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THE CAPACITY FOR ARYLSULFATASE SYNTHESIS IN SYNCHRONOUS AND SYNCHRONIZED CULTURES OF *CHLAMYDOMONAS REINHARDTI*

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Summary

The green algae *Chlamydomonas reinhardti* synthesizes arylsulfatase (arylsulfate sulfohydrolase EC 3.1.6.1) by derepression when the concentration of SO_4^{2-} in the growth medium is less than about $5 \cdot 10^{-5}$ M. The following observations indicate that the arylsulfatase enzyme is stable while its mRNA was unstable:

(1) The increase in enzyme activity stopped and remained constant after addition of cycloheximide to derepressed cells.

(2) After readdition of SO_4^{2-} the increase in enzyme activity continued at a lower rate whereafter it remained constant.

(3) No decay of radioactivity was observed after readdition of SO_4^{2-} in labelled enzyme protein isolated from pulse-labelled —S cells.

The maximum rate of arylsulfatase synthesis in derepressed cells was taken as a measure of their capacity for arylsulfatase synthesis. Measurements of this capacity in cells taken at different developmental stages from a selection synchronous and from a light-dark synchronized culture showed that:

(1) Arylsulfatase was derepressible at all stages of the life cycle.

(2) The same periodic capacity patterns were found, both with the synchronized and the synchronous cells.

Furthermore, the rate of accummulation of RNA and protein changed in the same periodic manner during the life cycle as did the enzyme capacity.

Introduction

We have previously reported very similar periodic patterns of capacity to synthesize nitrite reductase [1] and phosphatases [2,3] during the life cycle of the unicellular algae *Chlorella* and *Chlamydomonas*. Increase in arylsulfatase activity caused by SO_4^{2-} starvation has been reported in several microorganisms [4—6]. The present study was started with the main objective to investigate the

capacity to synthesize arylsulfatase on derepression (derepressibility) during the vegetative life cycle of *Chlamydomonas reinhardti.* Since light-dark synchronized cultures were used in our previous studies, it might be that the observed periodic pattern was a result of the synchronization method and not a function of the cell cycle per se. We have, therefore, compared the enzyme capacity pattern in synchronous and synchronized cultures of *Chlamydomonas*, this being possible by the development of selection synchrony of *Chlamydomonas* using a threshold centrifuge [7].

Materials and Methods

Culturing conditions and synchronization procedures

C. reinhardti No. 11-32 (90) from the Algal Collection of the Institute of Plant Physiology, University of Göttingen, Germany, was used in this study. Selection synchrony was obtained by starting the synchronous culture with zoospores selected from a non-synchronized continuous culture using the Rastgeldi Threshold Centrifuge [7,8]. The continuous non-synchronous culture was maintained at constant illumination in a plastic chamber with inner light path of 5 cm and with a culturing volume of 8 l. The cell density was kept at $1.0 \cdot 10^6$ cells per ml by continuous diluting with fresh medium. The selected zoospores were grown in 30-ml Kimax (Kimax Co., U.S.A.) test tubes [7]. Induced synchrony was obtained by alternating periods of 12 h light and 4 h dark [9].

The cells in the continuous culture and in the two different synchronous cultures were grown vegetatively at 35° C, and the cultures were aerated continuously with a mixture of filtered air and CO_2 (2%). The light intensity was approx. 20 000 lux incident on one surface of the growth vessels. The medium was composed according to Kuhl and Lorenzen [10]. This medium with a concentration of SO_4^{2-} of about $1.0 \cdot 10^{-3}$ M is called +S medium, and the cells grown in it +S cells. The +S medium was modified by replacing the $MgSO_4$, $FeSO_4$, $MnSO_4$ and $ZnSO_4$ by 246 mg $Mg(NO_3)_2 \cdot 6H_2O$, 695 mg $FeCl_2 \cdot 7H_2O$, 0.169 mg $MnCl_2 \cdot 4H_2O$ and 0.287 mg $ZnCl_2 \cdot 7H_2O$, respectively, per liter medium. This modified medium, with a concentration of SO_4^{2-} of about $1.0 \cdot 10^{-8}$ M, is called —S medium, and the cells grown in it —S cells.

The developmental stages of the cells are here given in hours from the beginning of the illumination of zoospores obtained either by selection or by light-dark induction. A more detailed account of the experimental system is found elsewhere [3,7,9].

Cell number and volume

Cell numbers and size distribution were measured electronically with a Celloscope, Model 302, particle counter (Ljungberg Co., Sweden) as previously described [11].

