Substrate-Dependent Arylsulfatase Activity in the Cyanobacterium *Plectonema* 73110

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Arylsulfatase activity of the cyanobacterium *Plectonema* 73110 is detected during growth on *p*-nitrophenyl sulfate as only sulfur source. A 60-fold purification of the soluble arylsulfatase yields a protein with an pH-optimum around 10 and K_m data for *p*-nitrophenyl sulfate of 9.5×10^{-5} and for nitrocatechol sulfate of 3.4×10^{-5}. The enzymatic activity is not effected by the cations Mg^{2+}, Mn^{2+}, K^+ or Na^+ used as chloride salts. The anions SO_3^{2-} and SO_4^{2-} did not inhibit this activity, however EDTA, thiols and triton X-100 decreased this activity to about 25% whereas SDS inhibited completely. Sulfate limitation is necessary for the development of arylsulfatase activity in the presence of its substrate *p*-nitrophenyl sulfate. This arylsulfatase activity is partly bound to membranes.

Introduction

Sulfate esters are widespread in nature, occurring in plants, animals and microorganisms. Especially phenolic and aromatic substances with an OH group are sulfonated yielding the corresponding sulfate esters to alter or detoxify specific compounds. Thus, simple phenylsulfates, tyrosine-O-sulfate, catecholamin-O-sulfate, arylsulfates based on the indole nucleus and flavonoidsulfates can be detected in living organisms [1].

Arylsulfatases are a group of enzymes catalyzing the desulfonation of arylsulfates. These sulfatases have been analyzed predominantly in bacteria (see [2] and [3] for a review) using artificial substrates, however they have been found also in green algae [4, 5] and higher plants [6]. So far, no information was available for the occurrence of arylsulfatase activity in cyanobacteria. Since *Plectonema* was able to grow on *p*-nitrophenyl sulfate as only sulfur source, this study was initiated to search for arylsulfatase activity in this organism.

Materials and Methods

Organism

The cyanobacterial strain *Plectonema* 73110 was obtained from the algal collection of Institut Pasteur and cultured under aseptic conditions according to Stanier *et al.* [7], using growth conditions of 27 °C and 10,000 lux. Magnesium sulfate was replaced when indicated by $MgCl_2$ and sulfur sources to be analyzed were added as indicated.

Preparation of algal extracts

Cells were harvested by centrifugation at $7{,}000 \times g$ for 10 min. 1 g of algae (wet weight) was taken up in 5 ml of 0.1 M Tris-HCl buffer containing 10 mM $MgCl_2$ and 10 mM 2-mercaptoethanol. Cells were disrupted in a french press at 12,000 PSI ($\sim 84 \times 10^6$ Pa). The extract was cleared by centrifugation for 10 min at $12{,}000 \times g$ and used as crude extract.

Analysis of arylsulfatase activity

This activity was determined using either *p*-nitrophenyl sulfate or *p*-nitrocatechol sulfate as a substrate. The following test conditions were used: 50 mM Tris-HCl buffer pH 8.0, 2 mM substrate (normally *p*-nitrophenyl sulfate), enzyme as needed in a total volume of 0.5 ml. After incubation for 45 min at 37 °C the reaction was stopped using 0.5 ml of 0.5 M NaOH. The desulfonated product was determined spectrophotometrically using an ϵ of $18.2 \times 10^6 \times cm^2 \times mol^{-1}$ for *p*-nitrophenol at 401 nm and an ϵ of $14.5 \times 10^6 \times cm^2 \times mol^{-1}$ for *p*-nitrocatechol at 514 nm with a Beckman spectral photometer DU 7. This test system was linear for either *p*-nitrophenyl sulfate or *p*-nitrocatechol sulfate from 1 to 200 nmol product formed under the conditions used.

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Purification of arylsulfatase activity

To 1 ml of crude extract 10 µl of a tenfold diluted Polymin P solution was added and the precipitate formed was removed by centrifugation. Arylsulfatase activity was concentrated by an ammoniumsulfate cut between 35% and 65% and dialyzed against 0.02 M Tris-HCl pH 8.0. This material was subjected to a diethylaminoethyl(DEAE)-cellulose column (2 × 10 cm) equilibrated with 0.02 M Tris-HCl pH 8.0. The column was developed using 200 ml of this buffer for reservoir 1 and 200 ml of the same buffer containing 0.4 M NaCl for the second reservoir. Arylsulfatase activity eluted between 118 and 146 ml. These fractions were concentrated on a membrane filter with a limit of 30,000 dalton. By this procedure a 60-fold purification was achieved with a yield of 23%.

Determination of thiosulfate reductase activity

This activity was determined according to Schmidt *et al.* [8].

Protein determination

Protein was determined using the Coomassie Blue Method according to Bradford [9] using the dye reagent preparation from Biorad. Bovine serum albumin was used as a reference.

Chemicals

The arylsulfates used were purchased from Sigma (München, W. Germany) whereas all reagents not mentioned were obtained from Merck (Darmstadt, W. Germany). Polymin P was a generous gift of BASF (Ludwigshafen, W. Germany).

