Molecular Relationships Between Closely Related Strains and Species of Nematodes

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Summary. Electrophoretic comparisons have been made for 24 enzymes in the Bergerac and Bristol strains of Caenorhabditis elegans and the related species, Caenorhabditis briggsae. No variation was detected between the two strains of C. elegans. In contrast, the two species, C. elegans and C. briggsae exhibited electrophoretic differences in 22 of 24 enzymes. A consensus 5S rRNA sequence was determined for C. elegans and found to be identical to that from C. briggsae. By analogy with other species with relatively well established fossil records it can be inferred that the time of divergence between the two nematode species is probably in the tens of millions of years.

The limited anatomical evolution during a time period in which proteins undergo extensive changes supports the hypothesis that anatomical evolution is not dependent on overall protein changes.

Key words: Enzyme Polymorphism – 5S rRNA – Caenorhabditis

Introduction

The genetic distance among populations and between species of higher organisms has been assayed by a variety of biochemical techniques. In general distant relationships have been explored using macromolecular sequencing while closely related species or even populations have been examined by electrophoresis, single copy DNA annealing and most recently by comparison of restriction enzyme digests (Upholt 1977; Upholt and Dawid 1977; Avise et al. 1979; Brown et al. 1979; Nei and Li 1979). Critical examination of the accumulating data led Wilson and co-workers to propose that evolution at the anatomical level is the result of changes in regulatory systems, especially embryogenesis, rather than to the general evolution of proteins (Wilson et al. 1974a, 1974b; Prager and Wilson 1974; King and Wilson 1975). If this hypothesis is correct one would expect that among organisms in which the developmental process is relatively simple one would expect to find species that were anatomically very similar in spite of large amounts of protein evolution. In a recent study utilizing restriction digests Emmons et al. (1979) encountered a surprising amount of diversity between two nematode species, Caenorhabditis elegans and Caenorhabditis briggsae. These nematodes are virtually indistinguishable by anatomical observation and are regarded as “twin species” (Nigon and Dougherty 1949). In view of the relevance of the Emmons et al. (1979) observation to evolutionary theory we have sought to obtain further molecular data documenting the amount of genetic diversity between these two nematode species. Herein we report electrophoretic characterizations of 24 enzymes and the consensus sequence of the C. elegans and C. briggsae 5S rRNAs.

Materials and Methods

Nematodes. Caenorhabditis elegans var. Bristol, strain N2, are descendants of a single hermaphrodite originally isolated near Bristol (Maupas 1900; Dougherty 1955; Nicholas et al. 1959; Brenner 1974). The other strain C. elegans var. Bergerac, was isolated in France and identified by Nigon (1949). These strains and C. briggsae (Dougherty 1985; Dougherty and Nigon 1949) were obtained from D. Hirsh, University of Colorado, Boulder. Nematodes were cultured asynchronously in an aerated liquid culture of E. coli or on standard NGM agar petri dishes (Brenner 1974) supplemented with hen’s egg media (Baillie and Rosenbluth, person, commun; Butler 1980).
Preparation of Nematode Extracts. The worms were collected and resuspended in cold 35% sucrose, followed by centrifugation at top speed in an IEC clinical centrifuge at 4°C to remove trace levels of bacteria which pellet (Sulston and Brenner 1974). The bacteria-free worms were removed from the top of the sucrose and washed three times in M9 buffer (Brenner 1974) followed by two washes in TEB sonication buffer containing 0.01 M Tris pH 7.6, 1 mM ethylenedinitrilto) tetraacetic acid (EDTA) and 1 mM β-mercaptoethanol. Prior to sonication, one half to an equal volume of TEB buffer was added to the washed worm pellet. All preparations were sonicated several times with a Heat Systems (Ultrasonics, Inc.) microtip at 20 watts for 30 s separated by 1 min intervals at 4°C until no visible carcasses were present. The extract was frozen overnight at –80°C and then clarified by centrifugation at 15,000 rpm at 4°C for 50 min.

Starch Gel Electrophoresis. The Siciliano and Shaw (1970) procedure was used to analyze 24 different enzymes. The details of this procedure and the optimal gel buffer for each enzyme and their respective histochemical detection assay are described briefly in Fig. 1 and in detail elsewhere (Butler 1980).

SS rRNA Sequence Determination. Bacteria free worms were prepared as outlined above and phenol extracted. The major RNA species were separated by electrophoresis on a 10% polyacrylamide slab gel (20 cm x 20 cm x 1.5 mm) and located by ultraviolet shadowing. The 5S rRNA was extracted from a gel slice as described (Donis-Keller et al. 1977) and then labeled with 32P in vitro. Intact 5S rRNA was labeled at either the 5′ (Donis-Keller et al. 1977) or in the 3′ end (England and Uhlenbeck 1978) while the large fragment produced by ribonuclease T2 digestion (Jordan 1971; Meyhack et al. 1977) was labeled exclusively at the 5′ end.

Sequencing gels were run on thin (40 cm x 20 cm x 0.02 cm) polyacrylamide (either 12% or 20%) slabs at 2.0 kv. The enzymatic sequencing method (Peattie 1979) was utilized on 3′ end labeled fragments. The sequences in the terminal regions were obtained by digestion of aliquots of end labeled material with ribonucleases T1, U2 or A which were then separated on DEAE paper in the presence of appropriate marker nucleotides (Uchida et al. 1974; Woese et al. 1976).

Results

Crude extracts of heterogeneously growing nematodes of Caenorhabditis elegans var. Bristol, var. Bergerac and the related species C. briggsae were subjected to electrophoresis in starch gels and assayed for twenty four different enzyme activities. The technique of starch gel electrophoresis was employed because it is capable of detecting single amino acid changes that alter the net charge of native enzymes (Siciliano et al. 1978). These enzymes were then detected histochemically. The resolution of this technique is demonstrated for 15 enzymes in Fig. 1, wherein nearly all of the enzyme mobilities of the two varieties of C. elegans (lanes 1 and 2) differed from the related species, C. briggsae (lane 3). Of the 24 enzymes studied 12 included two or more isoenzymes. Nearly all these enzyme mobilities differed between the two species (Butler 1980). Only arginine kinase (ArgK) and the leucyl-glycine peptidase (Pep GL) exhibited the same electrophoretic mobilities in all three species.