Derepression of arylsulfatase

In order to measure the capacity to synthesize arylsulfatase at different developmental stages of the cells, it was necessary that the derepressing treatment should be rapid. For this purpose the cells were rapidly sedimented in a

small, continuously operating centrifuge [12], and washed under centrifugation with —S medium at 35° C. Thereafter the cells were suspended in such medium giving about $1.5 \cdot 10^6$ cells per ml. The suspension was illuminated and aerated as usual at 35° C, and during a 5-h period of growth in —S medium, the increase in activity of arylsulfatase was recorded. Since each selection of zoospores gave enough cells for only one measurement of arylsulfatase production capacity, we had to perform as many parallel selections as the number of measurements to be done during the life cycle. Each selected portion of zoospores (15 ml containing about $1.5 \cdot 10^6$ cells per ml) was grown in a 30-ml Kimax test tube as mentioned above. At 2-h intervals after the start of the synchronous growth of the selected cells contained in the Kimax test tubes, they were given the derepression treatment. Similarly the cells in 50-ml samples of the light-dark synchronized culture were treated for derepression at 2-h intervals after start of illumination. Control experiments revealed proportionality between the number of cells per ml culture and the rate of increase in enzyme activity.

Arylsulfatase assay

The enzyme activity was measured in whole, intact cells and in cell-free enzyme solution as described in an accompanying paper [13]. The preparation of acetone powder of the cells and the measurement of arylsulfatase activity in the acetone-treated cells were done according to a method described previously [3]. *p*-Nitrophenylsulfate was used as substrate throughout this work. The properties of the enzyme were found to be closely similar in intact cells and in purified state [13]. And since we found that the activity of arylsulfatase was highest in washed living cells compared with that of the same number of broken cells and with that of acetone powder of the same number of cells, the arylsulfatase activity was measured in intact cells when we followed the time course of enzyme synthesis in the derepression experiments.

To establish the reproducibility of the enzyme assay procedure the arylsulfatase activity was measured in 30 identical samples of intact cells taken from the same cell suspension. The standard deviation of the enzyme assay from the mean value was ±4%. With incubation times shorter than 50 min the enzyme activity was directly proportional with time, and with numbers of cells per sample.

Determination of DNA, RNA and protein

Contents of DNA, RNA and protein were determined as previously reported [9].

Purification of arylsulfatase

Arylsulfatase was purified to homogeneity from acetone-powder extract by $(NH_4)_2SO_4$ precipitation, Sephadex G-200 filtration and ion-exchange chromatographies (DEAE and CM-Sephadex) as described in detail in an accompanying paper [13].

Polyacrylamide gel electrophoresis

Before electrophoresis the enzyme solutions were concentrated, desalted

and transferred to the spacer gel buffer by using a Minicon, Model B 15 ultrafiltration unit (Amicon, The Netherlands). The electrophoresis was done as described by Maurer [14] using a discontinuous acid buffer system consisting of a 7% separation gel, a 3.5% spacer gel, potassium acetate, pH 4.3 and 6.7, as gel buffers, and β-alanine acetate, pH 4.5, as electrode buffer [13]. After electrophoresis the arylsulfatase activity was located on the gel by using a histochemical staining method as described in an accompanying paper [13]. Scanning of the electropherogram was done with a MPS-50 L Shimadzu spectrophotometer.

By using an apparatus similar to that described by Matsumura and Noda [15], the gels were fractionated after the electrophoresis into 1-mm slices. Thereafter, each slice was put into a scintillation vial and 0.5 ml 30% H_2O_2 was added to each vial. The slices were solubilized by incubating the vials at 60°C for about 3 h during which time the vials were tightly capped to prevent evaporation. For measurement of radioactivity in each slice 1 ml scintillation liquid (Instagel) was added to each vial.

Isoelectric focusing

According to Vesterberg and Svensson [16] the isoelectric focusing was carried out in a 0 to 50% sucrose gradient and a pH gradient established by 1% carrier ampholytes, pH 7 to pH 10, contained in a 25-ml electrofocusing column [13]. The column was operated at 350 V for 24 h at 4°C. At the completion of the run, 1-ml fractions were collected and assayed for pH, arylsulfatase activity and radioactivity.

Incorporation of phenylalanine

Suspensions of −S and +S cells were incubated with [^{14}C]- or [^{3}H]phenylalanine according to the method previously described [17]. After phenylalanine addition, 1.0 ml samples were taken and treated for determination of radioactivity in trichloroacetic acid-insoluble fraction [18].

Radioactivity measurements

^{14}C or ^{3}H radioactivity in trichloracetic acid-treated cells on membrane filters and in liquid samples were counted in a Tri Carb (Packard Instrument Co., U.S.A.) scintillation counter, using Instagel (Packard) as the scintillation liquid. For measuring double-labelled samples (^{14}C and ^{3}H activity) appropriate corrections were made for ^{14}C counting in the ^{3}H channel.

