STABILITY OF THE PHYSICOCHEMICAL PARAMETERS OF DNA DURING PROLONGED KEEPING

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UDC 615.272.6:547.963.32].014.41

The physicochemical properties of purified high-polymer DNA isolated from calf thymus and sturgeon testis were investigated after its prolonged keeping (for 1.5-2 years) at 0-4°C in 1 M NaCl, as a precipitate under 70% ethanol, and in the freeze-dried state. DNA obtained from these sources can be kept for at least 1.5 years under the conditions tested without appreciable detriment to its degree of polymerization or its secondary structure. DNA from sturgeon testis, with similar initial physicochemical properties to those of thymus DNA, exhibits much greater stability when kept under the same conditions, indicating the strength of its molecular structure.

KEY WORDS: *DNA; calf thymus; sturgeon testis; stability of structure.*

Particular attention is being paid at the present time to the study of the biological effectiveness of DNA and its possible use as a therapeutic agent in the treatment of diseases of man and animals. For example, some interesting results have been obtained to show the beneficial effect of exogenous heterologous DNA in patients undergoing prolonged radiotherapy and chemotherapy for malignant tumors [12]. DNA also stimulates hematopoiesis under normal conditions and plays a role in the restoration of normal synthetic processes during recovery after irradiation [1, 9, 10]. The protective action of DNA preparations has been observed in rats irradiated with fast neutrons [5].

It was accordingly decided to study the stability of the basic physicochemical properties and biological effectiveness of high-polymer DNA after prolonged keeping under different conditions.

EXPERIMENTAL METHOD

The sodium salt of DNA obtained from freshly frozen calf thymus or sturgeon testis was used. DNA was isolated by the method of Kay et al. [7] with certain modifications. The preparations were kept for 1.5-2 years at 0-4°C in SSC (standard salt citrate) solution, in 1 M NaCl solution, as a precipitate under 70% ethanol, and in the freeze-dried state. The DNA concentration in the isolated preparations was measured spectrophotometrically [3], and the degree of polymerization (molecular weight) by a viscosimetric method using a three-ball low-gradient viscosimeter of the Ostwald type [4]. The mean velocity gradients for balls I, II, and III were 45, 26, 10 sec^{-1} respectively. Values of the characteristic viscosity (η) were found from the reduced viscosity by extrapolation to the ordinate. The native state of the DNA preparations was characterized by determination of the melting temperature (T_m), the coefficient of molar extinction at 260 nm [$E_{(p)260}$], calculated from the phosphorous concentration in the DNA, and the hyperchromicity (K_a), by means of the equation [6]

$$K_a = \frac{D_{270} \text{ DNA soln. in } 10^{-2} \text{ M NaCl, pH 3.0}}{D_{270} \text{ DNA soln. in } 10^{-2} \text{ M NaCl, pH 7.0}},$$

Research Sector, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Orekhovich.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 3, pp. 309-312, March, 1976. Original article submitted May 19, 1975.

