Formation of Benzoic Acid and *p*-Hydroxybenzoic Acid in the Blue Green Alga *Anacystis nidulans*: A Thylakoid-Bound Enzyme Complex Analogous to the Chloroplast System

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Membrane-Bound Enzyme Complexes, *Anacystis nidulans*, Thylakoids, Benzoate Synthase

The photosynthetic procaryote *Anacystis nidulans* converts L-phenylalanine and L-tyrosine into benzoic acid and *p*-hydroxybenzoic acid, respectively. Results obtained with thylakoid fractions support the hypothesis that the reaction sequence is catalyzed by thylakoid-bound enzyme complexes consisting of phenylalanine ammonia-lyase and benzoate synthase or tyrosine ammonia-lyase and *p*-hydroxybenzoate synthase, respectively. Both complexes do not accept phenylacetic acids as substrates, and cinnamic acids only at a small extent. These properties suggest a striking similarity to a benzoic acid-synthesizing enzyme system from higher plants which is situated at the thylakoid membrane of chloroplasts. The respective complexes of *Dunaliella marina* and *Porphyridium* sp. were included in this comparison.

Introduction

L-Phenylalanine ammonia-lyase (E.C. 4.3.1.5.) is the key enzyme of secondary aromatic metabolism in higher plants, certain fungi, eucaryotic algae and *Streptomyces* [1]. In higher plants, the enzyme is found in the soluble fraction, in microsomes [2] and in chloroplasts [3].

The occurrence of a multiple form in thylakoids is of particular interest, since flavonoids and hydroxycinnamic acid derivatives are found to be localized in so-called photosynthetic units [4]. Both compounds are derived, by the obligatory action of phenylalanine ammonia-lyase, from aromatic amino acids.

The occurrence in chloroplasts of an enzyme complex which converts L-phenylalanine via cinnamic acid into benzoic acid has recently been demonstrated [5]. Double-labelling experiments revealed that the benzoic acid-forming complex uses L-phenylalanine rather than externally supplied cinnamic acid as a substrate.

In extension of a previous study on the occurrence of phenylalanine ammonia-lyase in the green alga *Dunaliella marina* we attempted to investigate phenylalanine ammonia-lyase, the first enzyme in the metabolism of aromatic amino acids, in a blue-green alga. We failed to detect considerable amounts of phenylalanine ammonia-lyase, but found, in *Anacystis nidulans*, a membrane-bound complex which transforms L-phenylalanine into benzoic acid.

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Materials and Methods

Cultivation of algae

Anacystis nidulans (Sammlung von Algenkulturen, Pflanzenphysiologisches Institut, Universität Göttingen, Katalog Nr. L 1402-1) was grown at 37 °C in the medium C of Kratz and Myers [6] under continuous light and aeration (with approx. 0.5% CO_2 in air, v/v). Growth curves after addition of possible growth factors (*e. g.* benzoic acid) were performed at 34 °C in an illuminated incubator shaker. That was gassed with a CO_2-air mixture at a flow rate of 2 l/h. Cell density was measured using a photoelectric EEL-colorimeter.

Dunaliella marina (Sammlung von Algenkulturen, Pflanzenphysiologisches Institut, Universität Göttingen, Katalog Nr. LB 19-4) was grown at 20 °C in a synthetic sea water medium [7] in an atmosphere of 5% CO_2 in air. The unicellular red alga *Porphyridium* sp. was obtained from the Culture Collection of the Department of Botany, Indiana University, Bloomington, Indiana (catalogue no.

637) and was cultivated in a culture medium [8] at 25 °C under continuous light using a mixture of 5% CO_2 in air. All algae were used for the experiments when they were in the late logarithmic phase of growth.