Results

Arylsulfatase activity and the concentration of SO_4^{2-} in the growth medium

Fig. 1 shows the activity of arylsulfatase as a function of the concentration of SO_4^{2-} in the growth medium. At SO_4^{2-} concentrations lower than $1 \cdot 10^{-5}$ M, the activity of arylsulfatase increased both with time and with decreasing concentration of SO_4^{2-}. The increase in activity occurred after a lag period with a length depending on the concentration of SO_4^{2-}. The shortest lag period, 0.5 h, and the greatest rise in arylsulfatase activity (taking place during the first 4—5 h of growth) were found in the cells grown in the medium

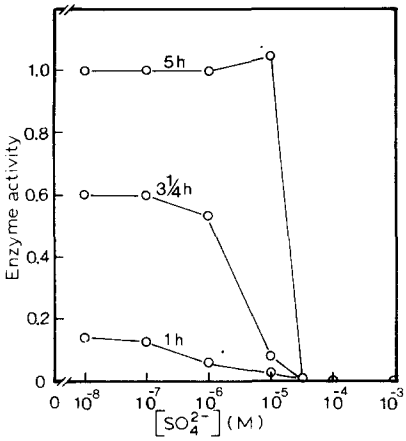

Fig. 1. The activity of arylsulfatase as a function of the concentration of SO_4^{2-} in the growth medium. Zoospores were collected by centrifugation, washed and then suspended in —S medium. The suspension of —S cells containing $1.5 \cdot 10^6$ cells per ml, was divided on several culture tubes. Different amounts of concentrated Na_2SO_4 were then added to the cultures giving a final concentration of sulfate ranging from $1 \cdot 10^{-8}$ M in that of the —S medium to $1 \cdot 10^{-3}$ M in that of the +S medium. At 1, 3¼ and 5 h of growth (indicated on the figure) samples were taken from each of the cultures for sulfatase determination expressed as A_{410nm} units per 1 ml culture and 30 min at 38° C.

containing $1 \cdot 10^{-6}$ M and less SO_4^{2-}. An optimum concentration of SO_4^{2-} for the rate of activity increase was found at about $1 \cdot 10^{-5}$ M. This optimum relation which appeared after about 5—6 h, became more pronounced after a longer period of growth in the presence of the different concentrations of SO_4^{2-}.

No arylsulfatase activity could be detected in cells grown in medium containing $5 \cdot 10^{-5}$ M or higher concentrations of SO_4^{2-} (Fig. 1), which was also the case at all other stages of the synchronous cycle. In those latter experiments variable amounts of washed living cells, of acetone-treated cells and of cells disrupted by sonication were used. We tested the effect of SO_4^{2-} on the growth of zoospores and 8-h cells, by using +S and —S medium, and from the time courses of dry weight and volume increase, as well as of protein accumulation, we found that the growth was uninfluenced by the absence of SO_4^{2-} from the medium during the 5-h test period. Since —S medium secured optimum growth rate with concomitant maximal rate of increase in arylsulfatase activity, this medium was used in our experiments on the cell's capacity to synthesize arylsulfatase.

Demonstration of de novo synthesis (derepression) of sulfatase in cells grown in —S medium

Derepression of sulfatase in —S cells implies de novo synthesis of the enzyme protein. According to Filner et al. [19], the existence of derepression can only be shown by directly demonstrating the production of the enzyme protein, and not by using more or less specific RNA and protein inhibitors. We, therefore, used [^{14}C]phenylalanine labelling of the protein fraction, combined with isolation of the enzyme protein for demonstration of incorporation of radioactivity into the enzyme. Previously we showed that all of the [^{14}C]phe-

nylalanine taken up by +S cells was incorporated into the protein fraction of the cells [17], this was also the case for —S cells.

As described in an accompanying paper [13] arylsulfatase was purified to homogeneity from acetone-powder extract of —S cells by $(NH_4)_2SO_4$ precipitation. Sephadex G-200 filtration and ion-exchange chromatography on DEAE- and CM-Sephadex. Polyacrylamide gel electrophoresis of the purified enzyme preparation gave a single protein band coinciding with a single band of enzyme activity located by histochemical staining [13]. If the enzyme was made de novo, polyacrylamide gel electrophoresis of a purified enzyme preparation from —S cells grown in the presence of [^{14}C]phenylalanine should give a radioactive peak with the same mobility as the enzyme activity (from added unlabelled enzyme). To test this we did the following experiment: —S cells and +S cells were grown for 5 h in the presence of the same amount of [^{14}C]phenylalanine (0.5 ml $1 \cdot 10^{-3}$ M [^{14}C]phenylalanine, 10 Ci/mole giving $4 \cdot 10^{-6}$ M concentration). Presumably the only difference in labelling should then be that the former cells contained radioactive sulfatase protein. Acetone powder was made of the labelled —S and +S cells. Each of these two samples of labelled powder was mixed with 4 g of acetone powder from unlabelled —S cells (carrier cells or carrier powder).

