DEOXYRIBONUCLEIC ACIDS IN CRABS OF THE GENUS CANCER

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The base compositions of the deoxyribonucleic acids (DNA) of microorganisms vary widely (Chargaff, 1955; Lee, Wahl and Barbu, 1956; Belozersky and Spirin, 1960; Sueoka, 1961a) and provide useful taxonomic information (Lee, Wahl and Barbu, 1956; Lanni, 1960; Sueoka, 1961a, b; Schildkraut et al., 1962a, b). Data presently available for higher plants and animals indicate a more limited range of base compositions (Chargaff, 1955; Sueoka, 1961a) and consequently taxonomic correlations are not immediately apparent. However, the techniques of gradient equilibrium centrifugation (Meselson, Stahl and Vinograd, 1957) and thermal denaturation as measured by changes in light absorption with temperature (Marmur and Doty, 1959) which allow the determination of base compositions (Sueoka, Marmur and Doty, 1959; Rolfe and Meselson, 1959; Schildkraut et al., 1962a) also provide more subtle information regarding DNA composition. Thus, organisms from protozoa to higher vertebrates have been shown to contain DNA's which are polymodal with respect to base composition (Schildkraut et al., 1962a, b; Szybalski, 1961; Sueoka, 1961a, b; Kit, 1961, 1962). Particularly interesting in this connection are the DNA's isolated by Sueoka from the crabs, Cancer borealis and Cancer irroratus (Sueoka, 1961a, Sueoka and Cheng, 1962a, b) where one of the two DNA components has the physical characteristics of a thymidylic acid-deoxyadenylic acid copolymer (Schachman et al., 1960). In Cancer borealis this component contains 97 mole

percent of adenine and thymine, these bases being predominantly arranged in alternating sequence (Swartz, Trautner and Kornberg, 1962). As part of a more extensive investigation of marine invertebrates, the DNA's of four more species of the same genus have been examined and the results are reported in the present communication.

Experimental

Crabs were collected during the summer of 1962 as follows: Cancer magister and Cancer productus in Burrard Inlet, Vancouver, and Departure Bay, Vancouver Island; Cancer gracilis at Ladysmith, Vancouver Island; and Cancer antennarius at Monterey, California. The testes and vas deferens (1-2 gm) were excised from living crabs and either frozen at -30°C until required or homogenized immediately in 8 ml of cold 0.1 M sodium chloride-0.05 M sodium ethylenediamine tetraacetate, pH 8.0 (saline-ETDA), using a glass tissue grinder. Two millilitres of 25% aqueous sodium dodecyl sulphate (Marmur, 1961; Sueoka, 1961a) were added to the suspension which was kept at 25°C for 1 hour. Sodium chloride (2.93 g)(Hamaguchi and Geiduschek, 1962) was added and the mixture shaken by hand with an equal volume of chloroform-isoamyl alcohol (24:1) for 5 minutes at 25°C, followed by centrifugation at 10,000 x g for 10 minutes at 0°C. The upper aqueous layer, containing DNA, was decanted from the insoluble interface and the organic phase. After three treatments with chloroform-isoamyl alcohol, the DNA solution was diluted with 10 ml of saline-ETDA, and the sodium deoxynucleate precipitated by addition of isopropyl alcohol (12 ml). The precipitated fibres were wound on a glass rod and dissolved in 0.15 M sodium chloride-0.015 sodium citrate, pH 7.0 (saline-citrate)(10 ml). Precipitation by 95% ethyl alcohol (20 ml), followed by dissolution in saline-citrate, was repeated three times, the sodium deoxynucleate solution being finally stored at 0°C over chloroform. Each crab yielded 0.1 mg to 2.5 mg of nucleic acid (estimated spectrophotometrically). In the case of Cancer antennarius the above procedure was applied to sperm contained in a female spermatheca to yield 1.5 mg of deoxynucleate.

For gradient centrifugation, crab sodium deoxynucleate (1-3 µg) and N^{15}-labelled Pseudomonas aeruginosa deoxynucleate (0.5-1 µg) in saline-citrate were added to 7 M cesium chloride, 0.01 M tris(hydroxymethyl)aminomethane hydrochloride, pH 8.5 (0.93 ml) and the volume adjusted to 1.10 ml with 0.01 M tris(hydroxymethyl)aminomethane hydrochloride, pH 8.5. Centrifugation was carried out as described by Schildkraut et al. (1962a). Thermal hypochromicity in 0.15 M sodium chloride, 0.015 sodium citrate, pH 7.0 was determined by the method of Marmur and Doty (1962).

Results

The mid-point temperature in the thermal denaturation curve (Tm) and the buoyant densities of DNA's are recorded in Table 1 together with the data of Sueoka (1961a) and Sueoka and Cheng (1962b). Also the densities of some of the nucleic acids after thermal denaturation

Table 1. Deoxynucleic acids of crabs of genus Cancer.

