformant, it must be smaller than this figure. Furthermore, interpretation of these results in terms of critical size models would require that, in the region below about 10% activity, transforming activity be a linear or convex function of molecular weight (2),

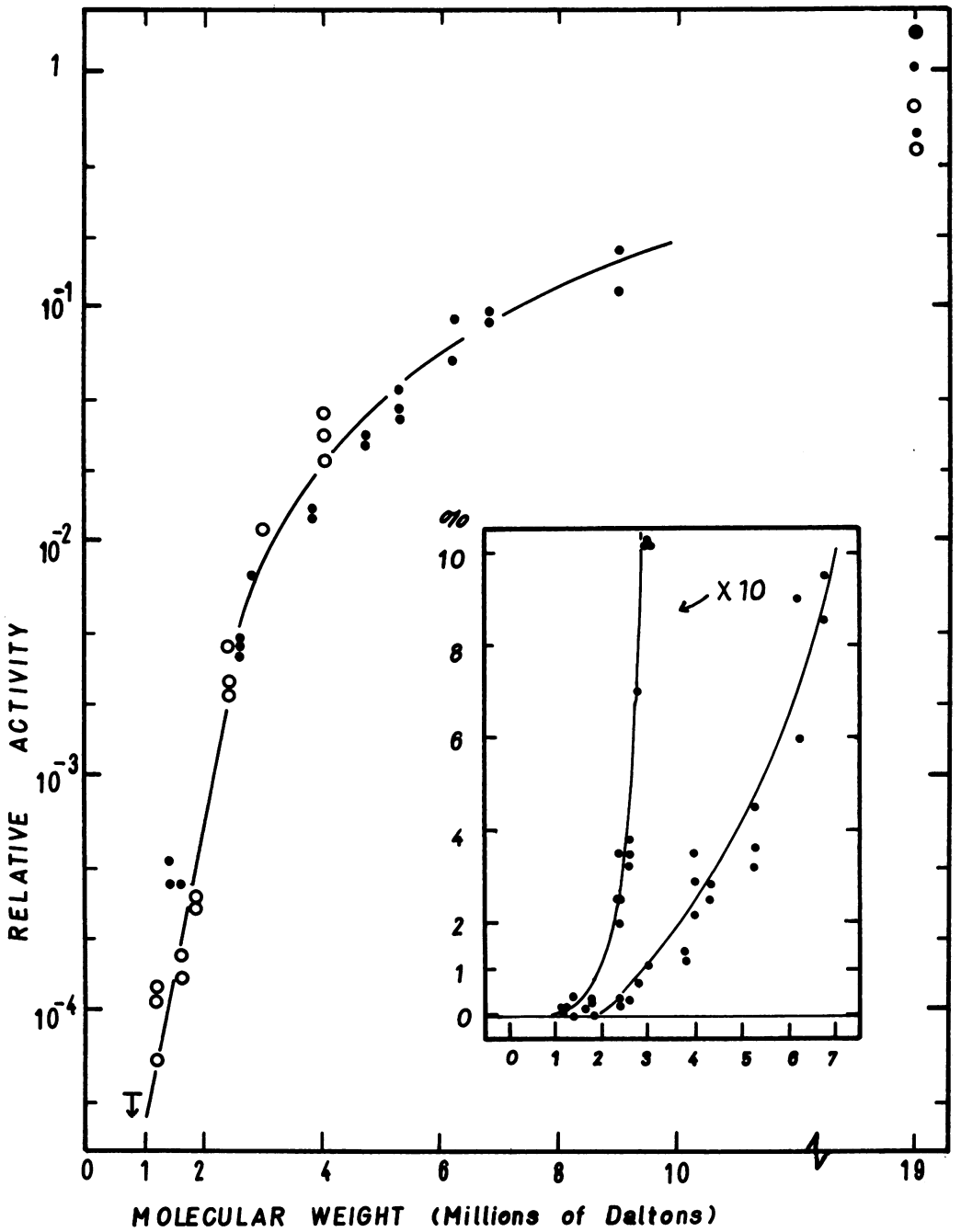

FIG. 1. *Relative transforming activity of sheared B. subtilis DNA as a function of molecular weight. Symbols: ●, unfractionated sheared samples; ○, peak fractions from sucrose gradients of sheared samples. Inset shows the lower 10% (●) and the lower 1% (○) of activity on a linear scale.*

extrapolating to zero activity at the critical size. It is clear from the inset in Fig. 1 that the observed function is concave, just as is the corresponding function observed for pneumococcus, and thus cannot be represented by such critical size models.

Our results are consistent with those of Bodmer for DNA larger than three million dal-

tons; below that size, we find a smooth concave activity function similar in shape to that found for pneumococcus. The data presented here give the relation of activity to size for DNA fragments smaller than ten million daltons; a description of the size dependence of integration for DNA inside cells and of the size dependence of the activity of larger DNA fragments must await a thorough study of the size dependence of the uptake step and of any other steps occurring prior to integration. Such a study for intracellular donor DNA in pneumococcus has shown (*manuscript in preparation*) that several early steps do exhibit size-dependent features.

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