Results

Arylsulfatase activity according to growth on different sulfur sources

The cyanobacterium *Plectonema* was grown on different sulfur sources and the corresponding arylsulfatase activity was measured as described in materials and methods. We did not find significant aryl-

Table I. Arylsulfatase activity according to sulfur nutrition. Conditions: *Plectonema* was cultured for 5 days on the sulfur sources listed using the concentrations indicated. The crude extract obtained from these algae as described in materials and methods was analyzed using the experimental conditions described for arylsulfate measurement in the same section. Data are given as nmol *p*-nitrophenol formed per hour and mg protein.

Sulfur source	Konzentration added [mM]	Arylsulfatase activity [nmol/mg prot × h]
Sulfate	none	4*
Sulfate	0.03	8
Sulfate	0.05	6*
Sulfate	0.075	4*
Sulfate	0.15	4*
Sulfate	0.3	6*
Sulfate	0.3	0
Thiosulfate	0.2	1*
Methanesulfonic acid	0.2	3*
Ethanesulfonic acid	0.2	19
Propansulfonic acid	0.2	2*
Taurine	0.2	5*
Aminoethylsulfate	0.2	16
Sulfanilic acid	0.2	1*
p-nitrophenyl sulfate	0.1	62
p-nitrophenyl sulfate	0.2	55
p-nitrophenyl sulfate	0.2 + sulfate (0.3)	0
p-nitrophenyl sulfate	0.5	0
p-nitrophenyl sulfate	1.0	0
p-nitrocatechol sulfate	0.2	1*

* Data not significant.

sulfatase activity during sulfur starvation conditions, in contrast to two green algal strains. However, significant arylsulfatase activity was detected during growth on ethanesulfonic acid and the sulfate ester aminoethyl sulfate. Best yields were obtained using the substrate *p*-nitrophenyl sulfate, whereas no activity was detected using the corresponding *p*-nitrocatechol sulfate as sulfur source for growth. It is evident that no arylsulfatase could be detected during "normal" sulfur starvation or on any sulfur compound in the presence of sulfate. Thus a sulfur starvation signal and the presence of the correct substrate (inducer?) are necessary for optimal arylsulfatase development. Algae could be grown either on normal air or using air enriched with 5% CO_2 without altering the results concerning arylsulfatase activity (data not shown).

Properties of the cyanobacterial arylsulfatase

For preparation and isolation of cyanobacterial arylsulfatase activity *Plectonema* was grown on 0.2 mM *p*-aminophenyl sulfate and the arylsulfatase activity was separated as described in materials and methods. This so far purified enzyme was used to characterize this cyanobacterial arylsulfatase activity. This test was linear up to 200 nmol substrate metabolized using either different incubation times or protein concentrations.

Table II. Influence of anions, cations and inhibitors. Conditions: Arylsulfatase activity was measured according to materials and methods adding the compounds indicated and stating the concentrations used. 100% are 1786 nmol pro mg protein and hour.

Compound used	Concentration [mM]	% Activity of control
none (control)		100
$MgCl_2$	1	102
$MnCl_2$	1	96
KCl	1	101
NaCl	1	101
Na_2SO_4	1	100
Na_2SO_3	1	100
KH_2PO_4	10	74
H_3BO_4	2	75
KCN	2	100
EDTA	2	100
Mercaptoethanol	10	84
Dithioerythritol (DTE)	10	71
Triton-X-100	0.5%	75
SDS	0.5%	5

Anion, cation, and inhibitor effects on arylsulfatase activity

Different anions, cations and inhibitors were tested for a possible inhibition of this arylsulfatase activity. These data are summarized in Table II.

It can be seen that none of the anions tested inhibited this activity significantly, whereas a partial inhibition was found using thiols at 10 mM concentrations and detergents (especially SDS). Cyanide and EDTA did not inhibit this enzymatic activity. A comparison of Tris-HCl buffer pH 8.0 with sodium phosphate and imidazol buffer of the same pH gave best results using imidazol buffer. Tris buffer was used, however, in this study for comparison with results obtained with crude extracts. This arylsulfatase had a broad pH-optimum of about 10 (data not shown).

K_m determination for *p*-nitrophenyl sulfate and *p*-nitrocatechol sulfate

The K_m data were determined to 9.5×10^{-5} M for *p*-nitrophenyl sulfate and to 3.4×10^{-5} M for *p*-nitrocatechol sulfate. Thus, this compound has a significant lower K_m towards *p*-nitrocatechol sulfate, although the enzyme has a higher velocity with *p*-nitrophenyl sulfate (Fig. 1).

Molecular weight determination

The molecular weight of this arylsulfatase was determined using a Biogel A 1.5 M column (2 × 80 cm) with bovine serum albumine (MW 68,000), egg albumin (MW 45,000), chymotrypsinogen (MW 25,000) and cytochrom *c* (MW 12,500) as references. A molecular weight for this arylsulfatase was determined to 60,000 daltons.