	Tm(°C)		%[1]	Density[2](gm/ml)		%[3]
Cancer antennarius Stimpson	65.8	84.0	30	1.677	1.700	26
Cancer borealis Stimpson[4]	64.2	85.0	30	1.681	1.702	30
				1.681	1.717[5]	
Cancer graciilis Dana	65.6	84.0	12	1.680	1.700	9
Cancer irroratus Say[4]				1.680	1.700	10
Cancer magister Dana	65.8	84.6	12	1.677	1.701	14
				1.681	1.719[5]	
Cancer productus Randall	66.4	83.2	31	1.679	1.701	32
				1.684	1.718[5]	

1 Percentage of the low "melting" DNA calculated from hyperchromicity.
2 All values relative to deoxyribonucleic acids of E. coli, density 1.710 (Schildkraut et al. 1962a) or P. aeuruginosa-N^{15}, density 1.742.
3 Percentage of low density DNA calculated from microdensitometer tracing.
4 Data of Sueoka (1961a) and Sueoka and Cheng (1962b).
5 After heating at 100°C in saline-citrate for 10 minutes followed by rapid cooling in ice.

and rapid cooling are recorded together with the approximate percentages of the low "melting", low density nucleic acids. Experimental error in the Tm values is estimated at ± 0.5°C and in the densities at ± 0.001 gm/ml.

Discussion

Six species of crabs of the genus Cancer, from widely separated North American habitats, are now known to contain DNA which is bimodal with respect to base composition. The DNA is usually isolated from testes and vas deferens but has also been found in a sperm-containing female spermatheca (in Cancer antennarius). Its presence in other crab tissue is not excluded.

The low density, low "melting", DNA constitutes approximately 30% of the total in Cancer antennarius, Cancer borealis and Cancer productus and 10% in Cancer gracilis, Cancer irroratus and Cancer magister (Table 1). On this basis it is suggested that the genus may be divided into two subgroups. It will be of interest if further species fall into these divisions and also if other taxonomic characteristics correlate.

The physical properties (Table 1 and Figure 1) of the minor DNA component from both subgroups are similar and presumably reflect a similarity of base composition and sequence in all six species. This implies structures analogous to that demonstrated for Cancer borealis DNA (Swartz, M.N., Trautner, T.A., and Kornberg, A., 1962). The latter is the only naturally occurring DNA for which a major portion of the base sequence is known. The existence of several species of crab with this type of DNA should greatly facilitate elucidation of its genetic significance.

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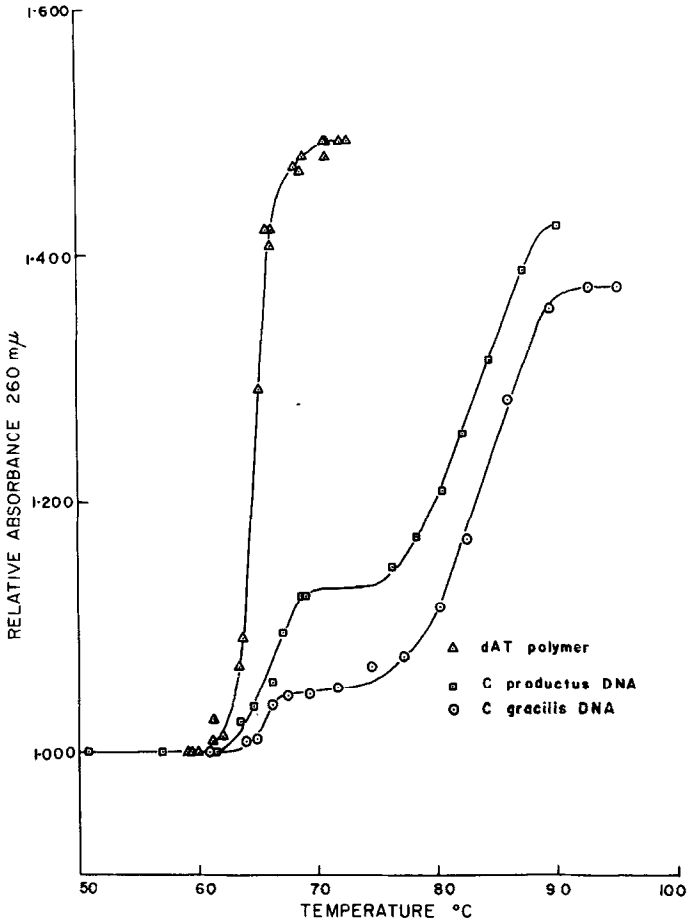

Fig. 1. Hyperchromic shifts of deoxyadenylic acid-thymidylic acid copolymer, C. gracilis DNA and C. productus DNA.

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