INCORPORATION OF METHIONINE-DERIVED METHYL GROUPS INTO FACTOR F_{430} BY *METHANOBACTERIUM THERMOAUTOTROPHICUM*

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1. Introduction

Factor F_{430} is a low M_r yellow compound with an absorption maximum at 430 nm present in all methanogenic bacteria [1,2]. It is probably the prosthetic group of methyl coenzyme M reductase [3], which catalyzes the reduction of methyl CoM to methane. In [4–6] factor F_{430} was found to contain nickel, which explained why methanogenic bacteria are dependent on this transition element for growth [7]. The mass/mol nickel was determined to be 1500 and ϵ_{430} to be near 23 000 $cm^{-1} \cdot l \cdot (mol\ Ni)^{-1}$ [4–6]. Labelling studies with [^{14}C]succinate [8] and δ-[^{14}C]-aminolevulinic acid (δ-ALA) [9] indicate that factor F_{430} has a nickel tetrapyrrole structure; 8 mol δ-ALA/mol Ni are incorporated into the factor. The arrangement of the tetrapyrrole is probably macrocyclic (as in the porphyrins) rather than linear (as in the bile pigments) because nickel does not dissociate from the factor, neither in strong acids (6 N HCl) nor under alkaline conditions (pH 13) [8]. The detailed structure of factor F_{430} is not known.

Chlorophylls, hemes, sirohemes and vitamin B_{12} are the macrocyclic tetrapyrroles of biological importance known to date. The 4 tetrapyrroles are either derived from protoporphyrin IX (chlorophylls, hemes and cytochromes) or from sirohydrochlorin (siroheme and vitamin B_{12}) (fig.1). Both protoporphyrin IX and sirohydrochlorin are synthesized from uroporphyrinogen III; the former by 6 consecutive decarboxylations, including oxidative decarboxylations; the latter by 2 reductive methylations with *S*-adenosyl methionine. Uroporphyrinogen III is formed from 8 δ-ALA.

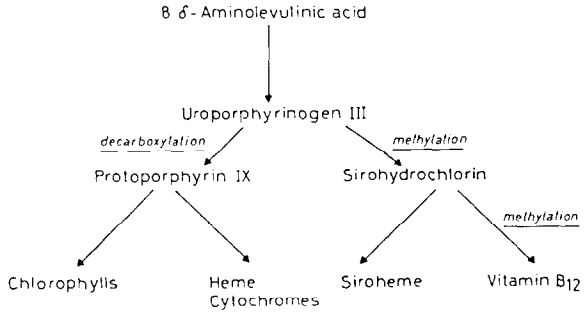

Fig.1. Biosynthesis of macrocyclic tetrapyrroles from δ-aminolevulinic acid [10].

Here we show that factor F_{430} contains 2 methyl groups/mol nickel, introduced from methionine. This finding suggests that the nickel tetrapyrrole could be derived from sirohydrochlorin.

2. Materials and methods

L-[*methyl*-^{14}C]Methionine and L-[*methyl*-^{3}H]-methionine were from Amersham Buchler (Braunschweig). *Methanobacterium thermoautotrophicum* was strain Marburg [11]. The cells were grown on H_2 and CO_2 as sole carbon and energy sources [12]. Where indicated, the medium was supplemented with methionine or δ-ALA. Factor F_{430} was isolated from the cells by the procedure in [9]. The concentration of factor F_{430} solutions (mol Ni/l) was calculated from the ΔA at 430 nm using an ϵ_{430} of 23 000 $cm^{-1} \cdot l \cdot (mol\ Ni)^{-1}$ [4–6].

The specific radioactivity of the methyl group of methionine was determined after cleavage with cyanogen bromide [13]. Methyl thiocyanate is formed from

This work is dedicated to Professor Dr Helmut Holzer, Albert-Ludwigs-Universität Freiburg, in honor of his 60th birthday

the methyl group with this reagent. About 2 μmol methionine or 25 mg protein were incubated in sealed tubes for 24 h at 30°C in 1.5 ml 0.1 M HCl containing 100 μmol BrCN. The methyl thiocyanate was extracted with 1 ml diethyl ether and quantitated in a gas chromatograph (Varian Model 2700) equipped with a flame ionization detecter. A stainless steel column (diam. 0.4 cm, length 2 m) filled with chromosorb W (80–100 mesh) + 5% SP 1000 was used to separate the methyl thiocyanate from BrCN and ether. Aliquots of the ether solution were added to 5 ml Aqualuma® (Baker Chemicals, Deventer) and assayed for radioactivity in a Beckman LS 7500 liquid scintillation counter. Protein was hydrolyzed and alanine, aspartate and glutamate were isolated and their specific radioactivities determined as in [11].

3. Results

Labelling experiments with L-[*methyl*-^{14}C]methionine, and L-[*methyl*-^{3}H]methionine were performed.

3.1. *L-[*methyl-^{14}*C]Methionine*

Methanobacterium thermoautotrophicum was grown on H_2 plus CO_2 in a mineral salts medium supplemented with L-[*methyl*-^{14}C]methionine (1–10 mM). Methionine was found to be incorporated by the growing cells; incorporation paralleled growth up to the stationary phase. The amount of methionine assimilated (μmol/g dried cells) increased when the methionine in the medium was increased up to 5 mM.