Arylsulfatase was purified from each of the two mixtures of acetone powder. After purification of the enzyme, several samples from each of the two enzyme solutions were subjected in parallel to polyacrylamide gel electrophoresis. Thereafter some gels were stained for arylsulfatase activity and the rest sliced for measurement of radioactivity. Fig. 2 shows the electrophoretic pat-

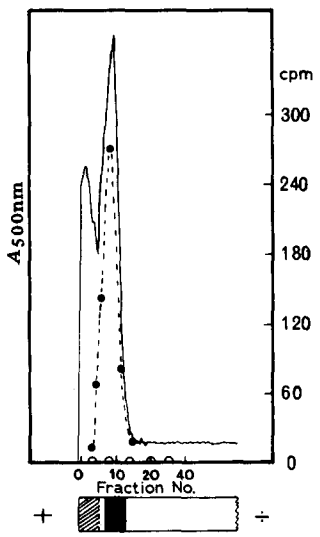

Fig. 2. Polyacrylamide gel electrophoresis of purified arylsulfatase from a mixture of ^{14}C-labelled and unlabelled —S cells (carrier cells) and from a mixture of ^{14}C-labelled +S cells and of unlabelled —S cells (carrier cells). Further details in the text. ———, spectrophotometric scanning of the gels stained for arylsulfatase activity; •- - - -•, ^{14}C activity in the gels on which arylsulfatase from the mixture of ^{14}C-labelled —S cells and unlabelled —S cells (carrier cells) was electrophoresed; o———o, ^{14}C activity in the gels on which arylsulfatase from the mixture of ^{14}C-labelled +S cells and unlabelled —S cells (carrier cells) was electrophoresed.

tern, with the spectrofotometric scanning of the stained gels. The electrophoresis of the enzyme purified from the ^{14}C-labelled —S cells and carrier acetone powder gave a radioactive peak coinciding with that of enzyme activity. But the electrophoresis of the enzyme purified from ^{14}C-labelled +S cells (containing no arylsulfatase activity) and carrier powder, gave no peak of radioactivity with the same mobility as the enzyme activity. The absorption peak of the anode side of the gel is not due to enzyme activity, but to the opaque spacer gel which absorbed at 500 nm.

Samples of 1 ml from each of the two purified enzyme solutions were also subjected to isoelectric focusing.

The focusing of the enzyme from ^{14}C-labelled —S cells, but not from the ^{14}C-labelled +S cells, gave (as the electrophoresis did) one peak of radioactivity coinciding with the peak of enzyme activity.

Hence, we conclude that derepression of arylsulfatase occurred upon removal of SO_4^{2-} from the growth medium.

Effect of SO_4^{2-} and cycloheximide on derepression of arylsulfatase. Stability of the enzyme in vivo

Derepressed enzyme synthesis can be stopped by addition of the corepressor and/or by a protein inhibitor (i.e. cycloheximide). Fig. 3 illustrates the effect of adding SO_4^{2-} and cycloheximide, respectively, to —S cells. It can be seen that during the first hour after readdition of SO_4^{2-} the increase in arylsulfatase activity continued at a lower rate than in —S cells, whereafter it remained constant. The increase in enzyme activity was rapidly inhibited by

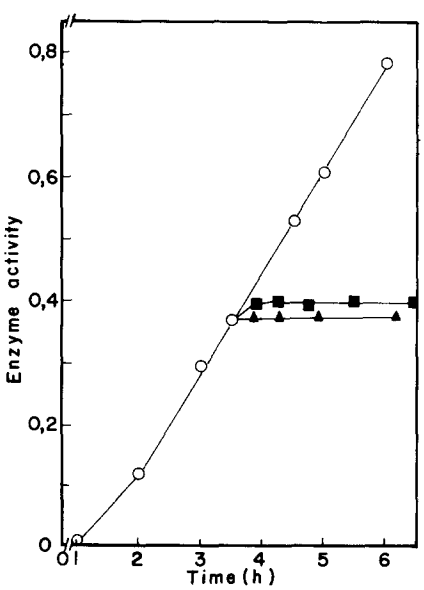

Fig. 3. Effect of cycloheximide and SO_4^{2-} on the synthesis of arylsulfatase. After 3½ h in the light period, the culture of —S cells was divided into three parts. Cycloheximide was added to one culture giving 10 μg/ml. Concentrated SO_4^{2-} solution (Na_2SO_4) was added to the second one, giving the same concentration of SO_4^{2-} as that of the +S medium, and the third culture was kept unchanged. Enzyme activities in —S cells (○———○), after the addition of SO_4^{2-} (■———■), and cylcoheximide (▲———▲). The enzyme activities per 1 ml culture are given as $A_{410\,nm}$ units per 30 min at 38° C.

