METABOLISM OF MACROMOLECULAR METHYL GROUPS IN *ESCHERICHIA COLI*

Whole cell NMR spectroscopy

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1. Introduction

Carbon-13 NMR spectra of ^{13}C-methyl-enriched, intact and viable *E. coli* cells have been obtained and shown to be useful in studies of the in vivo metabolism of this molecular group. Methyl resonances from whole cell spectra were shown to originate from non-dialyzable, macromolecular material present in subcellular fractions and not due to free cellular [*methyl*-^{13}C]methionine or other dialyzable compounds. Comparisons with the methyl resonances of several cellular proteins, membrane constituents, and other compounds have been made. ^{13}C-Enriched methyl groups were 'chased' from their original sites in late log phase cultures when cells were placed in minimal medium with [*methyl*-^{12}C]methionine. Methyl, originally assigned to locations in protein, as bound to the sulfur of methionine, was 'chased' to an exclusively membrane site bound to nitrogen in the quaternary amine of phosphatidyl choline.

Specific enrichment of carbon-13 is essential to carbon NMR studies of transient metabolites which may exist in the metabolic processes for only short periods of time and small molecules or macromolecules that exist at very low concentrations in the cell [1,2]. Thus, cell growth in media containing a macromolecular precursor or a metabolite that is 90+ atom % enriched in carbon-13 at a particular carbon is highly advantageous for studies in vivo. The label is restricted to a single site in the molecule and is extensive compared to the 1.1 atom % natural abundance of carbon-13. The present investigation represents the first study of the metabolism of a specific macromolecular moiety, methyl groups, by NMR spectroscopy of whole cells.

2. Materials and methods

A relaxed strain of *Escherichia coli C6 cys met* was grown as in [3] in minimal medium that had been supplemented with methionine enriched in ^{13}C in the methyl carbon (Merck, Canada). Enrichment was determined to be 90+ atom % ^{13}C and only in the methyl carbon [4]. The amount of methionine in the medium (0.10 mM) was not growth limiting for the auxotroph [3]. Isotopically enriched cells were harvested at the end of log-phase growth, frozen quickly with dry ice, and stored frozen until used for NMR spectroscopy. Cells were resuspended in culture medium (1.5 ml) containing unlabeled methionine for 'chase' experiments. Cell membrane and cytoplasmic fractions were prepared by grinding frozen cells (3 g, packed wet wt) with activated alumina (6 g) at 4°C for 15 min, suspending the material in 9 ml phosphate buffered saline and centrifuging at 1000 × *g* for 5 min to remove the alumina. The resulting supernatant material was centrifuged at 30 000 × *g* for 25 min to separate the membrane fraction (pellet) from cytoplasmic materials (supernatant fluid) which still contained large particles such as whole ribosomes. Membrane pellets were resuspended in 1.5 ml buffer.

Proton-decoupled CMR Fourier transform spectra were obtained at 25.2 MHz with a Varian XL-100-15

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spectrometer equipped with a Nicolet 1180 computer and at 22.63 MHz with a Bruker HFX-90 spectrometer equipped with a Nicolet 1080 computer. Measurements of NMR spectra of whole cell suspensions were accomplished with either a 10 or 12 mm (outer diameter) NMR sample tube kept at 25°C when acquiring spectra. The ^{2}H resonance of D_2O (10%) in the sample or ^{19}F resonance of a fluorine capillary insert were used for field-frequency stabilization of the Varian and Bruker instruments, respectively. Dioxane within the samples were used as a reference (67.4 ppm downfield from TMS). Natural abundance ^{13}C resonances of metabolites were not evident under the instrumental conditions used including the relatively short times for acquisition of spectra.

3. Results and discussion

A proton-decoupled NMR spectrum of whole *E. coli* cells is shown in fig.1. Virtually every resonance signal acquired was located in the methyl region of the NMR spectrum, 0–50 ppm. These signals must, therefore, have originated from the specifically enriched ^{13}C-methyl carbons. Natural abundance ^{13}C-signals were not evident in the spectrum with the possible exception of a resonance (signal 10) located downfield from dioxane (67.4 ppm) at 70.3 ppm. Carbon signals in this region of the spectrum are assigned to the structure >CHOH [5] occurring prevalently in all carbohydrates in cells.