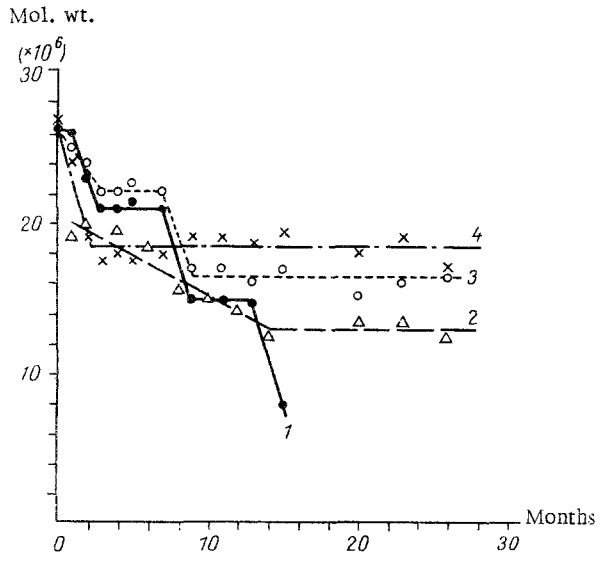

Fig. 1

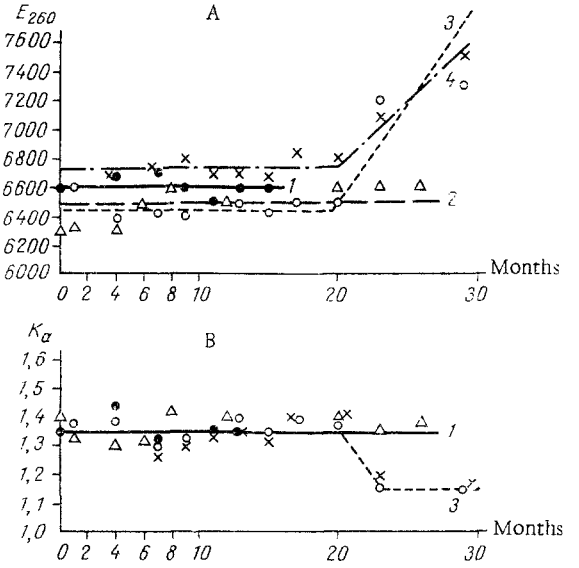

Fig. 2

Fig. 1. Degree of polymerization of samples of thymus DNA kept under different conditions as a function of duration of keeping : 1) keeping in SSC; 2) freeze-dried preparation; 3) under ethanol; 4) in 1 M NaCl. Abscissa, length of keeping DNA; ordinate, molecular weight of DNA (in daltons × 10^6).

Fig. 2. Changes in coefficient of molar extinction (E) and hyperchromicity (K_a) of thymus DNA during keeping. Abscissa, duration of keeping of DNA (in months); ordinate, value of E (A) or K_a (B). Remainder of legend as in Fig. 1.

where D_{270} is the optical density at 270 nm.

The accuracy of determination of the parameters under these conditions were: molecular weight ± 2 × 10^6, $E_{(p)260}$ ± 200, K_a ± 0.1 respectively.

The protein concentration in the DNA solutions was determined by the method of Lowry et al. [8]. The degree of contamination of the DNA with RNA was determined by the alkaline hydrolysis reaction [11].

Only those DNA samples whose molecular weight was not below 18 million, whose $E_{(p)260}$ was not more than 6500-6600, whose K_a was about 1.4, whose protein content did not exceed 1.5%, and whose RNA content did not exceed 2%, were chosen for keeping under the conditions specified above.

When DNA kept under 70% ethanol or in the freeze-dried state was dissolved, the yield of DNA was 100%.

EXPERIMENTAL RESULTS

It will be clear from Figs. 1 and 2 that all samples of thymus DNA studied exhibited definite instability at least during the first 3-4 months. Changes occurred not only in the more vulnerable secondary structure (characterized by the parameters K_a and $E_{(p)260}$), but also by the length of the biopolymer chain. The molecular weight of thymus DNA, reflecting its degree of polymerization, was found to be a more labile parameter than K_a and $E_{(p)260}$ during keeping. Curves showing changes in molecular weight during keeping of DNA samples from calf thymus in SSC and under 70% ethanol resembled staircases with many steps, whereas the curves showing the changes in $E_{(p)260}$ and K_a of calf thymus DNA during keeping were long plateaux with raised or depressed edges.

