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Geosmin production in the cyanobacterium *Oscillatoria brevis**

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Abstract. The cyanobacterium *Oscillatoria brevis* (Kütz.) Gom., strain NIVA CYA 7, was used to investigate how geosmin production is related to the synthesis of chlorophyll *a*, phycobiliproteins and β-carotene under nitrogen (NH_4^+) and light limiting conditions. Chemostat samples were used to inoculate batch cultures that were treated with inhibitors of isoprenoid synthesis, norflurazon and dimethazone, and gabaculine that inhibits tetrapyrrole synthesis. Dimethazone decreased and norflurazon increased geosmin production under light limited conditions, as was expected due to their sites of action in the isoprenoid pathway. This effect was not so pronounced in nitrogen limited cultures due to the additional effect of increasing nitrogen deficiency during the experimental period. Norflurazon was the only inhibitor that uncoupled geosmin production completely from β-carotene formation which indicates a strikt coupling between geosmin and β-carotene biosynthesis. From the observed increase of geosmin production relative to pigment synthesis after norflurazon treatment it was suggested that isoprenoid precursors are directed to geosmin synthesis when the demand for pigment precursors is very low. Within the framework of this study the data strongly support the hypothesis of geosmin formation via the isoprenoid pathway in *Oscillatoria brevis* as was found for actinomycetes.

Key words: Cyanobacterium – Geosmin synthesis – Isoprenoid pathway – *Oscillatoria brevis*

Geosmin is a compound that causes off-flavour problems in drinking water (Persson 1982; Weete et al. 1979). It has an intense earthy odour and is produced by numerous actinomycetes (Gerber 1967; Gerber and Lechevalier 1965) and some cyanobacteria (Kikuchi et al. 1973; Medsker et al. 1968; Rosen et al. 1970). In *Streptomyces* spp. geosmin is synthesized from a sesquiterpene precursor in the isoprenoid pathway (Bentley and Meganathan 1981). In cyanobacteria this pathway serves for the biosynthesis of phytol and carotenoids in addition to quinones. Light induced changes in Chl *a* and geosmin levels in the cyanobacterium *Oscillatoria brevis* suggested a control by the isoprenoid pathway over geosmin (Naes et al. 1985).

The pigments in cyanobacteria are carotenoids (in which β-carotene is the most abundant), PBPs consisting of tetrapyrrole bound to proteins and Chl *a* that has its tetrapyrrole (porphyrin) linked to phytol.

The nitrogen containing tetrapyrroles are synthesized via a completely different pathway than phytol, carotenoids and geosmin that all lack nitrogen, therefore, the pigments in cyanobacteria may be used as markers of cellular nitrogen levels (Chl *a* and PBPs), and of the net relative flows of isoprenoid precursors to phytol (Chl *a*) and phytoene (carotenoids). Figure 1 shows the pathway of carotenoid biosynthesis with the sites of action of dimethazone, and norflurazon, specific inhibitors in this pathway. Chl *a* synthesis depends on the availability of both phytol and porphyrins. The synthesis of the latter can be blocked by gabaculine by which PBP synthesis is also blocked. Since both light and nitrogen have marked effect on pigment levels and composition and since nitrogen is not at all required in the isoprenoid pathway that leads to essential components of the photosynthetic apparatus (phytol, carotenoids and quinones), it was hypothesized that differences in the stress induced by nitrogen and light as the growth limiting factor may result in different rates of geosmin production relative to pigment synthesis. This was investigated in two ways: 1) transient state analysis of the adaptation process of *O. brevis* shifted from nitrogen to light limiting conditions and *vice versa* (Naes and Post 1988) and 2) the objective of the study presented here was to investigate how geosmin synthesis is related to pigment synthesis under nitrogen limiting and light limiting conditions and it was questioned whether geosmin synthesis follows the isoprenoid pathway.