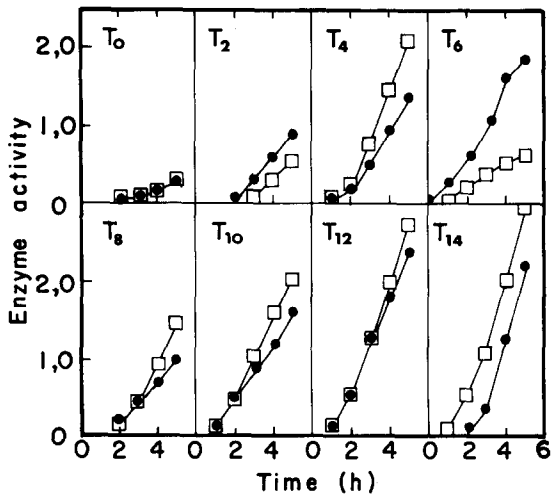

Fig. 4. Course of synthesis of arylsulfatase in cells of *C. reinhardti* after transfer of the cells to —S medium at eight different stages of development. ●———●, cells taken from the light-dark synchronized culture grown for 12 h in the light and 4 h in the dark; □———□, cells taken from the synchronous culture produced by separation of zoospores from a continuous asynchronous culture. The developmental stages of the cells when the different derepressions were started are given in hours (T_0, T_2, etc.) from the beginning of the illumination and growth of the selected zoospores (selection synchrony) and the beginning of the illumination period for the light-dark synchronized culture. During a 5-h period of derepression, the cells were placed in the light under the usual growth conditions. The enzyme activities are given per ml stock synchronous culture as A_{410nm} per 30 min at 38°C.

cycloheximide, and the activity remained constant in the cycloheximide-treated cells. Assuming that cycloheximide prevents translation [20] and SO_4^{2-} is acting at the transcriptional level by preventing synthesis of messenger RNA for arylsulfatase, the results presented in Fig. 4 indicate a stable enzyme and an unstable messenger RNA, the latter being degraded during the period with declining rate of enzyme synthesis.

To study the stability of sulfatase in vivo under non-drug conditions, the following experiment was done: The —S cells in two parallel cultures were labelled with [^{3}H]phenylalanine (25 μCi) and [^{14}C]phenylalanine (10 μCi), respectively. After 5 h of growth in —S medium, concentrated solution of Na_2SO_4 was added to the ^{3}H-labelled cells to repress further synthesis. The ^{14}C-labelled cells were then harvested and three equal portions of acetone powder were made of them. The ^{3}H-labelled cells were washed in unlabelled —S medium before the readdition of SO_4^{2-}.

At 0, 2 and 4 h after the readdition of SO_4^{2-} to the culture of ^{3}H-labelled cells, equal samples of these cells were harvested and acetone powder was made of them. One portion of ^{14}C-labelled acetone powder and one portion (4 g) of unlabelled carrier acetone powder of —S cells were mixed with each of the samples of ^{3}H-labelled acetone powder. Thereafter, arylsulfatase was purified (as described above in Materials and Methods) from each of the three mixtures of acetone powder. Samples from each of the purified enzyme solutions were then subjected to electrophoresis on polyacrylamide gels and to isoelectric focusing as described above. The ratio ^{3}H cpm:^{14}C cpm was calculated in the enzyme fractions obtained by isoelectric focusing and by slicing the gels after

TABLE I

STABILITY OF ARYLSULFATASE IN VIVO

Arylsulfatase was purified from three mixtures of acetone powder each of which contained an equal portion of ^{14}C-labelled —S cells; an equal portion of ^{3}H-labelled cells harvested at 0, 2 and 4 h after readdition of SO_4^{2-} to derepressed cells, which had grown in —S medium containing [^{3}H]phenylalanine for 5 h; and finally an equal portion of unlabelled —S cells (carrier powder). The ratio ^{3}H cpm:^{14}C cpm was calculated in the enzyme fractions obtained by isoelectric focusing and by slicing the gels after electrophoresis of the purified enzyme. The ratios ^{3}H cpm:^{14}C cpm are also presented for a similar experiment where concentrated acetone-powder extracts, and not solutions of purified enzyme, were subjected to isoelectric focusing.

Enzyme fractions obtained by:	Ratio ^{3}H cpm : ^{14}C cpm after readdition of SO_4^{2-} (h)			
	0	2	4	6
Isoelectric focusing of acetone-powder extracts	1.60	1.54	1.70	1.66
Electrophoresis of purified enzyme	1.65	1.54	1.64	—
Isoelectric focusing of purified enzyme	0.45	0.47	0.48	—

electrophoresis. These ratio remained almost constant during the period of 4 h after readdition of SO_4^{2-} (Table I). Constant ratios ^{3}H cpm:^{14}C cpm during a 6-h period after SO_4^{2-} readdition were also found in a similar experiment using concentrated acetone-powder extract and not purified enzyme solution (Table I).

These results strongly support the suggestion of a stable arylsulfatase in +S cells after readdition of SO_4^{2-}.

