BIOSYNTHETIC EVIDENCE FOR A NICKEL TETRAPYRROLE STRUCTURE OF FACTOR F_{430} FROM *METHANOBACTERIUM THERMOAUTOTROPHICUM*

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1. Introduction

Methanogenic bacteria have been shown to require nickel for growth [1]. From these organisms a yellow nickel containing compound can be isolated [2–6], that has been designated factor F_{430} [7]. Its mass/mol nickel was determined to be 1500 and ϵ_{430} to be near 23 000 cm^{-1} . l . $(mol\ Ni)^{-1}$ [5,6]. The structure and function of this compound is unknown. Incorporation studies with [^{14}C]succinate as labelled precursor indicate that factor F_{430} may be a tetrapyrrole compound. 8 mol succinate/mol nickel were found to be incorporated into the factor, which is the amount predicted for a tetrapyrrole [4].

Tetrapyrrole biosynthesis proceeds via δ-aminolevulinic acid (δ-ALA) and porphobilinogen as intermediates. Here we show with *Methanobacterium thermoautotrophicum* that factor F_{430} becomes labelled when the organism is grown in the presence of δ-[4-^{14}C]ALA. Dependent on the δ-ALA concentration in the growth medium up to 8 mol δ-ALA/mol nickel were incorporated. Only tetrapyrroles are known to be synthesized from 8 mol δ-ALA; therefore the incorporation data are taken as evidence that factor F_{430} has a nickel tetrapyrrole structure.

2. Materials and methods

δ-[4-^{14}C]Aminolevulinic acid, 0.05 mCi (0.16 mg)/0.5 ml 0.1 M HCl) was from NEN (Boston, MA). QAE–Sephadex A-25 was from Pharmacia Fine Chemicals (Uppsala), Bio-Gel P-6 (100–200 mesh) from Bio-Rad (München). *Methanobacterium thermoautotrophicum* was strain Marburg [8]. The cells were grown on H_2 and CO_2 as sole carbon and energy sources [9]. Where indicated the medium was supplemented with δ-[4-^{14}C]ALA.

2.1. *Isolation of factor* F_{430}

Cells of *M. thermoautotrophicum* were suspended in H_2O (5 ml/g wet cells). After addition of 0.5 mg deoxyribonuclease I the suspension was passed through a French pressure cell at 20 000 lb/in^2 (137 900 kPa) twice. Then a 0.5 M $HClO_4$ solution was added slowly to the cell extract under continuous stirring at 0°C. When pH 2.0 was reached, the suspension was stirred for another 45 min at 0°C. After centrifugation for 30 min at 27 000 × *g* the factor F_{430}-containing supernatant was adjusted to pH 7.0 with 1 M $KHCO_3$. The supernatant was diluted with the 2-fold volume of H_2O and subsequently applied to a QAE–Sephadex A-25 column (0.5 g QAE–Sephadex/g wet cells) pre-equilibrated with 50 mM glycine/KOH buffer (pH 9.5). Factor F_{430} was eluted with the same buffer containing 0.3 M NaCl [3]. The fractions containing factor F_{430} were pooled and diluted 5-fold to decrease the ionic strength. They were then applied to a QAE–Sephadex A-25 column (0.5 g QAE–Sephadex/g wet wells) pre-equilibrated with 50 mM Tris–HCl buffer (pH 7.5). The column was first washed with 1 mM HCl (10 ml/g wet cells) and factor F_{430} was then eluted with 2.5 mM HCl. The fractions containing factor F_{430} were pooled, diluted 2.5-fold and applied to a QAE–Sephadex A-25 column (0.5 g QAE–Sephadex/g wet cells) pre-equilibrated with 1 mM HCl. The column was washed with 10 ml 1 mM HCl/g wet cells and factor F_{430} was eluted with 3 mM HCl. The factor F_{430} containing fractions were subsequently pooled and

The term tetrapyrrole is used here also for compounds, the pyrrole ring(s) of which are reduced to the pyrroline or pyrrolidine level

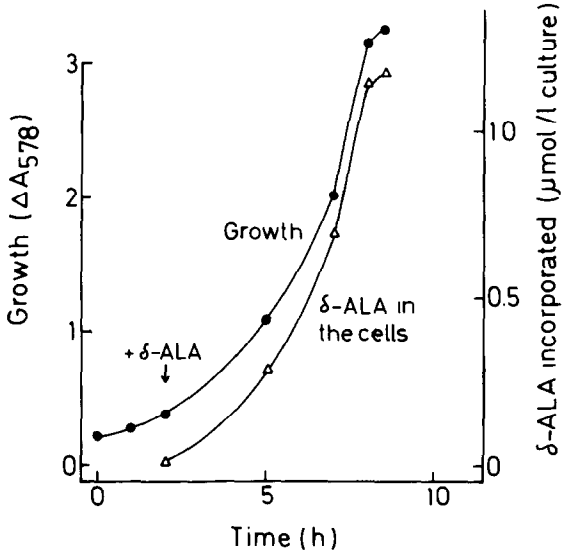

Fig.1. Incorporation of δ-ALA by *M. thermoautotrophicum* growing in the presence of δ-[4-^{14}C]ALA (50 μM; 8.8 × 10^6 dpm/μmol). ΔA_{578} = 1 corresponded to 0.4 g cells (dry wt)/liter.

lyophilized. The freeze-dried material was dissolved in 1 mM HCl (0.2–1 ml/g wet cells) and 1 ml yellow solution was loaded onto a Bio-Gel P-6 column (diam. 1.2 cm; length 50 cm) which was equilibrated with 1 mM HCl. The factor was eluted with 1 mM HCl.

2.2. *The nickel content of factor(s) F_{430} solutions*

This was calculated from the ΔA measured at 430 nm using an ϵ_{430} of 23 000 cm^{-1} . 1 . $(mol\ Ni)^{-1}$ [2–6].