Materials and methods

The cyanobacterium *Oscillatoria brevis* (Kütz.) Gom., strain NIVA CYA 7, were cultivated in 2 l continuous culture flasks using a mineral medium (Van Liere and Mur 1978) with a dilution rate of 0.010 h^{-1}. Continuous illumination was

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Abbreviations: Chl *a*, chlorophyll *a*; *O. brevis*, *Oscillatoria brevis*; PBPs, phycobiliproteins

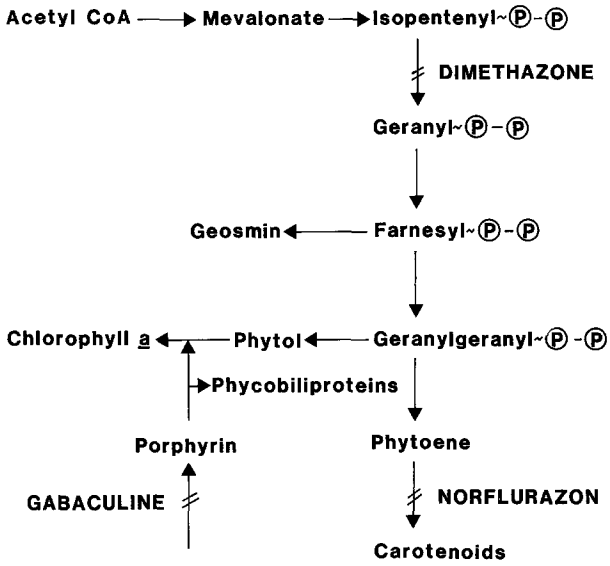

Fig. 1. Schematic representation of the biosynthetic pathway of geosmin, Chl *a* and carotenoids with the sites of action for the inhibitors dimethazone, norflurazon and gabaculine

provided by Philips TLE 32 W/33 and TLEM 40 W/33 lamps with an incident irradiance of 170 μmol m^{-2} s^{-1}. Temperature and pH were controlled at 20° C and 8.1 respectively and the culture was mixed by aeration at a rate of 150 l h^{-1}. Nitrogen limited and light limited cultures were obtained by allowing the cyanobacterial biomass to adjust to an input of 300 μM NH_4Cl or 170 μmol m^{-2} s^{-1}, respectively until steady state levels were reached.

Batch cultures for the inhibitor experiments were inoculated into Erlenmeyer flasks from a nitrogen limited and a light limited continuous culture, respectively. Batch cultures were maintained at the same growth conditions after the addition of the following inhibitors. Gabaculine (5-amino-1,3-cyclohexadienylcarboxylic acid) was used as a tetrapyrrole inhibitor in *O. brevis*. The compound is a naturally occurring amino acid known to inhibit γ-aminobutyric acid-α-ketoglutaric acid-transaminase (Rando 1977). Norflurazon (4-chloro-5-methylamino-2-(3-trifluoromethylphenyl)-pyridazin-3-(2H)one) which is reported to prevent the desaturation of phytoene (Kunert and Böger 1979; Clarke et al. 1982) was used to specifically inhibit the biosynthesis of carotenoids (β-carotene). Dimethazone (2-(2-chlorophenyl)methyl-4,4-dimethyl-3-isoxazolidinone) inhibits isopentenylpyrophosphate isomerase (Sandmann and Böger 1986; Sandmann pers. comm.). The inhibitors dissolved in methanol were added to give the following final concentrations in the cultures: 55 μM gabaculine, 200 μM dimethazone and 10 μM norflurazon, concentrations sufficient for maximal inhibition according to Rando (1977); Kunert and Böger (1979); Sandmann and Böger (1986); and Bramley et al. (1984), respectively.

Determinations of Chl *a*, PBPs, β-carotene, geosmin and biomass were outlined as described previously (Naes and Post 1988).

Results

Culture conditions had a marked effect on pigmentation and geosmin production in *O. brevis*. Under light limiting conditions the Chl *a* and PBP content, indicative of the nitrogen status of the cells, were at 3 and 44 mg·g dry wt.$^{-1}$, respectively, whereas β-carotene reached levels of 1.1 mg·g dry wt.$^{-1}$ (Table 1). Under nitrogen limiting conditions (Table 2) there was a 3-fold decrease in β-carotene compared to light limited cells. Chl *a* and PBP contents were affected even more and they decreased to 0.7 and 5 mg·g dry wt.$^{-1}$, respectively. Total amounts of geosmin decreased from 0.21 to 0.16 mg·g dry wt.$^{-1}$. Thus, the amount of β-carotene relative to Chl *a* and PBPs increased during nitrogen limited growth compared to the light limiting conditions. This resulted in an increase of the geosmin/Chl *a* and geosmin/β-carotene ratios by a factor of 3 and 2, respectively.