Labelled compounds

L-[U-^{14}C]Phenylalanine, L-[4-*ring*-^{3}H]phenylalanine, L-[U-^{14}C]tyrosine and L-[3′,5′-^{3}H]tyrosine were purchased from the Radiochemical Centre, Amersham. Compounds with high specific activities were diluted to about 10 mCi/mmol and purified by paper chromatography [3] immediately prior to use. [3-^{14}C] Cinnamic acid (50 mCi/mmol) was obtained from Schwartz & Mann (Orangeburg, New Jersey) and was freed from contaminating benzoic acid by paper chromatography and preparative gas-liquid chromatography [3].

[U-^{14}C]Phenylacetic acid was prepared from L-[U-^{14}C]phenylalanine (specific activity 10 mCi/mmol) by the following procedure. A solution (1.0 ml) containing the labelled amino acid and 1.25 units of L-amino acid oxidase (Boehringer, Mannheim) was brought to pH 7.5, and oxygen was allowed to bubble through the mixture for 150 min at 32 °C. After this period of time moistened indicator paper showed that ammonia was not evolved any more. Then 0.5 ml of a 5% solution of H_2O_2 in 2 N NaOH were added. After 3 hours the mixture was diluted to 20 ml, acidified with 1 ml of concentrated HCl and extracted four times with diethyl ether. [U-^{14}C]Phenylacetic acid was obtained in radiochemically pure form after paper chromatography in two solvent systems [3]. The purity of the product was checked by gas-liquid chromatography [3, 5].

[3′,5′-^{3}H]*p*-Hydroxyphenylacetic acid (specific activity 0.2 mCi/mmol) was isolated after incubation of sonicated cells of *A. nidulans* with L-[3′,5′-^{3}H]tyrosine. The product obtained after extraction with diethyl ether of the acidified solution was purified by paper chromatography in the two solvent systems mentioned [3].

[3′,5′-^{3}H]*p*-Coumaric acid was prepared from L-[3′,5′-^{3}H]tyrosine using a L-tyrosine ammonia-lyase preparation from *Avena sativa* [9].

Experiments with cell homogenates after sonication

Experiments with homogenates were performed by suspending the pelleted and washed agal cells of *A. nidulans* (approx. 3 g moist weight) in 3 ml 0.1 M borate buffer, pH 8.5, containing the labelled amino acid. After 4 × 60 sec sonication with a Branson sonifier (output 30 W) the suspension was incubated for 1 hour at 25 °C.

Experiments with purified cell fractions

Photosynthetic membranes of *A. nidulans* were prepared by sonication of the algal cells as described above, after removal of intact cells at 3000 × *g* for 5 min, and sedimentation at 15000 × *g* for 15 min. The pellet was suspended in buffer and incubated for 30 min with the labelled substrates. The supernatant was treated correspondingly. A blank either of the membrane or the supernatant fraction was boiled for 10 min and incubated in the same way.

For another set of experiments, membranes were purified by placing a homogenate of sonicated cells (in grinding medium containing sucrose [3]) on top of a linear sucrose density gradient ranging from 20 to 60% (w/w) sucrose (in 0.05 M phosphate buffer, pH 7.5). After centrifugation for 16 hours at 25000 rpm (Beckman rotor SW 27) a green band at 41% sucrose, a pair of yellow bands at 46% sucrose and a bulky green band at 54% sucrose were obtained.

Higher amounts of membranes were obtained by a brief sonication (4 × 30 sec). Thylakoids were suspended in grinding medium and layered on a discontinuous sucrose gradient consisting of 6 ml 65% (w/w) sucrose, 6 ml 55% sucrose, 5 ml 50% sucrose, 5 ml 44% sucrose and 10 ml 40% sucrose. Centrifugation for 3 hour at 27000 rpm yielded 3 green bands at 50% sucrose, 54% sucrose (major part), and 58% sucrose. All zones obtained were assayed for the corresponding enzymes.

