THE KINETIC COMPLEXITY OF *EUGLENA GRACILIS* CHLOROPLASTS DNA

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1. Introduction

The amount of double stranded DNA per *Euglena gracilis* chloroplast was estimated to be 1×10^{-14} g [1]. The question arises as to whether this analytical value has a correspondingly large kinetic complexity or whether *Euglena* chloroplast DNA, analogous to chloroplast DNA from some higher plants [2], contains a significant proportion of repeated nucleotide sequences.

To answer this question, we measured the reassociation rate of denatured chloroplast DNA using standardized conditions as given by Wetmur and Davidson [3]. From these results, we estimate *Euglena* chloroplast DNA to have a kinetic complexity equivalent to a nucleotide sequence of 1.8×10^8 daltons, which is approximately one-thirtieth of the analytical complexity.

2. Materials and methods

Euglena gracilis Klebs (z-strain) cells were grown routinely under autotrophic conditions, harvested, washed and stored at $-60°$ as reported earlier [4]. Chloroplast DNA and nuclear DNA were isolated and purified as described in detail [5]. The purity of chloroplast and nuclear DNA was tested by measuring the buoyant density in neutral CsCl (Spino Model E, An-D rotor, 12 cm centerpiece) and was considered satisfactory when, at a total load of 5 μg per ml, only one band was detectable either at $\rho = 1.685$ g/ml for chloroplast DNA or $\rho = 1.708$ g/ml for nuclear DNA [1, 6, 7]. The molecular weights of the various DNA components were calculated according to Studier [8] from S values determined by band-centrifugation [9]. The rate of DNA reassociation was monitored optically at 260 nm using a Gilford Model 2000 with temperature controlled cuvette chamber and automatic recording device.

E. coli DNA was isolated according to Marmur [10]. Bacteriophage T4 DNA was a gift from Dr. Hazelkorn, University of Chicago.

3. Results and discussion

It was already known from buoyant density measurements (CsCl) that *Euglena* chloroplast DNA, contrary to nuclear DNA, renatures rapidly and rather extensively [5]. The kinetics of the reassociation process are shown in fig. 1 where the relative change in hyperchromicity is plotted versus time. Under these experimental conditions *Euglena* chloroplast DNA and bacteriophage T4 reassociate at an almost identical rate. Increase in Na^+ concentration from 0.15 to 1.0 M, increases the reassociation rate as expected [3].

Euglena nuclear DNA renatures to a small extent and very rapidly at the beginning of the process but the reaction becomes very slow afterwards.

In fig. 2, a second order rate plot [3] of the reassociation data is shown. *Euglena* nuclear DNA is omitted but *E. coli* DNA is added for comparison. Over the time shown, *Euglena* chloroplast DNA yields a straight line under both ionic conditions indicating a rather homogeneous type DNA. This is contrary to results obtained with lettuce chloroplast DNA [2] where a fast and a slow component were found. However, for chloroplast DNA isolated from tobacco