After about 2 h in —S medium the enzyme activity increased in —S cells at an almost constant rate (Fig. 3). Such a course of accumulation of arylsulfatase activity was also observed from 2—12 h in a similar experiment starting with 0-h-old cells. Measurement of DNA content in —S cells revealed no synthesis of DNA throughout this period. Therefore, it seems likely that the synthesis of arylsulfatase, being fully derepressed after a lag period, depended on a continuous transcription of a constant amount of template for this enzyme.

Effect of methionine and cysteine on the synthesis of arylsulfatase in —S cells

The arylsulfatase activity in microorganisms has been shown to vary with the sulfur source used for growth. SO_4^{2-} has always been reported to cause repression, but different effects of sulfur-containing amino acids has been observed. In several fungi including *Aspergillus nidulans* [5,21] methionine as well as cysteine caused repression, while the enzyme in *Aerobacter aerogenes* is repressed by cysteine but not by methionine. The effect of methionine and cysteine on derepression of sulfatase was investigated by adding these compounds (to a final concentration of 1 mM) to cultures of —S cells which had grown in —S medium for 3 h. The activity of arylsulfatase was measured during a period of 4 h after the addition. The results of these measurements showed that the sulfatase activity increased with the same rate in the cells grown in the presence of methionine and cysteine as in the —S cells from a parallel control culture. Assuming that the uptake of methionine and cysteine are not inhibited in —S cells, the derepressed synthesis of arylsulfatase in *Chlamydomonas* is repressed neither by cysteine nor by methionine.

Capacity to synthesize arylsulfatase in synchronous and synchronized cultures

The maximum rates of arylsulfatase synthesis in —S cells, which are taken as a measure of the capacity for arylsulfatase synthesis, were measured during the derepression period in cells taken at different developmental stages from the synchronous and synchronized cultures.

We found that the arylsulfatase activity lost to the medium during sporulation was 9% of the activity in the cells. This loss was corrected for when we measured the capacity during the sporulation period, i.e. the derepression started with 8—10-h-old cells.

Typical results from derepression experiments with cells taken every 2 h from synchronous and synchronized cultures, are presented in Fig. 4. At all times of observation during the life cycle, depriving the cell of SO_4^{2-} caused increased activity of arylsulfatase. The average increase in activity over the last 2 h of the derepression period was taken to represent the arylsulfatase capacity. In Fig. 5 the capacities of the eight consecutive developmental stages of Fig. 5 are plotted versus the approximate cell age. The 2-h interval with maximal rate of synthesis is indicated by horizontal bars and curves are drawn through the midpoints of these bars. Both in the synchronous and in the synchronized cultures the total increase in capacity and in cell numbers during the sporulation were the same, namely about 16-fold. In summary the capacity patterns were the same with both types of synchronous cultures: both increased from the start of the life cycle to reach a peak around 8 h. Minimal values were found in the 9—13-h interval, whereafter the capacity increased again through the rest of the cycle.

From these results we conclude: (1) Arylsulfatase was derepressible at all stages of the life cycle. (2) Since the same patterns were found both with the synchronized and the synchronous cells, the pattern must be inherent in the life cycle and not a result of the synchronization method, i.e. light-dark shifts.

Comparison of the capacity pattern of arylsulfatase synthesis and the rate of RNA and protein synthesis during the life cycle

Do the variations in capacity pattern reflect corresponding variations in

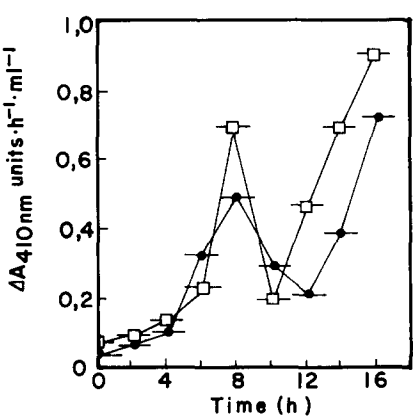

Fig. 5. Change of sulfatase capacity in synchronous culture of *C. reinhardti* produced by (●———●) light-dark shifts (12:4) and by (□———□) selection of zoospores from a non-synchronous culture. Ordinate: enzyme activity as A_{410nm} units per h per ml of stock synchronous culture.