Thylakoids from lysozyme-treated cells

In order to obtain intact thylakoids, protoplasts were prepared by treatment of the cells with lysozyme in 0.03 M phosphate buffer (pH 6.8) containing 0.5 M mannitol [10]. Amounts of lysozyme used depended on the Shibata units measured. After lysis of the washed protoplasts in hypotonic solution using a Potter homogenizer, thylakoids were obtained by centrifugation at 10000 × *g* for 15 min.

D. marina cells, lacking a rigid cell wall, were lysed by osmotic shock [11, 12]. Chloroplasts were obtained by sedimentation velocity centrifugation and thylakoids were prepared from them as described elsewhere [3].

We have not been able to isolate intact chloroplasts from *Porphyridium* sp.; treatment with the Merkenschlager homogenizer (4 × 45 sec) or grinding in a chilled mortar with quartz sand for 10 min yielded, after differential centrifugation, a fraction containing the main portion of chlorophyll. This pellet was subjected to sucrose density gradient

centrifugation (ranging from 30 – 60% sucrose) and yielded a chlorophyll-containing fraction at 54% sucrose which was used for incubations.

Identification of products

The incubation of labelled compounds with thylakoidal fractions was stopped after 30 min by acidification, extracted with ether, and purified by repeated chromatography in well established solvent systems [3, 13]. Finally, the products were identified by recrystallization to constant specific activity.

Determination of radioactivity

Radioactive samples were counted in the scintillation spectrometer (Betaszint BF-5000, Berthold, Wildbad); absolute radioactivity of single or double labelled compounds was calculated by a program based on channel ratio methods. Chromatograms were scanned on a chromatogram scanner (4π-geometry, 15% efficiency for ^{14}C).

Other determinations

Total chlorophyll and chlorophyll a were determined according to Strain [14]. The content of phycocyanin was measured at 625 nm using an extinction coefficient of $210000\ M^{-1} \times cm^{-1}$ for the monomer [15]. Phycocyanin accounts for about 36% of the soluble proteins of *A. nidulans*.

Sucrose density was determined refractometrically.

Results

Based on preliminary experiments with *Anacystis nidulans* and on analogous findings with other membranes, it could be anticipated that aromatic compounds other than the amino acids are not transported into the procaryotic cell. In order to avoid any permeability barriere the cell envelope was broken or weakened by ultrasonic treatment yielding homogenates; these were tested before work with subfractionated cells was started.

Experiments with total homogenates

Table I shows the conversion of L-phenylalanine into cinnamic acid and benzoic acid and of L-tyrosine in *p*-hydroxycinnamic acid and *p*-hydroxybenzoic acid, respectively. Unexpectedly, phenylalanine ammonia-lyase activity was very low. Instead of being present as "free" phenylalanine ammonia-lyase, this enzyme seems to be integrated in a benzoic acid-forming system. This benzoic acid-forming system is 10 times more efficient than that described in higher plants (*e. g. Nasturtium officinale* [5]).

With homogenates from cells of *A. nidulans*, *p*-hydroxybenzoate formation was found to be of about the same order of magnitude as the conversion phenylalanine → benzoic acid (Table I). A low L-tyrosine ammonia-lyase activity seems to be localized in the blue-green alga, too.

Experiments at the subcellular level

The following experiments were designed to reveal whether the enzymes responsible for the conversion observed were thylakoid-bound as demonstrated with chloroplasts of higher plants. Another important point was: Is there an enzyme complex – consisting of several enzymes, all associated with the same membrane – catalyzing the formation of benzoic acids from aromatic amino acids? Experiment No. 1 in Table II gives answers to both questions: First, thylakoids are capable of catalyzing the conversion phenylalanine → benzoic acid, and, secondly, externally supplied cinnamate is not converted as efficient as the amino acid. Phenylalanine ammonia-lyase activity is low, but significant. Thus, both enzyme activities responsible for the conversion phenylalanine → cinnamic acid → benzoic acid are present. But besides that, a complex even more efficient should exist for the total sequence.

