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Characterization of arylsulfatase activity in brine shrimp, *Artemia salina*

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Abstract

Arylsulfatase from *Artemia salina* exists in at least two forms (AS I and AS II). The paper presents characterization of the AS II form of the arylsulfatase. The enzyme was able to hydrolyze *p*-nitrocatechol sulfate (pNCS) as well as ascorbate sulfate. It exhibited maximum activity at temperature of 50 °C and was stable for 2 h at 4–10 °C. Optimum pH shifted from 6.2 at 4 mM pNCS (substrate) to 4.8 at 20 mM pNCS. The enzyme displayed linear kinetics. AS II arylsulfatase exists in two molecular forms (349 and 460 kDa) composed of identical subunits with molecular mass of 53 kDa. Sulfite and phosphate ions were the most potent inhibitors of the enzyme. Cyanide proved to be a weak inhibitor. Sulfate and low concentrations of silver ions had no effect on the enzyme activity. Based on the above results, modifications in the assay for determination of enzyme activity are proposed.

Keywords: *Artemia salina*; Arylsulfatase; Ascorbate sulfate; Invertebrate; Vitamin C

1. Introduction

Artemia salina is an invertebrate, which belongs to a group of few organisms that are able to synthesize ascorbate sulfate—a stable derivative of ascorbic acid (Mead and Finamore, 1969; Dabrowski, 1991). Only free ascorbic acid is biologically active, but because of its high degradation rates it has to be produced in a form of stable derivatives. The derivative, however, must not only be stable but also bioavailable. An enzymatic activity must be present which would make the given compound available in the amounts assuring the required vitamin C concentrations in tissues. Since *A. salina* synthesizes ascorbate sulfate and hydrolyzes it to release free ascorbic acid, information concerning the enzyme responsible for cleavage of this compound could be useful in studying its bioavailability including vertebrates. Our interest in ascorbate metabolism directed our attention to this organism and the enzymes that may participate in the hydrolysis of ascorbate sulfate.

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Arylsulfatases (arylsulfate sulfohydrolase, EC 3.1.6.1) are abundant among prokaryotes, invertebrates and vertebrates. This is a very diverse group of enzymes. Based on differences in solubility, substrate specificity and physicochemical properties two types of arylsulfatases were distinguished. Type I arylsulfatases are microsomal enzymes of low solubility, susceptible to cyanide and not affected by polyvalent ions such as sulfates and phosphates (Nicholls and Roy, 1971). Type II arylsulfatases, localized in lysosomes, are more soluble and susceptible to sulfates and phosphates. They were further divided into anionic arylsulfatase A and cationic arylsulfatase B. Arylsulfatase A is inhibited by silver ions and displays anomalous kinetics (Baum et al., 1958; Yang and Srivastava, 1976). It catalyzes hydrolysis of various sulfate esters including ascorbate sulfate (Roy, 1975). Arylsulfatase B is either unaffected or activated by silver ions and exhibits linear kinetics (Nicholls and Roy, 1971; Thompson and Daniel, 1988). Even crude preparations of arylsulfatases display these specific traits (Dabrowski et al., 1993).

Most of the studies on arylsulfatases have been conducted on mammals and therefore the classification is based on enzymes from these sources, minimal data is available on lower vertebrates and invertebrates. So far the best characterized have been arylsulfatases from Roman snail (*Helix pomatia*) (Roy, 1987), sea urchin (*Strongylocentrotus intermedius*) (Moriya and Hoshi, 1980) and knobbed triton (*Charonia lampas*) (Hatanaka et al., 1975). The invertebrate enzymes display the same traits as mammalian enzymes, albeit in different combinations, hence they do not follow such a strict classification. It is this unique combination of properties that often causes difficulties in applying methods developed for the purification of mammalian enzymes.

The purpose of this work was to characterize arylsulfatase from *A. salina* embryos and nauplii. The results of our studies could be helpful in creating the methods for purification of the enzyme and determining optimal assay conditions. The studies would have an impact on further research on the metabolism of ascorbic acid and hydrolysis of its stable forms. Our inquiries can also fill a gap in comparative studies on arylsulfatases and the present work is the first of its kind in respect to crustaceans.

2. Materials and methods

2.1. Artemia culturing

Lyophilized cysts of *A. salina* were incubated at 28 °C for 24 h in demineralized, aerated water bath containing 28 g/l NaCl. Throughout the hatching period the same conditions of light intensity and temperature were maintained. In order to study the effect of incubation time on arylsulfatase activity, incubation was terminated at various stages of *Artemia* development: after 6 and 12 h (embryonic development), 24 h (hatching), 48 and 72 h (post-hatching period). After the termination of incubation the activity of enzyme was determined as described in Enzymatic assays.

2.2. Homogenate preparation

After termination of the incubation *Artemia* was homogenized in 5 mM Tris–HCl, pH 7.0 using Omni homogenizer. Homogenate was centrifuged for 45 min at 19,000×*g* at 4 °C and filtered to remove the remnants of disrupted cells.

2.3. pH fractionation

The procedure was similar to that described by Carlson et al. (1977). The homogenate was divided into three portions and each of them was adjusted to pH 6.3, 6.1 or 5.9 with 2 M acetic acid. They were centrifuged at 13,000×*g* for 45 min at 4 °C. Sediments were suspended in small volumes of 5 mM Tris–HCl, pH 7.0. The protein content and arylsulfatase activity were analyzed. The results are presented as percents of total activity of homogenate in the given pH.

2.4. Ammonium sulfate fractionation

For the preparation of ammonium sulfate fractionation profile, a saturated, buffered solution of ammonium sulfate was added to the homogenate in a stepwise manner with 10% increments. After each addition of ammonium sulfate the mixture was allowed to stand for 30 min, and afterwards it was centrifuged for 30 min at 27,500×*g*. Pellet was stored for analysis (protein content and arylsulfatase activity determinations) and the supernatant was subjected to further fractionation procedure.