

Malate dehydrogenase and tetrazolium oxidase of scyphistomae of *Aurelia aurita*, *Chrysaora quinquecirrha*, and *Cyanea capillata* (Scyphozoa: Semaestomeae)*

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KURZFASSUNG: Malatdehydrogenase und Tetrazoliumoxydase der Scyphistomae von *Aurelia aurita*, *Chrysaora quinquecirrha* and *Cyanea capillata* (Scyphozoa: Semaestomeae). Im Gewebe der Scyphistomae von *Aurelia aurita*, *Chrysaora quinquecirrha* und *Cyanea capillata* wurde das Isozymmuster der Malatdehydrogenase (MDH) und der Tetrazoliumoxydase (TO) durch Anwendung der Polyacrylamidgel-Elektrophorese bestimmt. Entsprechend der Reihenfolge der genannten Arten betrug die Anzahl der gefundenen MDH-Isozymbanden 4,5 bzw. 1, während sich die der TO-Isozymbanden auf 2,1 bzw. 1 belief. Es wird darauf hingewiesen, daß das Isozymmuster für die taxonomische Zuordnung der schwer zu unterscheidenden Scyphopolypen neben anderen Merkmalen mit herangezogen werden kann.

INTRODUCTION

Three species of scyphozoan jellyfishes, *Aurelia aurita* (LINNAEUS 1758), *Chrysaora quinquecirrha* (DESOR 1848), and *Cyanea capillata* (LINNAEUS 1758) are commonly found in Chesapeake Bay. Because of the uncertainty in the identification of jellyfish scyphistomae (polyps) collected from the field, CALDER (1971) used the nematocyst complement for distinguishing polyps of these different species. BURNETT & GOULD (1971) used an immunological assay to differentiate polyps of *Aurelia aurita* and *Chrysaora quinquecirrha* from those of *Cyanea capillata*.

Scyphozoan jellyfishes exhibit alternation of generations in their life history. The sessile scyphistoma undergoes asexual reproduction by budding, podocyst formation, and metamorphosis (strobilation). In following the enzymes of carbohydrate metabolism during development, ZUBKOFF & LIN (1973a) reported differences in the malate dehydrogenase (MDH, 1.1.1.37) isozyme patterns in the developmental stages of Chesapeake Bay *Chrysaora quinquecirrha* and *Cyanea capillata*. The *Aurelia aurita* isozyme patterns of polyps from the Northern Atlantic Ocean (Woods Hole, Mass.) differ from those of Chesapeake Bay *Aurelia aurita* polyps (ZUBKOFF & LIN 1973b), an observation consistent with other reports that physiological and morphological

* Contribution No. 547 from the Virginia Institute of Marine Science.

differences also exist (RUSSELL 1970, WEBB et al. 1972, MORALES-ALAMO & HAVEN, unpublished observation).

In this study, the MDH and tetrazolium oxidase (TO) isozyme patterns of the polyps of Chesapeake Bay *Aurelia aurita*, *Chrysaora quinquecirrha*, and *Cyanea capillata* are reported. These isozyme patterns provide another method for distinguishing scyphozoan polyps of uncertain identity.

MATERIAL AND METHOD

Polyps used in this study were raised from planulae isolated from known medusae (CALDER 1971). Cultures of *Aurelia aurita* and *Chrysaora quinquecirrha* were maintained at room temperature (19–22° C) and those of *Cyanea capillata* at either 5° C or 15° C. Polyps maintained in filtered York River water (salinity, 20.5 ‰) were fed nauplii of newly hatched *Artemia salina* once a week. After washing, the polyps were ground with 3 ‰ (W/V) sucrose in Tris-glycine buffer, pH 8.3. In most experiments, the extract of two or three polyps of *Chrysaora quinquecirrha* or *Aurelia aurita* (5–9 µg of protein) was applied to each gel. Because of the small size of *Cyanea capillata*, six to eight polyps were used per gel. In order to assess the effect of enzyme concentration during electrophoresis, seven to nine polyps of *Chrysaora quinquecirrha* of a single clone were used.

Polyacrylamide gel electrophoresis in a vertical cell (BUCHLER Instruments, USA), using 7.5 ‰ gel made with acrylamide (EASTMAN) and Tris-HCl-TEAMED buffer system, pH 8.9, was performed (DAVIS 1964). All gels were polymerized with ammonium persulfate rather than photopolymerization. After the homogenates were centrifuged at 2000×g for 10 minutes, the crude extracts were applied directly to the tops of the gels without the aid of spacer gels. Electrophoresis was performed at 5 mA per gel in a water-cooled cell (usually 2–3 hours) with bromophenol blue as the electrophoresis dye marker.