Methyl carbon resonances of proteins and nucleic

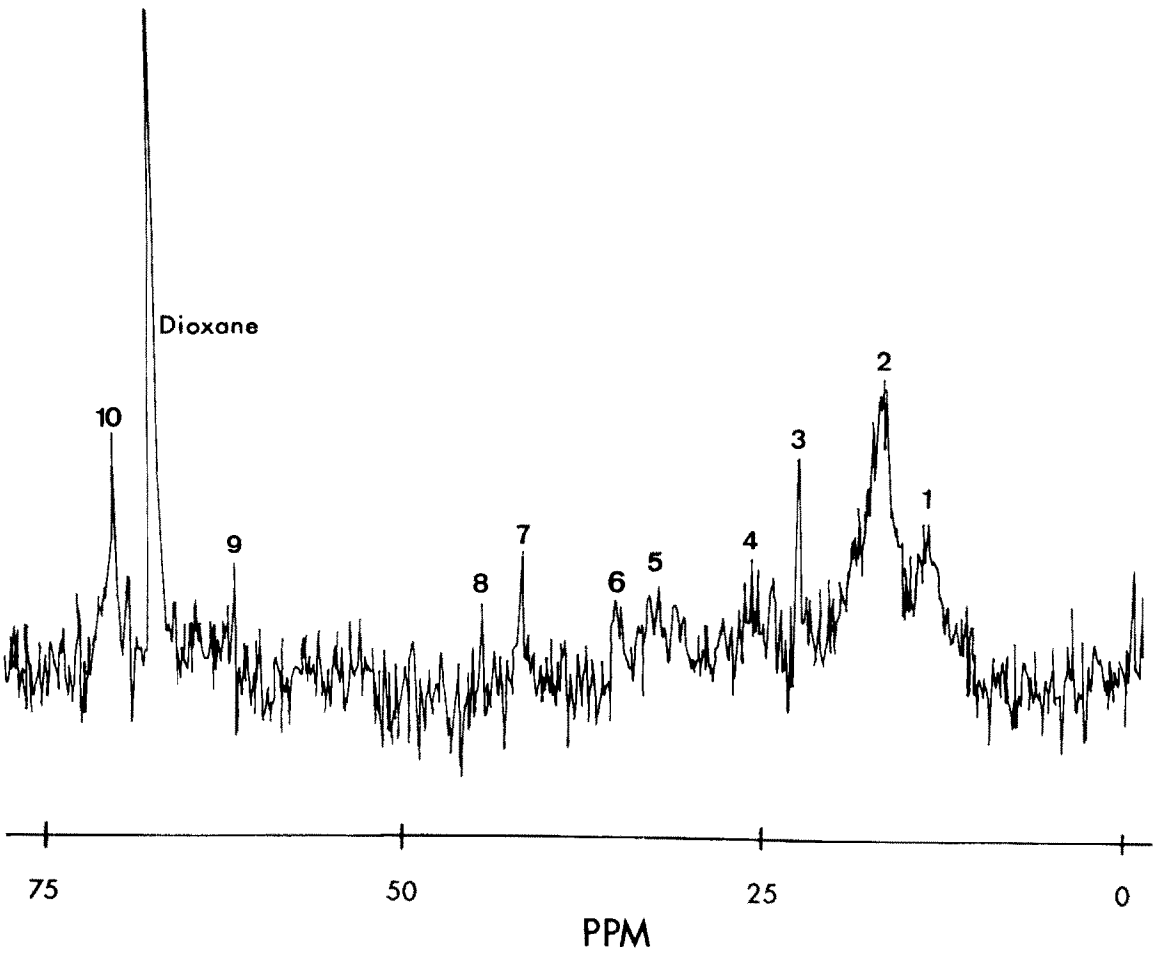

Fig.1. Proton-decoupled, Fourier transform NMR spectrum of ^{13}C-methyl-enriched, intact *E. coli* cells. The spectrum of intact *E. coli* cells (10 g packed wet wt; in 1.5 ml phosphate-buffered saline) was recorded at 25.2 MHz, with ~150 000 scans accumulated.

acids as well as those of small molecules occur in the high field region of the spectrum, 0–50 ppm [4,1–6]. Signals such as 1, 2 and 3 between 0 and 25 ppm are associated with methyl carbons bonded to other carbons or to sulfur, whereas signals 4–8 between 25 and 50 ppm are assigned to methyl carbons bonded to nitrogens, and signal 9 at 60 ppm, methyl bound to oxygen [5]. Location of the methyl groups in macromolecules was confirmed by NMR investigation of subcellular fractions.