Each inhibitor used in this study had a marked visual effect on pigmentation. In light limited cultures of *O. brevis* dimethazone and norflurazon caused a transition in culture colour from blue-green to deep blue. Cultures with a yellowbrown colour were characteristic after gabaculine treatment. Under nitrogen limiting conditions dimethazone treatment caused decreases in the content of β-carotene, Chl *a* and PBPs (Table 2). In the light limited cultures a similar effect on Chl *a* was shown, however, a marked increase of PBPs after dimethazone treatment was observed (Table 1). The effect of dimethazone on β-carotene synthesis was not detected as under nitrogen limiting conditions. In nitrogen and light limited cultures norflurazon inhibited β-carotene synthesis and a decrease in Chl *a* content was also observed. The PBP content also decreased during nitrogen limited growth, while cultures subjected to light limiting conditions markedly enhanced PBP synthesis after norflurazon treatment, a similar effect to that of dimethazone. Gabaculine was shown to be an effective inhibitor of Chl *a* and PBP synthesis in both light and nitrogen limited cultures. In light limited cultures, an inhibitory effect on β-carotene synthesis was also observed.

Table 1. The amount of β-carotene, Chl *a*, PBPs, and geosmin at the start (t = 0 d) and the end (t = 5 d) of inhibitor treatment of light limited batch cultures of *O. brevis*

Treatment	mg · g^{-1} DW			
	β-Carotene start–end	Chl *a* start–end	PBPs start–end	Geosmin start–end
Control	1.1–1.1	3.0–5.0	44– 45	0.21–0.35
Dimethazone	1.1–1.2	3.0–2.6	44– 70	0.21–0.20
Norflurazon	1.1–0.6	3.0–3.0	44–111	0.21–0.51
Gabaculine	1.1–0.8	3.0–1.7	44– 30	0.21–0.20

Table 2. The amount of β-carotene, Chl *a*, PBPs, and geosmin at the start (t = 0 d) and the end (t = 10 d) of inhibitor treatment of nitrogen limited batch cultures of *O. brevis*

Treatment	mg · g^{-1} DW			
	β-Carotene start–end	Chl *a* start–end	PBPs start–end	Geosmin start–end
Control	0.4–0.5	0.7–0.7	5.0–5.0	0.16–0.16
Dimethazone	0.4–0.2	0.7–0.3	5.0–0.9	0.16–0.14
Norflurazon	0.4–0.1	0.7–0.1	5.0–1.5	0.16–0.17
Gabaculine	0.4–0.5	0.7–0.1	5.0–0.8	0.16–0.17

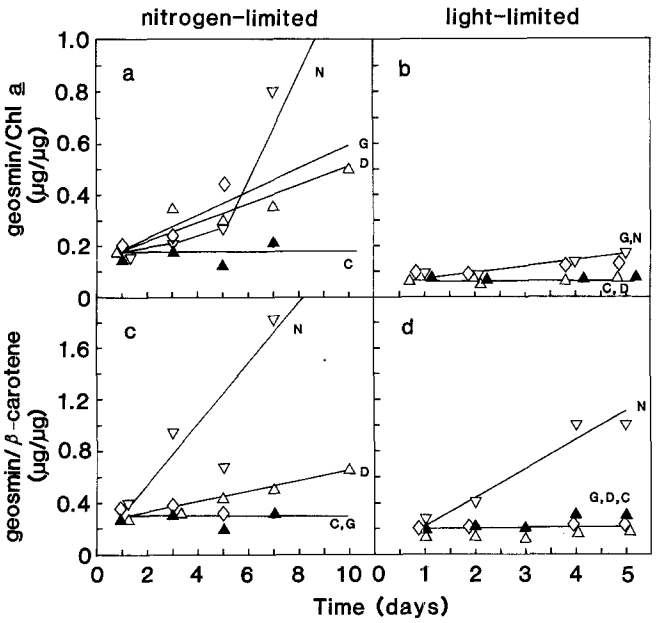

Fig. 2a–d. Effect of dimethazone (△), gabaculine (◇) and norflurazon (▽) compared to control (▲) on the production of geosmin relative to Chl *a* (**a, b**) and β-carotene (**c, d**) in nitrogen limited and light limited batch cultures of *O. brevis*

None of the inhibitors showed any marked effect on absolute amounts of geosmin under nitrogen limiting conditions (Table 2). Dimethazone might cause a slight decrease in geosmin content, however, this effect was more pronounced in light limited cultures. Gabaculine treatment caused a decrease in geosmin content during light limited growth (Table 1), while a marked increase in geosmin production was observed in cultures treated with norflurazon.