To study the coenzyme dependence of MDH, either 2 mg of nicotinamide-adenine dinucleotide (NAD) or 0.5 mg of nicotinamide-adenine-dinucleotide-phosphate (NADP) was added to 50 ml of gel, and either 30 mg of NAD or 10 mg of NADP was added to 300 ml of Tris-glycine, pH 8.3, in the cathode chamber. In the staining solutions, either 33 mg of NAD or 5 mg of NADP was used in 50 ml of solution. Staining solutions were made within 15 minutes before use (BREWER 1970). After electrophoresis, gels were incubated in the dark at room temperature until sharp bands were observed (usually 1/2–1 hour). For the detection of TO, gels were incubated in a 45° C water bath under ambient room light.

RESULTS

MDH isozyme patterns of the three species were quite different (Fig. 1). Polyps of *Chrysaora quinquecirrha* had a major band ($R_m = 0.53$) with a second band immediately below ($R_m = 0.54$) and three faint bands ($R_m = 0.40, 0.21, \text{ and } 0.22$). When extracts of seven to nine polyps of *Chrysaora quinquecirrha* either of the same

culture or of the same clone were used (20–30 μg protein), additional trace bands ($R_m = 0.31, 0.33, 0.37$) were observed that did not appear when extracts of 2–3 polyps were used. Polyps of *Aurelia aurita* had two isozyme bands, but the locations and relative intensities of the bands differed: one band ($R_m = 0.33$) was less intense than the faster moving band ($R_m = 0.43$); trace bands appeared immediately above these

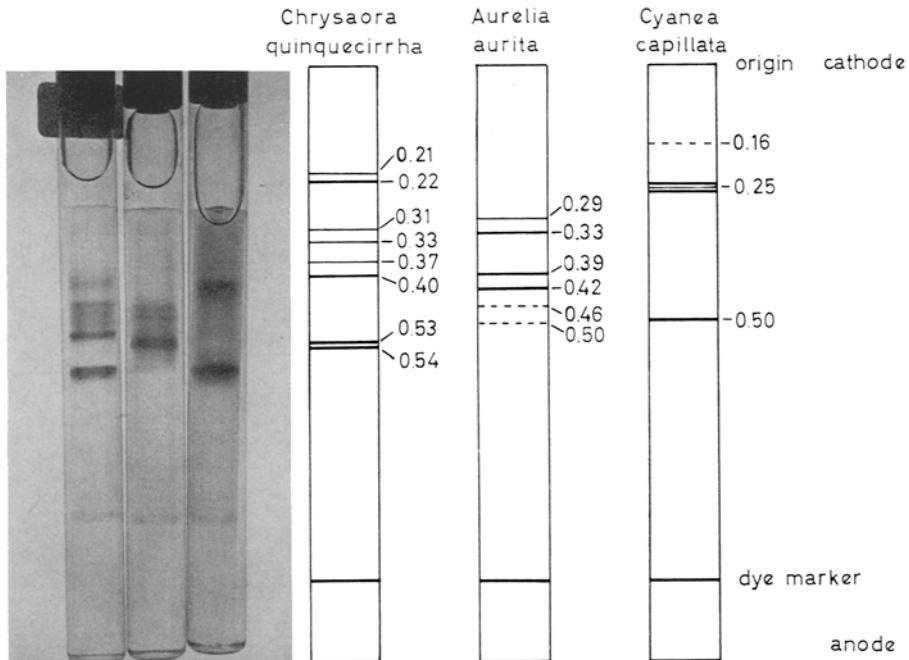


Fig. 1: MDH isozyme patterns of Chesapeake Bay scyphozoan polyps

bands ($R_m = 0.29, 0.39$) and sometimes elsewhere ($R_m = 0.46, 0.50$) when high concentrations were used. Polyps of *Cyanea capillata* usually had only one major band ($R_m = 0.50$), but sometimes a trace triplet ($R_m = 0.25$) and a single band ($R_m = 0.16$) appeared. Unlike glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (6PGDH, EC 1.1.1.44) (ZUBKOFF & LIN 1973c), MDH of the polyps of the three Chesapeake Bay jellyfishes was relatively stable after freezing and MDH was either NAD- or NADP-linked. The intensity of enzyme activities using either of these two cofactors was the same.