The molar ratio [^{3}H]benzoic acid to [^{14}C]benzoic acid is significantly higher than the molar ratio $^3H/^{14}C$ in the intermediate, cinnamic acid. That is

Exp. No.	Substrate	[M]	Product [nmol × (30 min)$^{-1}$ × (mg Chl)$^{-1}$]	
1	L-[4-ring-^{3}H]phenylalanine	2×10^{-4}	benzoic acid	1.04
			cinnamic acid	<0.01
2	L-[3′,5′-^{3}H]tyrosine	7×10^{-5}	*p*-hydroxybenzoic acid	0.48
			p-coumaric acid	0.07

Table I. Conversion of aromatic amino acids into benzoic acids by homogenates of *A. nidulans*.

Table II. Formation of Benzoic acid by subcellular preparations of *A. nidulans*. In experiment No. 2, two possible substrates are compared by incubating them simultaneously (with thylakoids). In the same way also the supernatant is tested (experiment No. 3). Conversion rates are given in nmol × (30 min)$^{-1}$ × (mg Chl a)$^{-1}$.

Exp. No.	Substrates	[M]	Preparation		Products: Cinnamic acid nmol	Cinnamic acid $^3H/^{14}C$	Benzoic acid nmol	Benzoic acid $^3H/^{14}C$
1	L-[^{3}H]phenylalanine + [^{14}C]cinnamic acid	3×10^{-4} 5×10^{-6}	US	thylakoids	0.05 (^{3}H) 5.00 (^{14}C)	0.01	0.26 (^{3}H) 0.05 (^{14}C)	5.2
2	L-[^{3}H]phenylalanine + [^{14}C]phenylacetic acid	3×10^{-4} 3×10^{-6}	US	thylakoids	0.02 (^{3}H) <0.01 (^{14}C)	—	0.33 (^{3}H) <0.01 (^{14}C)	30
3				supernatant	<0.01 (^{3}H) <0.01 (^{14}C)	—	0.08 (^{3}H) <0.01 (^{14}C)	> 8
4	L-[^{14}C]phenylalanine	3×10^{-4}	L	thylakoids	0.03	—	0.37	—
5				supernatant	<0.01		<0.01	

equivalent to the statement that the two pools of cinnamate – one at the enzyme complex (^{3}H-labelled) and one in the medium (^{14}C-labelled) – do exchange, but only at a very limited rate.

A possible role of phenylacetic acid as intermediate in the conversion phenylalanine → benzoic acid appears to be most unlikely as demonstrated in experiment No. 2 (Table II). Phenylacetic acid, which is known to be oxidized to benzoic acid in the parasitic fungus *Poria weirii* [16], is not utilized by photosynthetic membranes of *A. nidulans*. *p*-Hydroxybenzoic acid can be formed by α-oxidation of *p*-hydroxyphenylacetic acid in plant cell suspension cultures [17].

Using subcellular fraction obtained after lysozyme-treatment of the algal cells, it was observed that only thylakoids are able to convert L-phenylalanine into cinnamic acid [0.03 nmol × (30 min)$^{-1}$ × (mg chlorophyll)$^{-1}$] and benzoate [0.37 nmol × (30 min)$^{-1}$ × (mg chlorophyll^{-1})] while the supernatant exhibited an activity well below these figures [<0.01 nmol × (30 min)$^{-1}$ × (mg protein)$^{-1}$].

Data shown in Table III summarize the analogous experiments on the formation of *p*-hydroxybenzoic acid from L-tyrosine in *A. nidulans*. As the exogenously supplied intermediate, which competes with the complex, is ^{3}H-labelled and the substrate for the complex is ^{14}C-labelled, a low ratio $^3H/^{14}C$ stands for a tightly coupled reaction. The respective enzyme activities are thylakoid-bound but seem to be susceptible to solubilization (experiment Nos 1, 4 and 5). A large part of *p*-hydroxybenzoate formation is found in the supernatant (0.02 nmol; exp. No. 2) if a high percentage of thylakoids is damaged during ultrasonic-treatment of the cells. The amount of soluble enzyme activities decreases, therefore, when cells are broken after lysozyme-treatment (experiment No. 5). Analogous to the benzoic acid-forming system, *p*-hydroxyphenylacetic acid is not a precursor of *p*-hydroxybenzoic acid either (experiment No. 3). A complex of consecutive enzymes