In order to study geosmin production in relation to pigment synthesis, the data obtained during the experimental period were expressed as the ratio of geosmin/Chl *a* and geosmin/β-carotene as shown in Fig. 2. In nitrogen limited cultures of *O. brevis* dimethazone and gabaculine treatment caused an increase of geosmin relative to Chl *a*, while norflurazon markedly enhanced this ratio (Fig. 2a). This effect was due to changes in pigment content. In comparison to the nitrogen limited situation there were only minor effects of gabaculine and norflurazon in light limited cultures (Fig. 2b) due to minor changes in pigment content. The effect of norflurazon was, in addition, an effect of increased geosmin production (Table 1).

When the use of inhibitors of the isoprenoid pathway results in differences in the partitioning of precursors in the synthesis of phytol, *casu quo* Chl *a*, and geosmin, one would also expect effects on carotenoid levels. Dimethazone and norflurazon both affected an increase in geosmin production relative to β-carotene synthesis under nitrogen limiting conditions (Fig. 2c). In light limited cultures this effect was restricted to norflurazon only (Fig. 2d).

Discussion

Dimethazone inhibits early in the pathway (Fig. 1) and should therefore affect geosmin, phytol and β-carotene at the same level. This was not shown in the nitrogen limited situation (Fig. 2a, c), where geosmin increased relative to Chl *a* and β-carotene. Apparently, geosmin production always demands a fixed minimum of precursors to be taken from the route to carotenoids.

By inhibiting the desaturation of phytoene with norflurazon the flow of precursors can be directed to either phytol or geosmin with a preference for the latter. Since norflurazon also invoked a decreased Chl *a* content of the cells, the excess tetrapyrroles may flow into the PBP pool for norflurazon treated light limited cultures (see Table 1). This explanation may also account for the similar effect observed in light limited cultures after dimethazone treatment. This is consistent with the transition from blue-green to a deep blue colour observed in these cultures. That this effect was not shown in the nitrogen limited cultures (Table 2) can be due to a restricted synthesis of the protein part of PBPs caused by an increasing nitrogen deficiency during the experimental period.

Norflurazon did not increase absolute amounts of geosmin in nitrogen limited cultures (Table 2) as was observed in light limited cultures (Table 1). The increase in geosmin production caused by norflurazon treatment might have been compensated by a decreased production of geosmin due to nitrogen deficiency. This is consistent with the observed decrease of absolute amounts of geosmin in nitrogen compared to light limited cultures (Naes and Post 1988). The marked effect of norflurazon on geosmin relative to Chl *a* and β-carotene in nitrogen versus light limited *O. brevis* was caused by turnover of these two pigments (Fig. 2).

The stimulus of geosmin production after norflurazon treatment may indicate that isoprenoid precursors are directed to geosmin production when pigment synthesis is inhibited. This hypothesis is confirmed by the findings of an increased production of geosmin relative to Chl *a* and β-carotene during nitrogen limiting conditions.

Gabaculine and norflurazon were considered as effective inhibitors of tetrapyrrole pigments and carotenoids, respectively. The finding that norflurazon also decreased the level of Chl *a* and PBPs in nitrogen limited cultures was particulary striking. Possibly, carotenoid levels had reached such low levels in nitrogen limited *O. brevis* that blocking *de novo* synthesis of carotenoids also blocks the *de novo* assembly of operational photosystems in the thylakoid membranes and hence leads to Chl *a* and PBP turnover. However, destruction of photosynthetic pigments by photodamage due to low levels of protecting pigments must also be considered as a possible explanation (van Liere and Walsby 1982).