Figure 2 shows the TO isozyme patterns of polyps of *Chrysaora quinquecirrha*, *Aurelia aurita* and *Cyanea capillata*. *Chrysaora quinquecirrha* possesses only one band ($R_m = 0.58$) and *Cyanea capillata* has only one band with a slower movement ($R_m = 0.39$). *Aurelia aurita*, however, is quite different from the other two; it definitely has two distinct bands and sometimes three; the major band is fast-moving ($R_m = 0.51$); the slow-moving band is less intense ($R_m = 0.39$); occasionally a third trace band ($R_m = 0.62$) is observed.

DISCUSSION

MDH and TO isozyme patterns of polyps of these three species are distinct. The major differences in the TO system is that polyps of *Aurelia aurita* possess two distinct bands ($R_m = 0.39, 0.51$), with the fast-moving band ($R_m = 0.51$) as the major band and sometimes a third trace band ($R_m = 0.62$). Polyps of both *Chrysaora quinquecirrha* and *Cyanea capillata* possess single bands, but there is a great difference in their mobilities: the *Chrysaora quinquecirrha* TO band ($R_m = 0.58$) moves much faster than the *Cyanea capillata* band ($R_m = 0.39$).

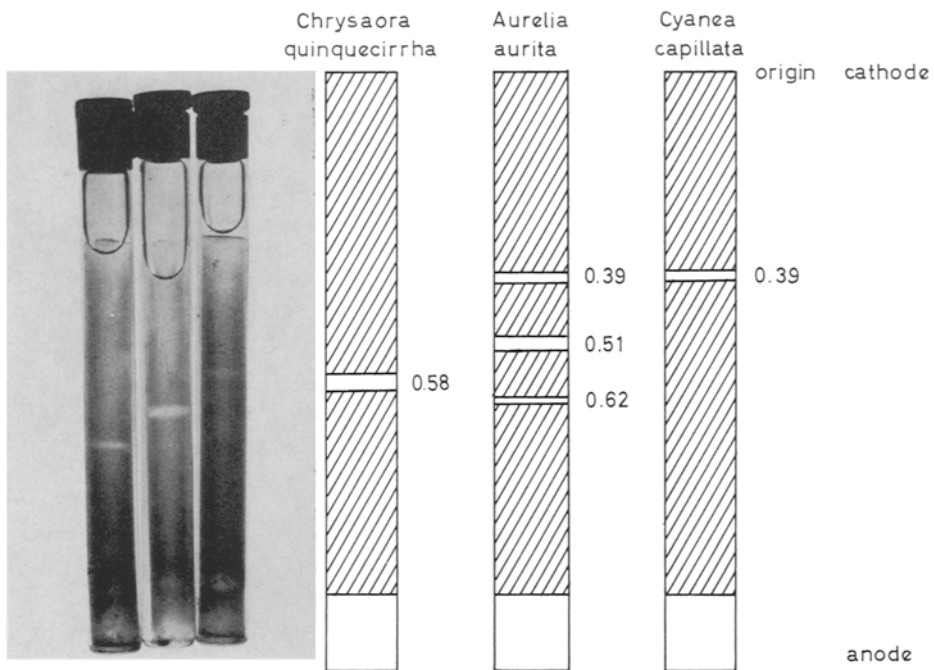


Fig. 2: TO isozyme patterns of Chesapeake Bay scyphozoan polyps

The *Chrysaora quinquecirrha* MDH isozyme pattern is the most complex isozyme pattern of the scyphozoans studied to date. There are 5 bands: a major fast-moving band ($R_m = 0.53$) and a somewhat less intense band ($R_m = 0.54$) close to it; a slower-moving band ($R_m = 0.22$) and a less intense band ($R_m = 0.21$) close to it; and a band of intermediate mobility ($R_m = 0.40$). When three-fold concentrated extracts of polyps of *Chrysaora quinquecirrha* are used, three additional trace bands ($R_m = 0.31, 0.33, \text{ and } 0.37$) are observed. It is possible that these additional trace components can be observed only at high concentrations. *Cyanea capillata* MDH has only one band ($R_m = 0.50$), although faint bands ($R_m = 0.16, 0.25$) were occasionally detected by increasing the concentration three-fold. *Aurelia aurita* has two major MDH bands