Sulfur status of the cell and arylsulfatase activity

The data collected so far suggested that sulfate limitation and the presence of the substrate *p*-nitrophenyl sulfate are necessary for the development of arylsulfatase activity. This was tested using thiosulfate reductase as a marker for sulfur starvation and comparing thiosulfate reductase and arylsulfatase activities using different growth conditions given in Table III. It can be seen that sulfur starvation and thiosulfate reductase correspond, however sulfur starvation is not the inducer of arylsulfatase activity although it is necessary for arylsulfatase activity. Addition of sulfate abolishes the sulfur starvation signal

Table III. Sulfur status and arylsulfatase activity. Conditions: *Plectonema* was grown as described in materials and methods using the sulfur compound indicated. Thiosulfate reductase and arylsulfatase were measured as described in the method section.

Sulfur compound used for growth	Concentration [mM]	Thiosulfate-reductase activity*	Arylsulfatase activity*
none	–	420	0
sulfate	2×10^{-5}	274	6
sulfate	2×10^{-4}	29	0
p-nitrophenyl sulfate	2×10^{-4}	82	52
sulfate + *p*-nitrophenyl sulfate each	2×10^{-4}	32	0
p-nitrocatechol sulfate	2×10^{-4}	38	1

* Data are given as nmol/mg protein and hour.

and arylsulfatase activity. Obviously *p*-nitrocatechol sulfate did not activate arylsulfatase activity under the concentrations used, and no signal of sulfur starvation was observed suggesting that this compound is metabolized quickly enough (Table III). These data clearly confirm the relationship between sulfate limitation and arylsulfatase activity only in the presence of the corresponding substrate.

Occurrence of membrane bound arylsulfatase activity

The distribution of membrane-bound and free arylsulfatase activity was analyzed using *Plectonema* cells grown at two different temperatures and measuring soluble and bound activity. These data are summarized in Table IV. In parallel experiments arylsulfatase activity was determined using living, not broken cells. To our surprise about 60% of the total arylsulfatase activity could be found using intact cells, thus the situation seems to be comparable to *Chlorella* [4] suggesting that arylsulfatase is at least partly available at the outside of the controlling uptake barrier.

Table IV. Free and membrane-bound arylsulfatase activity in *Plectonema*. Conditions: Cells were grown at the temperatur indicated, harvested and the crude extract was prepared as described in materials and methods. The pellet fraction after the french-press treatment was resuspended in the identical volume and analyzed separately for arylsulfatase activity using the conditions stated in the same section.

Growth temperatur [°C]		Activity [nmol/ml extract]	Specific activity [nmol/mg prot × h]
27	crude extract	67	34
27	pellet fraction	65	27
32	crude extract	28	15
32	pellet fraction	130	54

Discussion

Arylsulfatase activity is found in bacteria, fungi, animals, green algae and higher plants, however reports for arylsulfatase activity in cyanobacteria were missing. The data presented here demonstrate that arylsulfatase activity can be found in the cyanobacterium *Plectonema* 71110, however only under specialized growth conditions. Arylsulfatase activity in *Chlamydomonas* and *Chlorella* can be induced by sulfate starvation conditions even in the presence of either cysteine or methionine [4, 5]. Sulfate starvation, however, did not enhance arylsulfatase activity in *Plectonema* significantly indicating a different metabolic regulation. Growth experiments summarized in Table I revealed that arylsulfatase was found only using *p*-nitrophenyl sulfate as sulfur source for growth in the absence of sulfate. Even higher concentrations of *p*-nitrophenyl sulfate prevented appearance of arylsulfatase activity, suggesting that this compounds was metabolized at higher concentrations quickly enough to assure normal sulfate availability. This possibility was tested using a second enzyme thiosulfate reductase as a marker, which is enhanced during sulfur starvation in this alga (data of Table III). Obviously *p*-nitrocatechol sulfate did not enhance thiosulfate reductase activity thus demonstrating that the sulfur status of the cell is normal during growth on 2 mM *p*-nitrocatechol sulfate.

The arylsulfatase of *Plectonema* catalyzes the hydrolysis of *p*-nitrophenyl sulfate and *p*-nitrocatechol sulfate in a ratio of about 3:1 and this ration remained constant during the purification procedure. The observed data summarized in Table I allow to characterize this arylsulfatase according to Dogson and Spencer [10] as an arylsulfatase typ I, since this activity was not inhibited by sulfate or sulfite, nor by KCN, and it could use both substrates *p*-nitrophenyl sulfate and *p*-nitrocatechol sulfate. Arylsulfatases from the green algae *Chlamydomonas* [5] and *Chlorella* [4] have been characterized also as arylsulfatases of type I. The arylsulfatases of the two green algae are, however, induced by sulfate starvation in the absence of its substrate, whereas in *Plectonema* sulfate limitation and the presence of the substrate seems to be necessary for arylsulfatase activity.

The properties of the enzyme investigated using intact cells were similar to the activity found in disrupted cells. This suggests that the arylsulfatase of *Plectonema* is at least partly accessable from the outside of the cell and has to be attached therefore to the cell surface as found for *Chlamydomonas* [5]. We have found a derepressible arylsulfatase also in *Synechococcus* 6301 using the same growth conditions; thus arylsulfatases are present in cyanobacteria.

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