Metabolic pathways of δ-aminolaevulinic acid in *Rhodopseudomonas spheroides*

R. Couso[1], L. Gorriño, G. A. Locascio and H. A. Tigier[2]

Universidad de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Departamento de Química Biológica, Ciudad Universitaria, Buenos Aires (Argentina), 20 December 1976

Summary. Incorporation of δ-aminolaevulinic acid 5 ^{14}C and 4 ^{14}C into the inosine monophosphate pool and into porphyrins, was studied in cell suspensions of *R. spheroides*. The results contradict a direct incorporation of δ-aminolaevulinic acid into the purine ring of nucleotides through γδ-dioxovaleric acid. It would suggest a nonspecific incorporation after degradation of δ-aminolaevulinic acid without a transamination as a first reaction.

It is generally recognized that δ-aminolaevulinic acid (δALA) is a precursor in porphyrin biosynthesis through its conversion to prophobilinogen. Moreover, it is known that a transamination reaction involving δALA and γδ-dioxivaleric acid (DOVA) is also possible.

Nemeth et al.[3] demonstrated the incorporation of the 5 C of δALA into the purine ring through its transamination to DOVA. Braunstein et al.[4] found the 5 C of δALA inclusion into inosine monophosphate (IMP) and hypoxanthine in pidgeon liver. In addition δALA transamination was studied in different tissues[5,6]. However Neuberger and Turner[7] working with R. spheroides pointed out that the reverse reaction, namely δALA formation from DOVA, is favoured. Furthermore, according to Lascelles[8], R. spheroides grown anaerobically and in the presence of light, excreted porphyrins, mainly coproporphyrin III. Such excretion did not take place in aerobiosis and dark.

In this paper ^{14}C incorporation from δALA 5 ^{14}C and δALA 4 ^{14}C into IMP and porphyrins was studied in cell suspensions of R. spheroides.

Materials and methods. δALA 4 ^{14}C and δALA 5 ^{14}C, and glycine 2 ^{14}C were purchased from New England Nuclear. Media, growth and harvesting of cultures were performed according to Lascelles[8].

Cells were grown in anaerobiosis and light or aerobiosis and dark. The incubations were performed in aerobiosis and dark at 36–37 °C, suspending them in a mixture indicated in the different experiments. After incubation, the cell suspensions were centrifuged at 40,000 × g.

The cell pellets containing the intracellular pool of IMP were carefully washed with saline and resuspended in a carbonate-bicarbonate buffer 0.1 M, pH 10.7, with unlabelled IMP as carrier. Proteins were eliminated by heating and centrifugation. Supernatants were passed through columns of 2 ml of cationic resin AG 1 × 8. A solution of 6 mM HCl was added in order to reach pH 8.0. Finally the IMP fractions were evaporated and radioactivity was measured. The recovery of IMP from the resin was 90%; the corresponding fractions contain some guanosine monophosphate and adenosine monophosphate. The IMP fraction was hydrolyzed to hypoxanthine in 0.5 M HCl for 10 min at 100 °C. IMP and hypoxanthine were identified by thin layer chromatography using plates of cellulose MN 300 G and developed with a mixture of isopropanol:HCl:water; 65:16.5:100 (v/v).

Porphyrins were isolated from the supernatants with a new batch method using a permutit anionic resin[9]. According to this method, nonporphyrinic radioactive compounds and the excess of labelled δALA were discarded. The supernatants were mixed with 1 ml of the resin. After washing carefully with distilled water, the porphyrins were eluted and esterified with a mixture of methanol-sulphuric acid 19:1. The suspensions were left 24 h in the dark and finally porphyrins were extracted with chloroform. The amount of porphyrins were determined spectrophotometrically using the extinction coefficient for coproporphyrin III[10]. After evaporation, radioactivity was measured in a flow counter.