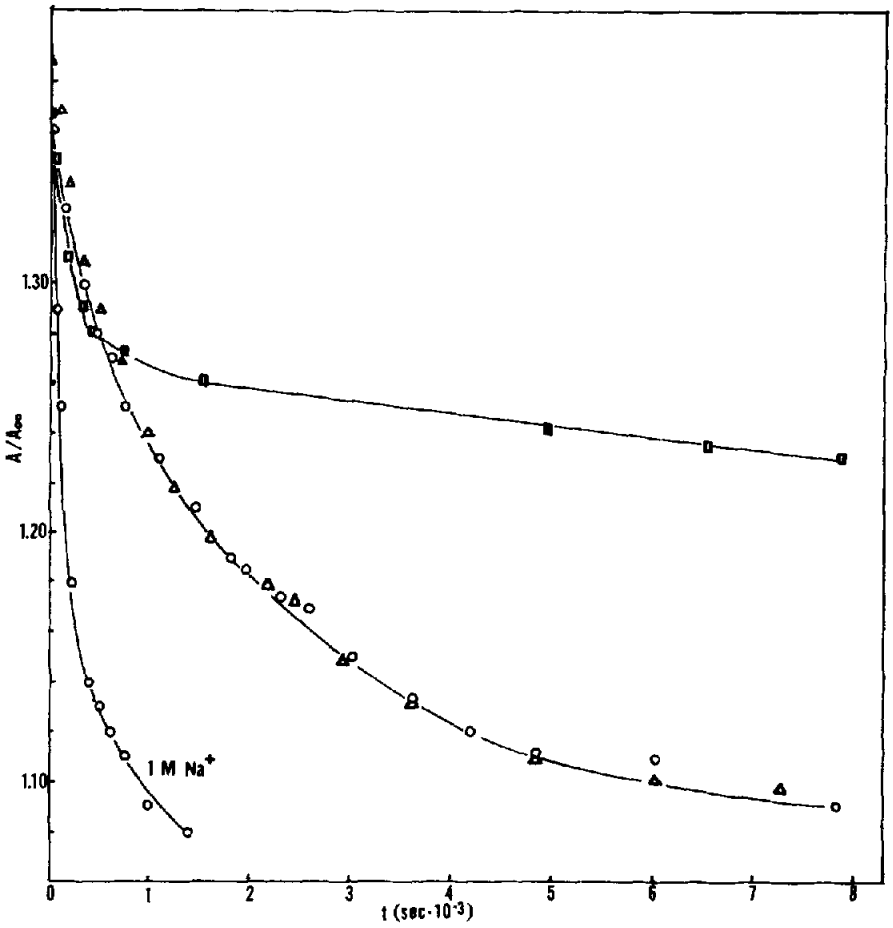

Fig. 1. Reassociation curves for various DNAs. The DNA samples were sheared prior to denaturing by passing through a 27-gauge needle. The stoppered cuvettes with the samples (1 ml) were placed in the heat controlled chamber and equilibrated at the renaturing temperature for about 15 min. The absorbance reading (260 nm) at renaturing temperature was taken as the (A_{∞}) value. (The absorbance readings at room temperature and at renaturing temperature were not significantly different). The samples were heat denatured by submerging the stoppered cuvettes in boiling water for 10 min. The first absorbance reading at renaturing temperature was usually taken 8 to 10 sec after removal from the water bath. At the end of the experiment, the samples were heated up to 95°C. The absorbance at this temperature corrected for heat expansion was taken as (A_t) value for zero times. Chloroplast and bacteriophage DNA were renatured at 60° in either 0.15 M NaCl or 1 M NaCl (see graph) buffered with 0.015 M Na citrate to pH 7.0. *Euglena* nuclear DNA was renatured at 65° in 1 M NaCl + 0.015 M Na-citrate. Chloroplast DNA (29 μg), – ○ –; bacteriophage T4 DNA (30 μg), – △ –; nuclear DNA (20 μg), – □ – .

plants only one type of DNA was found which renatured at a rate equivalent to a nucleotide sequence complexity of approximately 2×10^8 daltons [11].

In table 1 the pertinent data are summarized. The kinetic complexity is calculated according to Wetmur and Davidson [3] using the k_2 from the reassociation experiments at 1 M Na^+. According to this, *Euglena* chloroplast DNA (G + C = 26%, ρ = 1.685 g/ml, neutral CsCl) has an average kinetic complexity of 1.8×10^8 daltons. This value is about 30 times smaller than the reported analytical value [1]. We believe that a gross error in our renaturing experiment can be excluded especially since our results for both bacteriophage T4 and *E. coli* DNA match well