Table III. Formation of *p*-hydroxybenzoic acid by subcellular preparations of *A. nidulans*. Symbols are the same as used in Table II. Experiments Nos. 4 and 5 were both carried out with a mixture of ^{14}C-labelled L-tyrosine and ^{3}H-labelled *p*-coumaric acid as substrates. The same conditions were used for incubating thylakoids and supernatants as well.

Exp. No.	Substrates	[M]	Preparation		Products: *p*-Coumaric acid nmol	*p*-Coumaric acid $^3H/^{14}C$	*p*-Hydroxybenzoic acid nmol	*p*-Hydroxybenzoic acid $^3H/^{14}C$
1	L-[^{3}H]tyrosine	1×10^{-4}	US	thylakoids	<0.01	—	0.19	—
2				supernatant	<0.01	—	0.02	—
3	*p*-[^{3}H]hydroxy-phenylacetic acid	1×10^{-4}	US	thylakoids	—	—	<0.01	—
4	L-[^{14}C]tyrosine +*p*-[^{3}H]coumaric acid	2×10^{-4} 2×10^{-4}	L	thylakoids	<0.01 (^{14}C) 200 (^{3}H)	>200	0.25 (^{14}C) <0.01 (^{3}H)	<0.1
5				supernatant	<0.01 (^{14}C) 200 (^{3}H)	>200	0.01 (^{14}C) <0.01 (^{3}H)	—

should also be involved here, because externally added *p*-coumaric acid is not used as a precursor of *p*-hydroxybenzoic acid (experiment No. 4).

In order to make sure that the activities reported are in fact connected with our thylakoid preparations we purified them by density gradient centrifugation (Fig. 1). The results were in good agreement with those obtained by Sigalat and Kouchkovsky [15]. Five bands (as labelled in Fig. 1) were collected from the centrifuge tubes and assayed for benzoic acid-forming systems (Table IV).

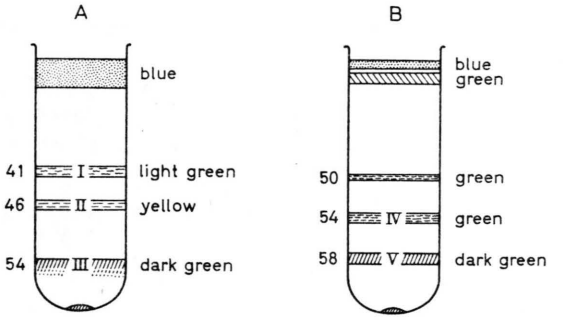

Fig. 1. Pattern of zones obtained by density gradient centrifugation of a homogenate (A) or a thylakoid preparation (B) from *A. nidulans*. A: Homogenate separated on a gradient from 20–60% sucrose, centrifuged at 25000 rpm for 16 h. B: Purification of a thylakoid preparation on a gradient from 40–63% sucrose (27000 rpm, 3 h).

Benzoate and cinnamate were found to be labelled only with ^{14}C, whereas *p*-hydroxybenzoate and *p*-coumaric acid showed only ^{3}H-labelling. Thus no hydroxylation at these levels seem to occur on *A. nidulans* thylakoids, in contrast to chloroplasts of eucaryotic algae [12]. The part of L-tyrosine which was not converted could be reisolated and was checked for ^{14}C-labelling: the membranes investigated did not catalyze the hydroxylation of phenylalanine into tyrosine either.