1 Present address: Instituto de Investigaciones Bioquímicas, Obligado 2490, 1428 Buenos Aires (Argentina).
2 Present address: Departamento de Ciencias Biológicas, Universidad Nacional de Río Cuarto 5800, Río Cuarto, Prov Córdoba (Argentina).
3 A. M. Nemeth, C. S. Russell and D. Shemin, J. biol. Chem. *229*, 415 (1957).
4 A. E. Braunstein, A. A. Poznanzkaia, R. A. Sprishkova and N.V. Gnuchev, Proc. Acad. Sci. USSR *157*, 982 (1964).
5 E. Kowalsky, A. M. Dancewicz, Z. Szot, B. Lipinsky and O. Rosiek, Acta biochim. pol. *6*, 257 (1959).
6 M. Gassman, J. Pluscec and L. Bogorad. Pl. Physiol. *43*, 1411 (1968).
7 A. Neuberger and J. M. Turner, Biochim. biophys. Acta *67*, 342 (1963).
8 J. Lascelles, Biochem. J. *62*, 78 (1956).
9 R. C. Garcia, L. C. San Martin de Viale, J. M. Tomio and M. Grinstein, Biochim. biophys. Acta *309*, 203 (1973).
10 J. M. Tomio, R. C. Garcia, L. C. San Martin de Viale and M. Grinstein, Biochim. biophys . Acta *198*, 353 (1970).

^{14}C incorporation from labelled aminolaevulinic acid into IMP fractions and porphyrins

Experiment No.	Culture	Substrate	IMP recovery (cpm)	δALA conversion into IMP (nmoles)	Porphyrins recovery (cpm)	δALA conversion into porphyrins (nmoles)
1	Aerobic	δALA 5 ^{14}C	140	111	1202	956
2	Anaerobic	δALA 5 ^{14}C	481	381	12496	9847
3	Aerobic	δALA 4 ^{14}C	250	198	1248	983
4	Anaerobic	δALA 4 ^{14}C	235	186	12598	9998

Each tube contained: sodium bicarbonate 200 μmoles; radioactive ALA 100 μmoles (spec. act. 1260 cpm/μmole), Medium up to 5 ml. Bacterial concentration 6 mg/ml (dry wt/ml). Aerobiosis and dark incubation: Time of incubation 4 h; incubation temperature 37 °C.

Results and discussion. In a first series of experiments, the incorporation of ^{14}C from glycine into IMP was investigated. In these experiments, each tube contained: sodium bicarbonate: 200 µmoles, sodium formiate: 120 µmoles, glycine 2 ^{14}C: 120 µmoles (spec. act.: 2000 cpm/µmole). Furthermore all acidic fractions containing IMP, co-chromatographied with unlabelled IMP. In order to ascertain that radioactivity was located in the purine ring, each labelled spot was eluted from the plate and hydrolyzed. Then the hydrolysate was run under identical conditions and all radioactivity was found at the same position as unlabelled hypoxanthine. According to our results, cell suspensions of *R. spheroides* (1.8 mg dry wt/ml) incubated in aerobiosis and dark were able to synthesize labelled IMP using glycine 2 ^{14}C as substrate. 2% of the radioactivity was incorporated within 4 h (data not shown) instead of only 1% in Schulman's experiments[11] working with animal tissues.

In order to study the biosynthesis of the purine ring through δALA transamination to DOVA, similar experiments were performed using δALA 5 ^{14}C and δALA 4 ^{14}C as substrates. The table shows the comparative incorporation of ^{14}C from δALA 5 ^{14}C and δALA 4 ^{14}C into IMP fractions and porphyrins.

When δALA 5 ^{14}C was the substrate, anaerobic and light-grown bacteria incorporated more radioactivity into porphyrins (10%) than cells grown aerobically in the dark (1%). However, the ^{14}C incorporation into IMP was low in both conditions, in spite of the fact that cells grown aerobically and in the dark could favour this incorporation due to their low rate of porphyrin biosynthesis. Also with 4 ^{14}C, δALA incorporation of radioactivity into IMP is low in both conditions of growth.