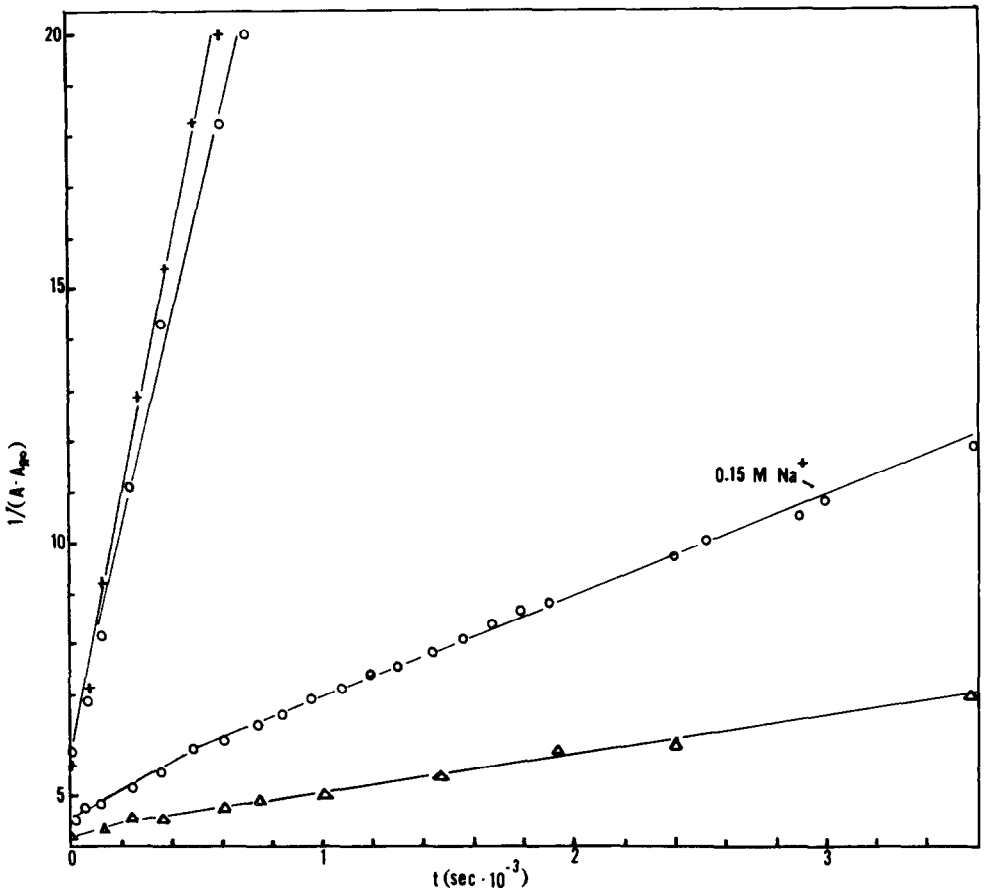

Fig. 2. k_2-Plot of partly reassociated DNAs. The reciprocal value of the remaining hyperchromicity is plotted versus time according to Wetmur and Davidson [3]. The experimental conditions were as given in the legend to fig. 1. Chloroplast and bacteriophage DNA are reassociated to 73% after 700 and 600 sec, respectively, (1 M NaCl) and to 60% after 3600 sec (0.15 M NaCl). Chloroplast DNA, – o –; bacteriophage T4 DNA, – + –; *E. coli* DNA – △ –. The NaCl is 1 M if not indicated otherwise in the graph.

the published values [3]. We may, therefore, conclude that either the reported analytical value is too high by at least one order of magnitude or the chloroplast DNA has a high frequency of repetitious nucleotide sequences (e.g., polyploid).

The question as to the length of the chloroplast DNA molecule(s) remains open. It was concluded from UV-irradiation studies [12] that mature chloroplasts of *Euglena* contain three DNA entities. Provided these three "chromosomes" are equal in size, an average moelcular weight of 2×10^9 results. Ray and Hanawalt [6] using sucrose gradients calculated that *Euglena* chloroplast DNA had a molecular weight of 2×10^7 (largest component). We measured the S value by band-centrifugation in 1 M NaCl and calculated a molecular weight of 1.2×10^7. However, we have good reasons to believe that our chloroplast DNA preparations are fragmented [13] and some of our DNA/RNA hybridization results can best be explained by assuming that intact *Euglena* chloroplast DNA has a molecular weight in the range of 10^8 daltons. Finally, it is noteworthy that *Euglena* chloroplast DNA contains between 20 to 30 cistron copies coding for 23 S/16 S chloroplast rRNA [5, 14]. This figure matches, maybe accidentally, the number of nucleotide sequence repetitions.

Table 1
Complexity in molecular weight units calculated from reassociation rates [3].

DNA	pH 13 $S_{20,w}$	k_2 ($mol^{-1}.sec^{-1}$)	$\frac{5.5 \times 10^8 (S_{20,w}^{pH13})^{1.25}}{k_2}$
Euglena chloroplast	16.8	101	1.8×10^8
Bacteriophage T4	15.1	120	1.4×10^8
E. coli	14.5	5	3.0×10^9

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