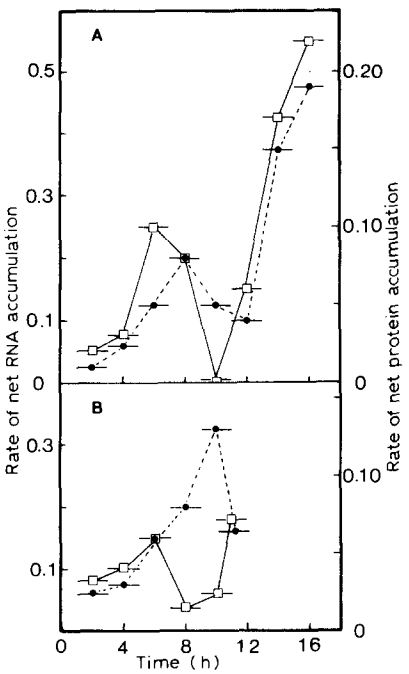

Fig. 6. Rate of net RNA (□——□) and protein (●——●) accumulation, (A) in —S and (B) in +S cells. (A) Amounts of RNA and protein were measured in samples of cells taken from the suspension with cells in —S medium undergoing derepression. The average increase in RNA and protein during the last 2 h of each of the derepression periods are plotted versus the approximate cell age. (B) Rate or "capacity" plots of the data from RNA and protein determinations during the light period of the light-dark synchronized cultures (+S cells). Rates of RNA accumulation are given as ΔRNA per h per 100 ml of stock synchronous culture, where ΔRNA is the difference between content of RNA expressed as $A_{552\,nm} - A_{510\,nm}$ at two time points. Rates of protein accumulation are given as $\Delta A_{610\,nm}$ per h per ml of stock synchronous culture.

the general protein and/or RNA synthesis? To answer this we compared arylsulfatase production capacity with the rate of RNA and protein synthesis. The net amounts of these macromolecules were measured concomitantly with enzyme synthesis in samples taken from the suspensions with cells in —S medium undergoing derepression. In the same way as for the enzyme capacity, the average increase in RNA and protein during the last 2 h of each of the derepression periods was taken to represent the rate of net RNA and protein synthesis. In Fig. 6A these rates of eight different developmental stages are plotted versus the approximate cell age. It can be seen that the rate of RNA and protein synthesis changed in the same periodic manner during the life cycle as did the enzyme capacity. The pattern for the enzyme coincided with that of protein, both having maximum values around 8 h and minimum values around 12 h. But the RNA pattern showed maximum and minimum values 2 h earlier than the foregoing ones, namely at 6 h and 10 h, respectively.

It might be that the transfer of the cells to —S medium caused changes in the pattern of synthesis of RNA, which in turn caused similar variation in the two other patterns. However, as previously reported [3,10], the rate of net RNA synthesis in the light-dark synchronized cultures (complete medium) also followed a periodic pattern during the light period with minimum rate from

8—10 h. The rate of the net protein synthesis in those cultures reached maximum around 10—11 h, whereafter it decreased during the last hours of illumination. Constructing rate plots or "capacity" plots of RNA and protein from these accumulation curves during the light period (Fig. 6B), revealed rate patterns almost identical to the corresponding patterns found with —S cells during the same period of the life cycle. The only significant differences were a larger amplitude for —S cells than for +S cells in the periodic variation of the rate of RNA synthesis, and the time for maximum rate of protein synthesis which was at 10 h and 8 h for +S and —S cells, respectively. Therefore, the observed patterns of rate of RNA and protein synthesis of cells transferred to —S medium, were not caused by the absence of SO_4^{2-}. Hence, we can conclude that the capacity for arylsulfatase synthesis follows the rates at which the cells synthesize RNA and protein.

Discussion

The observed appearance and increase of arylsulfatase activity in *C. reinhardti* after lowering the concentration of SO_4^{2-} in the growth medium, could either be due to an activation of preexisting enzyme molecules or to an increased amount of enzyme molecules. If the last possibility was the case, the appearance and increase in enzyme protein concentration could result from an increased rate of synthesis or from a decreased rate of degradation. Combination of these possibilities are also possible.

There was no detectable arylsulfatase activity in cells grown in medium with a concentration of SO_4^{2-} higher than $5 \cdot 10^{-5}$ M. If the appearance and increase of sulfatase activity in —S cells and in cells grown in medium with less than $5 \cdot 10^{-5}$ M SO_4^{2-} are solely due to inhibition of enzyme degradation, the turnover of sulfatase in +S cells must be so high that all of the enzyme has to be degraded immediately after being synthesized. This possibility is unlikely since there was no decrease in enzyme activity when protein synthesis was inhibited by cycloheximide and no decay of radioactivity in the radioactive enzyme protein after readdition of SO_4^{2-}.

If the increase in activity is solely due to activation of preexisting enzyme, there should be no incorporation of ^{14}C-labelled amino acid into the enzyme protein. The close correspondance between the location of enzyme protein, enzyme activity and radioactivity after both electrophoresis and isoelectric focusing of purified enzyme from —S cells incubated with radioactive phenylalanine, shows that removal of SO_4^{2-} from the cells resulted in de novo synthesis of arylsulfatase enzyme protein. Hence, we conclude that arylsulfatase in *Chlamydomonas* is a derepressible enzyme which is synthesized when the concentration of SO_4^{2-} in the cells is reduced below a certain level. The lag period in enzyme synthesis, the length of which depended on the concentration of SO_4^{2-} in the medium, may be due to the internal SO_4^{2-} pool which had to be depleted before the triggering level was reached. A similar trend is found for phosphate and phosphatase derepression (unpublished result).