Table 1
Isozyme patterns in three Chesapeake Bay polyps

Species	Tetrazolium oxidase			Malic dehydrogenase		
	Number of bands	Rm* of bands	Relative intensity	Number of bands	Rm of bands	Relative intensity
<i>Chrysaora quinquecirrha</i>	1	0.58	+++	5	0.21 0.22 0.31‡ 0.33‡ 0.37‡ 0.40	+ ++ ++ +
					0.53 0.54	+ + + +
<i>Aurelia aurita</i>	2	0.39	++	4	0.29 0.33	+ + + +
		0.51	+++		0.39 0.43	+ + + +
		0.62‡	+		0.46† 0.50†	+ + + +
<i>Cyanea capillata</i>	1	0.39	++	1	0.16† 0.25‡ 0.50	+ + + +

* Rm = distance from origin to band/distance from origin to dye marker

‡ observed only in high concentration preparation

† observed only occasionally in high concentration preparation

($R_m = 0.33$ and 0.43) and two minor MDH bands ($R_m = 0.29$ and 0.39) slightly less mobile than the major bands.

The MDH isozyme pattern of *Chrysaora quinquecirrha* can be easily distinguished from that of *Cyanea capillata* and *Aurelia aurita* by (1) the number of MDH bands and (2) the relative mobilities. The *Chrysaora quinquecirrha* MDH slow-moving doublet is unique ($R_m = 0.21$ and 0.22); *Cyanea capillata* has a single band ($R_m = 0.50$) while *Chrysaora quinquecirrha* has two bands ($R_m = 0.53$ and 0.54) of this approximate electrophoretic mobility; and the four MDH bands of *Aurelia aurita* are intermediate between those of *Chrysaora quinquecirrha* and less mobile than that of *Cyanea capillata* ($R_m = 0.50$).

The function of MDH is either to reduce NAD (or NADP) for biological processes or to further metabolize the end products of glycolysis through the tricarboxylic acid cycle. BLANQUET (1972) suggested that polyps of *Chrysaora quinquecirrha* tend to maintain high levels of NADPH for the synthesis of lipid energy stored within the cyst. MDH has also been shown to dissociate and reassociate under various conditions (SIEGEL 1967, MURPHEY et al. 1967, MURPHEY & KAPLAN 1967, WOLFENSTEIN et al. 1969), and in anemones to respond to changes in lower oxygen tensions (BEATTIE & O'DAY 1971). NAD or NADP does not play an important role in MDH conformational changes. MDH in the jellyfish polyps studied did not require NAD or NADP stabilization during electrophoresis as did G6PDH (ZUBKOFF & LIN 1973c). Either NAD or NADP were effective cofactors for these three scyphozoan MDH enzymes, and their intensities, as detected by electrophoresis, were similar. Unlike G6PDH and 6PGDH of jellyfish whose activities decrease considerably after freezing (ZUBKOFF & LIN 1973c), MDH is relatively stable after freezing and thawing.

Two forms of MDH are commonly recognized: the mitochondrial form is differentiated from the extra-mitochondrial form by chemical, catalytic, and immunochemical properties (MURPHEY et al. 1967). In this investigation no attempt was made to separate them. The MDH isozyme pattern observed in this study was assumed to be a combination of the two. There is a possibility that the detected bands may be due to differences of the original location of the enzyme within the cell.

The MDH and TO isozyme patterns of the 3 species are quite variable, in contrast to those of G6PDH and 6 PGDH which are almost identical. We also observed a difference in MDH isozyme patterns in the different developmental stages of *Cyanea capillata* and *Chrysaora quinquecirrha* (ZUBKOFF & LIN 1973a) and noted different MDH isozyme patterns of polyps of *Aurelia aurita* obtained from different geographical locations (ZUBKOFF & LIN 1973b), an observation consistent with other morphological and physiological observations (RUSSELL 1970, WEBB et al. 1972, MORALES-ALAMO & HAVEN, unpublished observation).

CALDER (1971) indicated that scyphozoan polyps were difficult to identify because of their simple anatomy and variable morphology. As an alternative to the identification of polyps based on the ephyrae they release, CALDER (1971) used the nematocyst complement of polyps for identification. The electrophoretic studies of the MDH and TO isozyme patterns reported here are a useful alternative to the use of either nematocyst complement, culturing, or immunological assay for identifying Chesapeake Bay jellyfishes in the polyp stage.