These results are not in agreement with a direct incorporation of δALA into the purine ring of nucleotides, through its conversion to DOVA. According to this pathway, at least more than 2% incorporation into IMP would be expected, instead of 0.1–0.3% obtained when using δALA 5 ^{14}C as substrate, an intermediate closer to the end product than glycine.

Our results suggest a nonspecific incorporation of ^{14}C from δALA into IMP, after δALA degradation; without a direct transamination. In addition, our data support the results of Neuberger and Turner[7] and Lohr and Friedman[12] that the transamination works in the reverse sense from DOVA to δALA.

11 M. P. Schulman, J. C. Sonne and J. M. Buchanan, J. biol. Chem. *196*, 499 (1952).

12 J. B. Lohr and H. AC. Friedmann, Biochem. biophys. Res. Commun. *69*, 908 (1976).

Electrophoretic variation in esterases in 3 wild-type and respective mutant strains of *Aspergillus flavus*[1]

H. M. L. de Souza, I. R. Baracho, C. L. Messias and J. L. de Azevedo

Department of Genetics and Evolution, Institute of Biology, University of Campinas, 13100 Campinas, São Paulo (Brazil), 28 March 1977

Summary. Wild-type strains and auxotrophic mutants of *Aspergillus flavus*, differing regarding aflatoxin production, were tested for esterases isozymes. Esterases variation was found in all strains used, and a possible correlation between the pattern of esterase bands and aflatoxin production is suggested.

The present work was designed to obtain preliminary information about the extent and the nature of electrophoretic variation of esterases isozymes within 3 wild-type isolates of the filamentous fungus *Aspergillus flavus*. Mutants derived from such strains were also tested for esterases isozymes in an attempt to study the influence of such mutants in relation to esterase production.

Material and methods. 3 wild-type strains (A5a, A6e and B2d) were used. The strain A5a did not produce aflatoxin, either in culture medium or mycelia. A6e has a high production of aflatoxin in mycelia (400 ppm of Bl-aflatoxin and 300 ppm of Gl-aflatoxin) but a low production in culture medium. B2d produces a high amount of aflatoxin both in culture medium (40 ppm of Bl and 30 ppm of Gl) and in mycelia (400 ppm of Bl and 300 ppm of Gl). Mutants strains A5a arg, A5a w, A6e arg, A6e pur, A6e y and B2d lys were also used. Mutant alleles were designated as follows: w and y, white and yellow conidia respectively; arg, lys and pur, requirement respectively for arginine, lysine and purines. The isolation of mutants and the aflatoxin determinations were reported elsewhere[2]. Stock cultures were mantained on complete medium slants[3]. Cultures were transferred to liquid minimal medium and, after 7 days incubation at 28 °C, they were used for electrophoretic analysis both in mycelia and culture medium. The electrophoretic technique was as follows: mycelia were homogeneized in 0.05 ml of distilled water at 4 °C. The homogenate and the

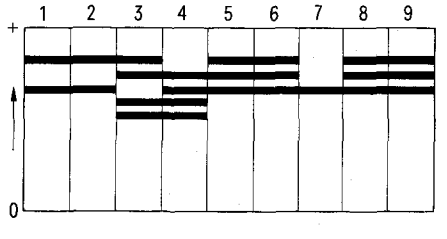

Fig. 1. Schematic representation of esterases zymograms from mycelia of wild-type strains: 2(A5a), 3(B2d), 8(A6e) and of mutants strains: 1(A6e y), 4(A5a arg), 5(A6e arg), 6(A5a w), 7(B2d lys) and 9(A6e pur).

1 Acknowledgment. The authors are thankful to the National Council for the development of Science and Technology (CNPq) and Assistance to Research for São Paulo State (FAPESP) for financial assistance.

2 C. L. Messias, A. Martinelli Fº, H. Fonseca and J. L. Azevedo, Relat. cient., ESALQ–USP, p. 100 (1974).

3 G. Pontecorvo, J. A. Roper, L. M. Hemmons, K. D. MacDonald and A. W. J. Bufton, Adv. Genet. *5*, 141 (1953).