3. Results

Cells of *M. thermoautotrophicum* growing in a mineral salts medium supplemented with δ-[4-^{14}C]-ALA were found to incorporate δ-ALA; incorporation paralleled growth (fig.1). Between 1.5–3% of the δ-ALA added to the medium was taken up by the cells independent of whether the δ-ALA concentration in the medium was low (50 μM) or high (5 mM).

Factor F_{430} isolated from cells grown in the presence of δ-[4-^{14}C]ALA was found to be radioactive. The factor eluted from the Bio-Gel P-6 column with a constant absorbance % radioactivity ratio (fig.2A) indicating that δ-ALA was incorporated into factor F_{430}. It has been shown that 3 nickel containing

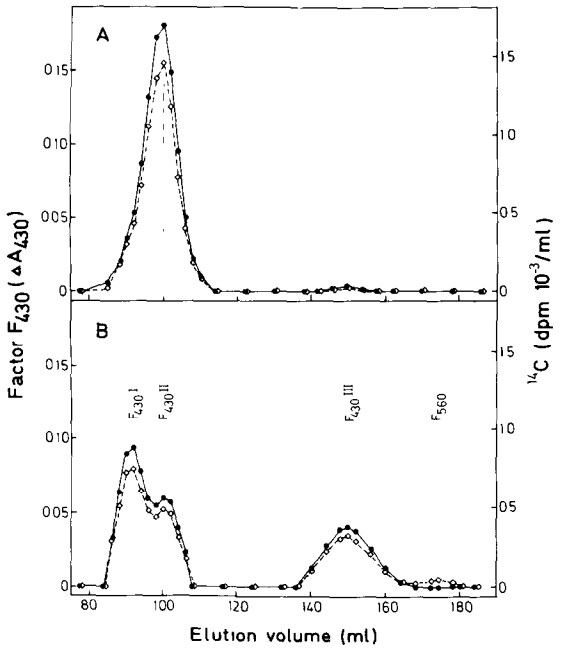

Fig.2. ^{14}C (◇) and factor(s) F_{430} (F_{560}) (●) elution profiles from Bio-Gel P-6 column (1.2 × 50 cm). The factor(s) were eluted with 1 mM HCl. 2 ml fractions were collected. δ-ALA in the growth medium was 2 mM, the spec. radioact. 22 × 10^3 dpm/μmol). (A) Factor F_{430}; (B) nickel containing degradation products of factor F_{430}.

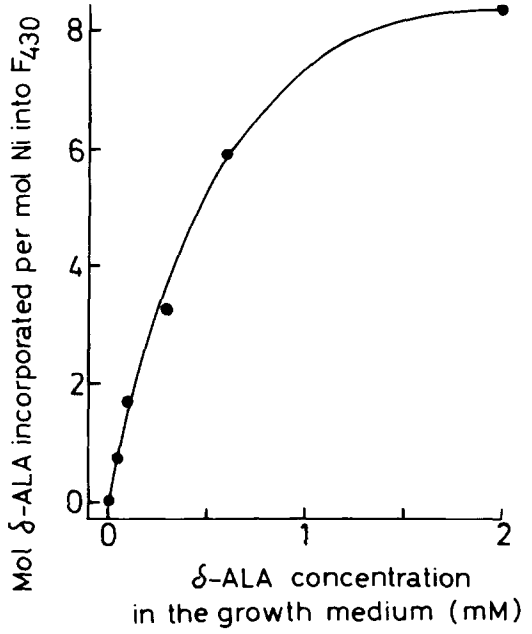

Fig.3. mol δ-ALA incorporated/mol nickel into factor F_{430} of *M. thermoautotrophicum* in relation to the [δ-ALA] in the growth medium.

degradation products of factor F_{430} are formed when the cells are extracted with H_2O at 100°C rather than with $HClO_4$ at 0°C [5]. The 3 degradation products (factors F_{430} I, F_{430} III, and F_{560}) contained the same amount of radioactivity/mol nickel as factor F_{430} (F_{430} II) (fig.2B). The amount of δ-ALA incorporated/mol nickel into factor F_{430} increased with increasing concentrations of δ-ALA in the growth medium (fig.3). At 2 mM δ-ALA and 5 mM SALA, respectively, the specific radioactivity of factor F_{430} (dpm/μmol Ni) was 8 times as high as that of δ-ALA. This indicates that factor F_{430} incorporated 8 mol δ-ALA/mol nickel under these conditions. The same stoichiometry was found for the 3 nickel containing degradation products of factor F_{430}.

The specific radioactivity of factor(s) F_{430} (dpm/μmol Ni) was calculated using an ϵ_{430} of 23 000 $cm^{-1} . l . (mol\ Ni)^{-1}$. Dependent on the method used, ϵ_{430} was determined to be 21 500–24 500 $cm^{-1} . l . (mol\ Ni)^{-1}$ [2–6]. Thus the mol δ-ALA incorporated/mol nickel into factor(s) F_{430} are known only within an accuracy range of ±7%.

4. Discussion

δ-ALA is a specific intermediate in the synthesis of tetrapyrroles. 8 mol δ-ALA are required for the formation of 1 tetrapyrrole. Depending on the δ-ALA concentration in the medium, factor F_{430} incorporated up to 8 mol δ-ALA per mol nickel. Therefore, factor F_{430} has a tetrapyrrole structure.

Nickel has been shown to be an essential component of urease [10,11] in plants and of carbon monoxide dehydrogenase in Clostridia [12,13]. In urease, nickel is bound to protein via functional groups of amino acids [10]. In carbon monoxide dehydrogenase, nickel is a component of a low molecular weight factor the structure of which has not yet been elucidated.

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