After a longer period, (6—8 h), of growth in medium containing different SO_4^{2-} concentrations, an optimum relation was found between the SO_4^{2-} concentration and the arylsulfatase activity. This relation can be explained by

assuming that the overall protein synthesis including arylsulfatase enzyme protein is retarded because of sulfur becoming a limiting factor of protein synthesis at an external concentration of SO_4^{2-} below $1 \cdot 10^{-5}$ M. At concentrations of SO_4^{2-} higher than $1 \cdot 10^{-5}$ M, the synthesis of arylsulfatase is repressed reaching complete repression at about $5 \cdot 10^{-5}$ M.

Patterns of enzyme synthesis during the cell cycle, have been compared in synchronous and synchronized cultures of *Chlorella* [22] and yeast [23,24]. The conclusion of these comparisons was the same as in the present report for the pattern of capacity to synthesize arylsulfatase, namely that the pattern was not a manifestation of the rythm imposed on the cells by the light-dark synchronization procedure.

The variation in the rate of enzyme synthesis, or in the capacity for such synthesis (derepressibility) during the cell cycle, is due to variation in the capacity of the rate-limiting step of the protein production. In short, the machinery for protein synthesis consists of the general part being the translational apparatus, and the special part consisting of the transcriptional mechanism and the mechanism of transport of the RNA species from the nucleus to the cytoplasm. It is conceivable that during the life cycle not one, but different steps might in turn be rate limiting in the process of protein synthesis. In the case of the capacity for arylsulfatase synthesis three phases were found. During the first one no detectable DNA accumulation took place, and hence the gene level was constant. Despite this the capacity increased, which can be explained if the general machinery was the rate-limiting factor throughout this phase, as an increase in its capacity. From 9 to 12 h the DNA was replicated, but despite the increase in gene level for the sulfatase the capacity for the enzyme synthesis fell from 8 to 10 h. This decrease can be explained as reduced availability of template for transcription due to condensed chromosomes during the mitoses following each of the S-phases. The capacity was lowest when the DNA synthesis was at its highest rate, but the capacity did not drop to zero as should be expected. The remaining capacity might reflect the lack of complete synchrony of the culture. After 10 h the capacity again increased presumably as a result of increased gene level. That the capacities for synthesis of sulfatase varied in parallel with the net RNA and protein accumulation indicated that a causal relationship existed between these parameters. But only a thorough analysis of the interrelationships of the different processes making up the general and special machinery for protein synthesis and the dynamic behaviour of this apparatus can show the possible existence of such a causal connection. We are, at the moment, undertaking an analysis of this kind which will be published elsewhere. However, we will discus at some length some factors which can determine the amount of ribosomal RNA of *Chlamydomonas* and thereby determine the capacity of the general part of the apparatus of protein synthesis.

Since rRNA makes up the larger part of the cell's RNA, the observed rate variation of RNA to a large extent is that of rRNA. Therefore, much of the increase in rate of RNA accumulation during the 0—6-h interval can conceivably be due to replication of the rRNA cistrons. Since these represent a smaller part of the total amount of DNA their synthesis would be unnoticed in the detection of total DNA, but would be shown by labelling experiments.

Alternatively the RNA polymerase or the amount of nucleoside triphosphates might limit the RNA synthesis, and an increase in these factors manifesting itself as increased rate of rRNA production. From 6 to 10 h the rate of RNA (rRNA) synthesis decreased almost to zero. This period coincided with a period where the nucleus lost much of its stainability with brillian cresyl blue [10], giving a diffuse picture of the nucleus in contrast to the sharp and condensed ones at other times during the cell cycle. In *C. mouwusi* var. rotunda Vaage [25] detected the same, and he found from electron microscopy that concurrent with the "diffuse state" of the nucleus the nucleolus had almost disappeared. Others [26] have found that in *C. reinhardti* a single large nucleolus disintegrates before mitosis and reforms after. The nucleolus has been shown to be the site of rRNA synthesis [27], and Howell [28] found during the life cycle of *Chlamydomonas* a correlation between the patterns of degradation and synthesis of the rRNA cistrons on the one hand, and break-down and formation of the nucleolus on the other. The increase in rate after 10 h can be the result of production of new rRNA cistrons.

The amount of rRNA and ribosomes in the cytoplasm can be further regulated by the process of transport of rRNA through the nuclear pores.

Assuming that the amount of rRNA in the cytoplasm determined the capacity of the protein synthesis throughout the cell cycle, the variation in the rate of synthesis of rRNA would generate a corresponding variation in the rate of net protein accumulation. The degree of similarity between the rate variations would of course depend on the stability of the different enzyme proteins because the net accumulation is the result of production and degradation.

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