SUMMARY

1. The malate dehydrogenase (MDH) isozyme patterns of the three most common polyps of Chesapeake Bay (USA) differ in number and in electrophoretic mobilities: *Aurelia aurita*: 4 bands ($R_m = 0.29, 0.33, 0.39, 0.43$); *Chrysaora quinquecirrha*: 5 bands ($R_m = 0.21, 0.22, 0.40, 0.53, 0.54$); *Cyanea capillata*: 1 band ($R_m = 0.50$) or sometimes a second ($R_m = 0.25$).
2. The tetrazolium oxidase (TO) isozyme patterns differ as follows: *Aurelia aurita*: 2 bands ($R_m = 0.39, 0.51$); *Chrysaora quinquecirrha*: 1 band ($R_m = 0.58$); *Cyanea capillata*: 1 band ($R_m = 0.39$).
3. Isozyme components of MDH and TO, either singly or in combination, may be used to distinguish the common Chesapeake Bay polyps of unknown origin.

Acknowledgements. We thank Dr. D. R. CALDER for the polyps, Dr. R. E. BLACK for stimulating discussions, Mr. W. F. EANES for aid with the electrophoresis techniques, and Mrs. P. CREWE for maintaining the cultures. This study was conducted as part of the Virginia Jellyfish Research Program under Public Law 89-720, the Jellyfish Act, Contract No. N-043-226-72(G) from the National Marine Fisheries Service of the National Oceanic and Atmospheric Administration, U. S. Department of Commerce and the Commonwealth of Virginia.

LITERATURE CITED

- BEATTIE, C. W. & O'DAY, D. H., 1971. Alternations in coelenterate malic dehydrogenase isoenzymes during temporary anaerobiosis. *Comp. Biochem. Physiol.* **40** (B), 917-922.
- BLANQUET, R. S., 1972. Temperature acclimation in the medusa, *Chrysaora quinquecirrha*. *Comp. Biochem. Physiol.* **43** (B), 717-723.
- BREWSTER, G. J., 1970. An Introduction to Isozyme Techniques. Acad. Press, New York, 186 pp.
- BURNETT, J. W. & GOULD, W. M., 1971. Immunodiffusion - a technique for coelenterate polyp identification. *Comp. Biochem. Physiol.* **40** (A), 855-857.
- CALDER, D. R., 1971. Nematocysts of polyps of *Aurelia*, *Chrysaora*, and *Cyanea*, and their utility in identification. *Trans. Am. microsc. Soc.* **90**, 269-274.
- DAVIS, D. J., 1964. Disc electrophoresis. II. Method and application to human serum proteins. *Ann. N. Y. Acad. Sci.* **121**, 404-427.
- MURPHEY, W. H. & KAPLAN, N. O., 1967. Malate dehydrogenases. IV. Alternation catalytic properties during purification of *Bacillus subtilis* malate dehydrogenases. *J. biol. Chem.* **242**, 1560-1565.
- BARNABY, C., LIN, F. J. & KAPLAN, N. O., 1967. Malate dehydrogenases. II. Purification and properties of *Bacillus subtilis*, *Bacillus stearothermophilus*, and *Escherichia coli* malate dehydrogenases. *J. biol. Chem.* **242**, 1548-1559.
- RUSSELL, F. S., 1970. The medusae of the British Isles. Cambridge Univ. Press, Cambridge, **2**, 1-284.
- SIEGEL, L., 1967. The effect of inorganic ions on the structure and function of mitochondrial malic dehydrogenase from bovine heart muscle. *Biochemistry, U. S.* **6**, 2261-2267.
- WEBB, K. L., SCHIMPF, A. L. & OLMON, J., 1972. Free amino acid composition of scyphozoan polyps of *Aurelia aurita*, *Chrysaora quinquecirrha* and *Cyanea capillata* at various salinities. *Comp. Biochem. Physiol.* **43** (B), 653-663.
- WOLFENSTEIN, C., ENGLAND, S. & LISTOWSKY, I., 1969. Bovine heart malic dehydrogenase. VIII: Subunit structure and molecular weight of the supernatant enzyme. *J. biol. Chem.* **244**, 6412-6419.

- ZUBKOFF, P. L. & LIN, A. L., 1973a, Malate dehydrogenase isozymes of developing *Chrysaora quinquecirrha* and *Cyanea capillata* (in prep.).
- — 1973b. Isozymes of *Aurelia aurita* obtained from different geographical locations (in prep.).
- — 1973c. Glucose-6-phosphate and 6-phospho-gluconate dehydrogenases of *Chrysaora quinquecirrha* polyps (in prep.).

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