Only the chlorophyll-containing thylakoid membranes catalyzed the formation of benzoic acids from their corresponding amino acid precursors: The yellowish coloured double band at 46% sucrose (Fig. 1, II) showed no activity at all; thylakoid bands at higher densities (III, V) proofed to be more active than those at lower densities (I, IV), presumably because they represent rather intact membrane lamellae. In general, the incubations using membrane fractions from gradient B, where the algae were sonicated only for half the time compared with gradient A, gave better yields of benzoic acid and *p*-hydroxybenzoic acid. The phenylalanine ammonia-lyase and tyrosine ammonia-lyase activities of the thylakoids were very low.

Comparison of photosynthetic membranes from procaryotic and eucaryotic algae

Dunaliella marina was shown to contain the same coupled L-phenylalanine ammonia-lyase – benzoate synthase system as the chloroplasts of higher plants (Table V). That can be seen if one compares the $^{3}H/^{14}C$ ratio in the product, benzoic acid, with the respective value in the possible intermediate, cinnamic acid: 5.4 *versus* 0.12. In the column which outlines the amount of [^{3}H]cinnamic acid produced a value of 2.65 nmol is given. As 13 nmol [^{14}C]cinnamic acid was already added in the incubation mixture (1.0 ml), a ratio $^{3}H/^{14}C$ of 0.20 results from these data. The fact that the corresponding ratio is much higher in benzoic acid, leads us to suggest that "coupling" between the functioning of phenylalanine ammonia-lyase and benzoate synthase takes place. However, it can be seen, that at *D. marina* chloroplasts (a) coupling is not as efficient as at the photosynthetic membranes of the procaryotic alga and (b) phenylalanine ammonia-lyase is present in significant amounts.

The benzoate-forming enzyme system from *Porphyridium* sp. thylakoids turned out to be more

Table IV. Products formed upon simultaneous incubation of purified membrane fractions (see Fig. 1 for designation) with L-[U-^{14}C]phenylalanine (2.5×10^{-4} M) and L-[3′,5′-^{3}H]tyrosine (2.5×10^{-4} M). Conversion rates are given in nmol × (30 min)$^{-1}$ × (mg Chl)$^{-1}$.

Products	Label	Conversion rates, using the following fractions: I	II	III	IV	V
benzoic acid	[^{14}C]only	0.14	<0.01	0.21	0.75	0.84
cinnamic acid	[^{14}C]only	<0.01	<0.01	0.08	<0.01	0.11
p-hydroxybenzoic acid	[^{3}H]only	0.09	<0.01	0.12	0.34	1.19
p-coumaric acid	[^{3}H]only	<0.01	<0.01	0.02	<0.01	0.06

Table V. Formation of cinnamic acid and benzoic acid by thylakoids from *D. marina* and *Porphyridium* sp. The amounts of products formed are given in nmol × (30 min)$^{-1}$ × (mg Chl)$^{-1}$. The number in parenthesis [] is the molar ratio $^{3}H/^{14}C$.

Exp. No.	Preparation	Substrate	[M]	Products: Cinnamic acid	Products: Benzoic acid
1	*D. marina* chloroplasts	L-[^{3}H]phenylalanine	4×10^{-5}	2.65 (^{3}H) [0.20]	0.09 [^{14}C) [5.4]
2	thylakoids	+ [^{14}C]cinnamid acid	1.3×10^{-5}	2.60 (^{3}H) [0.20]	0.08 (^{14}C) [5.1]
3	*Porphyridium* sp. thylakoids (crude prep.)	L-[^{14}C]phenylalanine	1×10^{-4}	0.22	0.65
4	*Porphyridium* sp. thylakoids (purified prep.)	L-[^{14}C]phenylalanine	1×10^{-4}	0.25	0.78

active than that of the green alga (Table V, Exp. No. 1) or the photosynthetic procaryot (Table II, Exp. No. 1). In that respect *Porphyridium* sp. resembles *A. nidulans* with the exception that L-phenylalanine ammonia-lyase activity could be demonstrated unequivocally only in the red alga.