This instability of thymus DNA during keeping may have been due to the activity of nucleases which remained with the DNA during isolation, to the action of microorganism as a result of disturbance of the conditions of sterility during keeping, or to thermo-oscillatory movement of fragments of the DNA chain relative to each other. The first of these

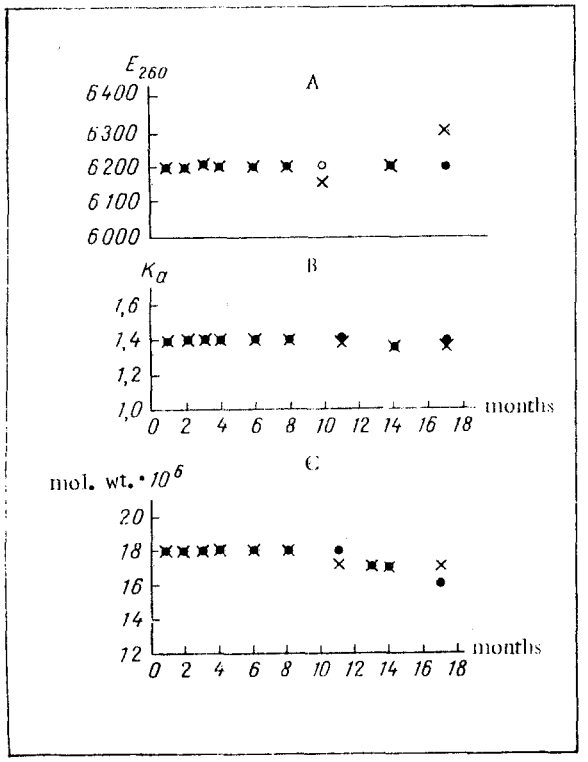

Fig. 3. Changes in physicochemical parameters of sturgeon testis DNA during prolonged keeping. Ordinate (C) molecular weight of DNA (in daltons × 10^6). Remainder of legend as in Figs. 1 and 2.

suggested causes is unlikely, for the method used to obtain DNA was based on virtually complete inhibition of nucleases (keeping the homogenate for 3 h at 60-65°C with sodium dodecylsulfate and NaCl). The second cause cannot be completely ruled out, although the absence of any appreciable changes in the secondary structure during keeping for 20 months (Fig. 2) suggests that the instability of molecular weight of thymus DNA observed during keeping was due primarily to thermo-oscillatory movements of fragments of the DNA chain relative to each other. Along the DNA chain possible sites exist at which, for various reasons (for example, the appearance of single ruptures during isolation of the DNA), bonds between neighboring polynucleotide fragments, themselves of fairly high molecular weight, are weakened. Oscillatory movements of these heavy fragments leads to depolymerization of the original chain during keeping. So far as the greater stability of DNA during keeping in 1 M NaCl is concerned, this could be the result of the more compact and more stable structure of the DNA. This explanation seems all the more likely because no appreciable changes in secondary structure of DNA were found on the curves showing changes in the values of $E_{(p)260}$ and K_a of the DNA preparation during the same period (from the 4th to the 20th month of keeping).

Despite the relative instability of the indices studied, their value, as is clear from Figs. 1 and 2, for DNA samples kept for 20 months under ethanol, in 1 M NaCl, and in the freeze-dried state, does not go beyond the limits corresponding to high-molecular-weight undenatured DNA (molecular weight not below 12×10^6, K_a not below 1.37, $E_{(p)260}$ not above 6800).

It is interesting to note that DNA obtained from sturgeon testis, with closely similar initial physicochemical properties to thymus DNA, was much more stable during keeping under the same conditions, indicating greater stability of its molecule (Fig. 3). Differences in the stability of DNA molecules (at least in their secondary structure) are associated with higher or lower values of the (G + C)/(A + T) ratio. However, according to data in the literature, the content of G + C bases in sturgeon and calf DNA is approximately equal [2]. The greater stability of DNA from sturgeon testis on keeping is probably due to other as yet unknown causes.

The results described above thus show that DNA obtained from sturgeon testis and calf thymus can be kept for at least 1.5 years at 0-4°C in 1 M NaCl, as a precipitate under 70% ethanol, and in the freeze-dried state without appreciable detriment to its state of polymerization or its secondary structure. These observations will prove useful during the study of problems connected with the production of therapeutic forms of DNA.

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