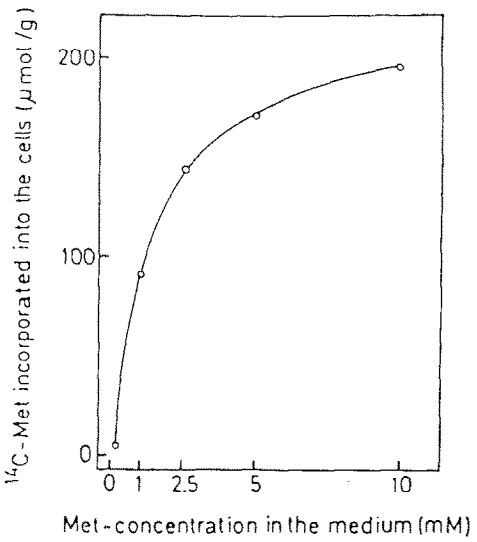

Fig.2. L-[*methyl*-^{14}C]Methionine incorporation into *M. thermoautotrophicum* cells as a function of the methionine concentration in the growth medium.

At >5 mM, a constant value of 200 μmol/g was reached (fig.2). Analyses of the cell composition of *M. thermoautotrophicum* indicate that this is about the amount required for the synthesis of 1 g cells. The methyl-group of methionine was not reduced to methane.

The specific radioactivity of the methyl-group of methionine in the cell protein (intracellular methionine) was determined and compared with that of methionine in the medium (extracellular methionine) (table 1). It was found that at low concentrations of

Table 1
Incorporation of ^{14}C and ^{3}H into factor F_{430} during growth of *M. thermoautotrophicum* in the presence of L-[*methyl*-^{14}C]-methionine or L-[*methyl*-^{3}H]methionine, respectively

Methionine (mM) in the medium	Methyl group labelled with	Specific radioactivity of the methyl group of methionine (dpm/μmol)		Specific radioactivity of factor F_{430} (dpm/μmol Ni)	Methyl groups incorporated into factor F_{430} (mol/mol Ni)
		Extracellular	Intracellular		
1	^{14}C	42 500	19 000	36 600	1.92
2.5	^{14}C	19 600	14 600	32 200	2.2
5	^{14}C	9100	7000	16 600	2.37
10	^{14}C	18 800	18 200	37 900	2.08
10	^{3}H	94 000	84 400	176 400	2.09

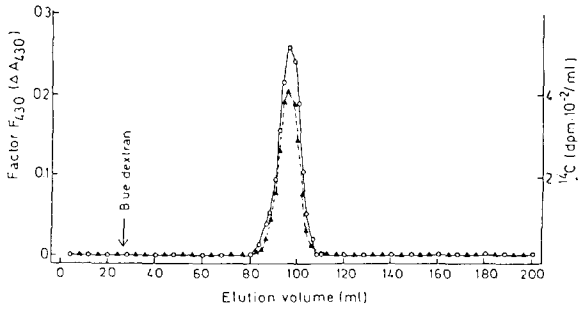

Fig.3. ^{14}C (▲) factor F_{430} (○) clution profiles from Bio-Gel P6 column (1.2 × 50 cm). The factor was eluted with 1 mM HCl; 2 ml fractions were collected. L-[*methyl*-^{14}C]Methionine in the growth medium was 10 mM, the specific radioactivity 18 800 dpm/μmol.

methionine in the medium the specific radioactivity of the methyl group of intracellular methionine was considerably lower than that of extracellular methionine. At high methionine concentrations (>5 mM), however, the specific radioactivities became almost identical. This indicates that under these conditions the endogenous synthesis of methionine was suppressed.

Factor F_{430}, which was isolated from the cells grown in the presence of L-[*methyl*-^{14}C]methionine, was radioactive. Fig.3 shows an elution profile from a Bio-Gel P6 column, which has proven useful in the separation of factor F_{430} from other compounds [8,9]. The factor eluted with a constant absorbance to radioactivity ratio. The specific radioactivity of the factor was found to be twice that of the methyl group of intracellular methionine at all methionine concentrations in the medium tested (table 1). Factor F_{430}, thus must contain two carbon atoms derived from the methyl group of methionine.

Alanine, aspartate and glutamate in the cell protein were not labelled by L-[*methyl*-^{14}C]methionine. An incorporation of the methyl group of methionine into factor F_{430} via pyruvate, oxaloacetate or α-ketoglutarate can therefore be excluded.

3.2. *L-[*methyl-^{3}H*]Methionine*

When *M. thermoautotrophicum* was grown in the presence of L-[*methyl*-^{3}H]methionine (10 mM), the specific radioactivity of isolated factor F_{430} was twice as high as that of the methyl group of intracellular methionine (table 1). This finding indicates that two methyl groups are introduced intact from methionine into factor F_{430}, i.e., without the loss of H^+ from the methyl group.

4. Discussion

Methanogenic bacteria have been shown to contain corrinoids [14,15], *Methanosarcina* species also contain cytochromes [16]. This indicates that these organisms synthesize uroporphyrinogen III from δ-ALA. It is therefore reasonable to assume that factor F_{430} is derived from uroporphyrinogen III as are the other tetrapyrroles of biological importance.

From the presence of corrinoids, it further can be deduced that methanogens can synthesize sirohydrochlorin, which contains 2 methionine-derived methyl groups. The finding that factor F_{430} contains 2 methyl groups introduced from methionine suggests that the nickel tetrapyrrole could be derived from sirohydrochlorin as are siroheme and vitamin B_{12} (fig.1). Labelling studies with L-[*methyl*-^{13}C]methionine and δ-[2-^{13}C]ALA are under way to prove or disprove this hypothesis.

Acknowledgements

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