Conclusion

Definite evidence for geosmin formation by *O. brevis* via the isoprenoid pathway, as was found for *Streptomyces* spp., can not be concluded from the data presented here, but they strongly support this hypothesis. Norflurazon was the only inhibitor effective to uncouple geosmin production from β-carotene synthesis, which indicated a strict coupling between geosmin and β-carotene biosynthesis. The increased and decreased production of geosmin with norflurazon and dimethazone treatment, respectively, fits very well within the suggested pathway for geosmin synthesis in *O. brevis*. The responses in geosmin production after norflurazon treatment indicate that isoprenoid precursors are directed to geosmin synthesis during restircted pigment synthesis. This

was further confirmed by the increased production of geosmin relative to Chl *a* and β-carotene during nitrogen limited growth. Changes in total geosmin production as responses to changes in growth limiting factors reveal geosmin production interesting from an ecological point of view. This has been dealt with in another paper where the adaptation process of geosmin production after transitions from light to nitrogen limiting growth conditions and *vice versa* have been investigated (Naes and Post 1988).

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References

Bentley R, Meganathan R (1981) Geosmin and methylisoberneol biosynthesis in *Streptomyces*. FEBS Lett 125:220–222

Bramley PM, Clarke IE, Sandmann G, Böger P (1984) Inhibition of carotene biosynthesis in cell extracts of *Phycomyces blakesleeanus*. Z Naturforsch 39c:460–463

Clarke IE, Sandmann G, Bramley PM, Böger P (1982) Carotene biosynthesis with isolated photosynthetic membranes. FEBS Lett 140:203–206

Gerber NN (1967) Geosmin, an earthy-smelling substance isolated from actinomycetes. Biotechnol Bioeng 9:321–327

Gerber NN (1968) Geosmin, from microorganisms, is trans-1,10-dimethyl-trans-9-decalol. Tetrahedron Lett 25:2971–2974

Gerber NN, Lechevalier HA (1965) Geosmin, an earthy-smelling substance isolated from actinomycetes. Appl Microbiol 13:935–938

Kikuchi T, Mimura T, Harimaya K, Yano H, Arimoto T, Masada Y, Inoue T (1973) Odorous metabolite of blue-green algae: *Schizothrix muelleri* nageli collected in the southern basin of Lake Biwa. Identification of geosmin. Chem Pharm Bull 21:2342–2343

Kunert K-J, Böger P (1979) Influence of bleaching herbicides on chlorophyll and carotenoids. Z Naturforsch 34c:1047–1051

Medsker LL, Jenkins D, Thomas JF (1968) Odorous compounds in natural waters. Environ Sci Technol 2:461–464

Naes H, Aarnes H, Utkilen HC, Nilsen S, Skulberg OM (1985) Effect of photon fluence rate and specific growth rate on geosmin production of the cyanobacterium *Oscillatoria brevis* (Kütz) Gom. Appl Environ Microbiol 49:1538–1540

Naes H, Post AF (1988) Transient states of geosmin, pigments, carbohydrates and proteins in continuous cultures of *Oscillatoria brevis* induced by changes in nitrogen supply. Arch Microbiol 150:333–337

Persson P-E (1982) Muddy odour: a problem associated with extreme eutrophication. Hydrobiologia 86:161–164

Rando RR (1977) Mechanism of the irreversible inhibition of γ-aminobutyric acid α-ketoglutaric acid transaminase by the neurotoxin gabaculine. Biochemistry 16:4604–4610

Rosen AA, Mashni CI, Safferman RS (1970) Recent developments in the chemistry of odour in water, the cause of earthy/musy odour. Water Treat Exam 19:106–114

Sandmann G, Böger P (1986) Interference of dimethazone with formation of terpenoid compounds. Z Naturforsch 41c:729–732

Van Liere L, Mur LR (1978) Light-limited cultures of the blue-green algae *Oscillatoria agardhii*. Mitt Int Verein Limnol 21:158–167

Van Liere L, Walsby AE (1982) Interactions of cyanobacteria with light. In: Carr NG, Whitton BA (eds) The biology of cyanobacteria, vol 19. Blackwell Scientific Publication, pp 34–35

Weete JD, Huang WY, Laseter JL (1979) *Streptomyces* sp.: a source of odorous substances in potable water. Water Air Soil Pollut 11:217–223

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