Effect of benzoic acids on the growth of A. nidulans

Both benzoate and *p*-hydroxybenzoate caused a slight stimulation of growth of *A. nidulans* (Fig. 2). This effect was more pronounced on increasing the concentrations in the range of $1-5\times10^{-5}$ M.

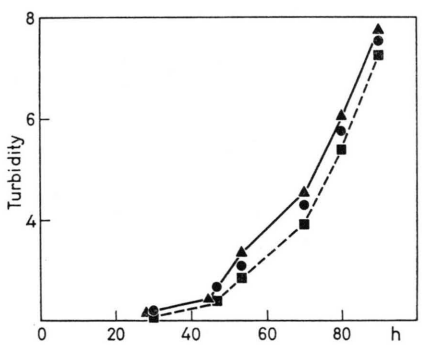

Fig. 2. Effect of benzoic acid and *p*-hydroxybenzoic acid on growth of *A. nidulans*. 4 days old cultures (1.5 ml) were transferred into a fresh medium [6] (100 ml) which contained no further additions (—■—■—) for 1×10^{-5} M benzoate (●●●), or 1×10^{-5} M *p*-hydroxybenzoate (—▲—▲—). Turbidity (EEL-reading) is plotted against time (h).

Discussion

A low L-phenylalanine ammonia-lyase activity, absent in heterotrophic bacteria, could be detected on thylakoids of a blue-green bacterium, *A. nidulans*. The major part of membrane-bound phenylalanine ammonia-lyase in this organism seems to form a tight complex with benzoate synthase being responsible for chain-shortening of the C_6C_3-compound to a C_6C_1-compound. Double-labelling experiments indicate that externally added cinnamate is hardly accepted by the enzyme complex. That has a strong resemblance to complexes found in the case of the biosynthesis of hydroxycinnamic acids or benzoic acid at the thylakoids of eucaryotic plants. In all these events the postulated intermediate is not accepted as substrate as efficient as L-phenylalanine. Problems and implications related to this hypothesis have already been discussed elsewhere [12].

The parallelism between chloroplasts of eucaryotic plants and photosynthetic membranes of procaryotic algae is the more striking as it is not shared by other compartments of eucaryotic cells or by bacteria.

The activity of the benzoate-forming enzymes in *A. nidulans* is 10 times higher than in the green alga *D. marina* which seems to be more related to higher plants; also in respect of the thylakoid-bound phenylalanine ammonia-lyase activity.

Porphyridium sp. shows, although being an eucaryotic organism, several similarities to blue-green algae, *e. g.* the occurrence of biliproteins, as light harvesting pigments, the pathway of biosynthesis of unsaturated fatty acids [18] *ect.* In the case of secondary metabolism of L-phenylalanine the red alga possesses a similar level of benzoate-formation as *A. nidulans* and a comparatively low activity of uncoupled phenylalanine ammonia-lyase. Unlike chloroplasts, the benzoate-forming system on *A. nidulans* thylakoids converts L-tyrosine into *p*-hydroxybenzoate, without using *p*-coumarate as a substrate. This is possible due to the lack of cinnamate hydroxylase in the blue-green alga. Tyrosine ammonia-lyase activity was very low and could not always be demonstrated.

In higher plants, *p*-hydroxybenzoate, the precursor of ubiquinone, is synthesized by a mitochondrial enzyme system that utilizes *p*-coumarate [19]. Since blue-green algae do not contain ubiquinone, there is no obvious physiological function of the benzoate and *p*-hydroxybenzoate forming-enzyme systems except that they are part of a catabolic pathway. However, our experiments showed a growth promoting activity of both acids for *A. nidulans*. Possibly their role is an ecological one, because brominated derivatives of *p*-hydroxybenzaldehyde and *p*-hydroxybenzyl alcohol have recently been detected in marine blue-green [20] and red algae [21]. They seem to enhance the growth rate of the organisms producing them whereas they are toxic to other unicellular algae.

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