Cytoplasmic and membrane subcellular fractions were isolated from ^{13}C-enriched *E. coli*. The spectra illustrated at the top of fig.2 are those of the cytoplasm (——) and membrane (– – –) fractions. These spectra have better signal-to-noise ratios than that of the spectrum of the corresponding whole cells. Again, most of the resonance signals appeared in the methyl region of the spectrum. The highly prominent and broad signal between 16 and 20 ppm was common to both the membrane and cytoplasm fractions (resonance 2) and was also seen in the whole cell spectrum (signal 2 of fig.1). A spectrum of dialyzed cytoplasm yielded resonances at the same locations and of the same relative magnitudes as non-dialyzed material. Therefore, we conclude that the methyl resonances of the whole cell sample originated from non-dialyzable macromolecular methyl groups of the cytoplasm as well as similar methyl groups of the particulate membrane fraction. Protein methionine residues in the cytoplasm and membrane most likely contribute these resonances to the spectrum. Some resonances were not found to be common to both the spectrum from the cytoplasm and that of the membrane fraction. Signals 4,5 and 11 were rather well-resolved in the spectrum of the cytoplasm but were apparently absent from that of the membrane fraction (fig.2). In contrast, signals 7 and 10 were easily discerned in the membrane fraction spectrum but were not present in the spectrum from the cytoplasm.

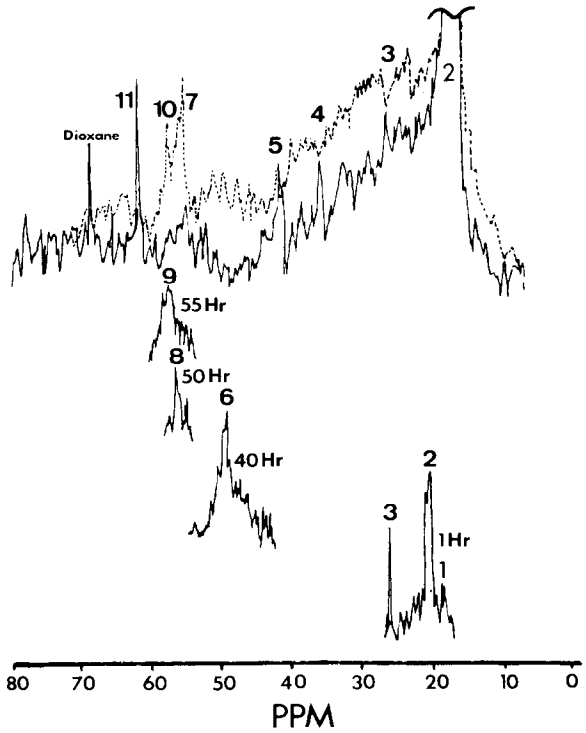

Fig.2. Metabolism of methyl groups by NMR spectroscopy. The upper portion of the figure shows proton-decoupled, Fourier-transformed NMR spectra of cytoplasmic (——) and membrane (– – –) fractions isolated from ^{13}C-methyl-enriched *E. coli* cells. The lower portion of the figure shows metabolism of macromolecular methyl groups. ^{13}C-Methyl groups in intact *E. coli* were 'chased' by natural turn-over during incubation at 37°C with [*methyl*-^{12}C]methionine. Periodic spectral acquisitions were obtained at 1, 40, 50 and 55 h after incubation of cells at 37°C. Each spectrum represents the predominant signal acquired from signal averaging of ~1800 scans.

Metabolism of the macromolecular methyl groups could be analyzed by NMR spectroscopy of whole *E. coli* cells. Late logphase cells that had given the spectrum seen in fig.1 were incubated in medium containing [*methyl*-^{12}C]methionine. The ^{13}C-enriched methyl groups were to be effectively 'chased' from their present sites by natural turnover of the macromolecules during incubation of the culture. Periodic acquisition of whole cell spectra gave results shown in fig.2. Resonance signals 1, 2 and 3 in the lower part of fig.2 were the most prominent signals present after 1 h incubation at 37°C and corresponded to signals 1, 2 and 3 of fig.1. However, these resonances were extinct after several hours of 'chase' and predominant resonance signals appeared downfield from the original resonances. As shown in fig.2, after a 40 h incubation the most prominent whole cell resonance (signal 6) was located at 49 ppm; after 50 h, at 55 ppm (signal 8); and after 55 h, at 57 ppm (signal 9). Each of the spectra acquired during the 'chase' experiment took ~30 min, and therefore represented averaged signals from carbons that may have been undergoing some biochemical change during the acquisition. However, the 30 min duration for the NMR measurements was considered insignificant when compared to the 55 h length of the entire experiment.

Incubation of the cells for times >55 h did not change the location of signal 9 in fig.2. The chemical shifts of signals 8 and 9 coincided approximately with resonances 7 and 10 of the cellular membrane fraction. These findings indicated that the dominant methyl resonance from the in vivo 'chase' experiment moved downfield and may finally be attributed to compounds residing in the cellular membrane fraction. Major components of *E. coli* cellular membrane include phosphatidyl ethanolamine, which becomes methylated through reaction with *S*-adenosyl-L-methionine to produce phosphatidyl choline [12,13]. The whole cell resonance numbered 9 at 57 ppm after 55 h incubation and the corresponding signal 10 of the membrane fraction do indeed coincide with the methyl resonance of phosphatidyl choline. $RN^{+}(CH_3)_3$, where R is phosphatidyl ethanolamine, has been reported to have a signal at 57 ppm [14–17]. The up-field shoulder of resonance 9 corresponds in chemical shift to membrane signal 7 and is also suspected to emanate from the methyls of quaternary nitrogen in the membrane. Therefore, we conclude that the formation of this methylated membrane component could be one of the end products of methyl group metabolism.

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References

[1] Ugurbil, K., Brown, T. R., Den Hollander, J. A., Glynn, P. and Shulman, R. G. (1978) Proc. Natl. Acad. Sci. USA 75, 3742–3746.
[2] Tompson, J. G., Hayashi, F., Paukstelis, J. V., Loeppky, R. N. and Agris, P. F. (1979) Biochemistry 18, 2079–2085.
[3] Tompson, J. C. and Agris, P. F. (1979) Nucleic Acids Res. 7, 765–779.
[4] Agris, P. F., Fujiwara, F. G., Schmidt, C. F. and Loeppky, R. N. (1975) Nucleic Acids Res. 2, 1503–1512.
[5] Stothers, J. B. (1972) Carbon-13 NMR Spectroscopy, Academic Press, New York.
[6] Glushko, V., Lawson, P. J. and Gurd, F. R. N. (1972) J. Biol. Chem. 247, 3176–3185.
[7] Allerhand, A., Childers, R. F. and Oldfield, E. (1973) Biochemistry 12, 1335–1341.
[8] Keim, P., Vigna, R. A., Nigen, A. M., Morrow, J. S. and Gurd, F. R. N. (1974)
[9] Blakley, R. L., Cocco, L., London, R. E., Walker, T. E. and Matwiyoff, N. A. (1978) Biochemistry 17, 2284–2293.
[10] Fujiwara, F. G., Tompson, J., Loeppky, R. N. and Agris, P. F. (1978) in: Biomolecular Structure and Function (Agris, P. F. ed) pp. 527–533, Academic Press, New York.
[11] Richarz, R. and Wüthrich, K. (1978) Biochemistry 17, 2263–2269.
[12] Kaneshiro, T. and Marr, A. G. (1962) J. Lipid Res. 3, 184–189.
[13] Kaneshiro, T. and Law, J. H. (1964) J. Biol. Chem. 239, 1705–1713.
[14] Behr, J.-P. and Lehn, J.-M. (1972) Biochem. Biophys. Res. Commun. 49, 1573–1579.
[15] Levine, Y. K., Birdsall, N. J. M., Lee, A. G. and Metcalfe, J. C. (1972) Biochemistry 11, 1416–1421.
[16] London, R. E., Hildebrand, C. E., Olson, E. S. and Matwiyoff, N. A. (1976) Biochemistry 15, 5480–5486.
[17] Deber, C. M., Moscarello, M. A. and Wood, D. D. (1978) Biochemistry 17, 898–903.