Thymidine Metabolism in *Mycoplasma hominis*

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Production of extracellular deoxyribonuclease (Randall, Gafford, Gentry & Lawson, 1965) by *Mycoplasma hominis* suggested that this organism might use a scavenger pathway (Fig. 1) to supply thymidine (TdR) nucleotides for deoxyribonucleic acid synthesis as has been suggested for *M. laidlawii* (Razin, 1962; Smith, 1964). To determine if this was a reasonable possibility we assayed in fractions of *M. hominis* the activity of several enzymes involved in the synthesis of thymidylate (dTMP) and its incorporation into DNA (as the triphosphate).

The methods used to grow, harvest and fractionate the organisms have been described (Stock & Gentry, 1969). Briefly, washed cell pellets were lysed by osmotic shock and freezing and thawing, and then centrifuged. The supernatant was used as the cell-free extract.

Thymidine (TdR) kinase (EC 2.7.1.21) was assayed essentially as described by Takahashi, Ueda & Ogino (1966). The aqueous reaction mixture contained 22.7 μ -moles KCl, 2.5 μ moles ATP, 3 μ moles 3-phosphoglycerate, 2.5 μ moles MgCl₂, 1.5 μ moles trishydroxymethylaminomethane (tris), pH 7.8, 16.7 μ moles TdR [2-¹⁴C] (5 nCi), and 0.1 ml. enzyme preparation, all in 0.5 ml. final volume.

DNA nucleotidyl transferase (EC 2.7.7.7) was assayed by the method of Magee (1962). The reaction mixture contained 10 μ g. highly polymerized, heat-denatured L-cell DNA as primer; 75 nmoles each of the triphosphates of TdR, deoxyadenosine, and deoxyguanosine; 1.3 nmoles [³H]deoxycytidine triphosphate (dCTP) (2.5 μ Ci); 0.5 μ moles 2-mercaptoethanol; 15 μ moles tris, pH 7.8; 2.5 μ moles MgCl₂; and 0.1 ml. enzyme preparation in 0.5 ml. final volume.

Deoxycytidylate (dCMP) deaminase (EC 3.5.4.12), was assayed with the following reaction mixture: 2.5 μ moles tris, pH 7.4; 32 nmoles [2-¹⁴C]dCMP (12.5 nCi); and 0.1 ml. enzyme preparation in 0.25 ml. final volume. The reaction was stopped by freezing at -20°; after thawing, 50 μ l. of the mixture was spotted on Whatman no. 3 paper, which then was developed in a mixture of 75 ml. 95 % ethanol + 30 ml. 1 M-ammonium acetate, pH 7.5 (Solvent system 29, Schwarz Bioresearch Catalogue 1966). The chromatogram was scanned (Vanguard Autoscanner) to locate the peak of [2-¹⁴C]deoxyuridine monophosphate (dUMP). The peak was eluted with water and counted in a liquid scintillation spectrometer (Packard Instrument Co.).

dTMP synthetase (EC 2.1.1.11) was assayed by the method of Lomax & Greenberg (1967). The reaction mixture contained 40 μ moles tris, pH 7.4; 26 μ moles MgCl₂; 1.06 μ moles ethylenediamine-tetraacetate; 106 μ moles 2-mercaptoethanol; 15.8 μ moles

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formaldehyde; 37.6 nmoles tetrahydrofolic acid; 0.5 nmoles [^3H] dUMP (0.5 μCi); and 0.1 ml. enzyme preparation in 1.0 ml. final volume.

The method of Lowry, Rosebrough, Farr & Randall (1951) was used for measurement of protein.

All incubations were for 30 min. at 37°. The values found, each representing the average of assays of four separate cell-free extracts, were, in nmoles of product formed/min./mg. protein, DNA nucleotidyl transferase, 0.002 nmole dCTP (made acid-insoluble); TdR kinase, 14.6 nmoles dTMP; dCMP deaminase, 5.4 nmoles dUMP; and dTMP synthetase, 0.093 nmole dTMP.

Excepting dTMP synthetase, considerable variation in enzyme activity was noted from sample to sample, but this may stem from the uneven distribution of activity between the soluble fraction and the membranes. The latter contained variable amounts of the several activities, although with few exceptions less than the corresponding cell-free extracts. The data do, however, indicate that *Mycoplasma hominis* has

Fig. 1. Suggested pathway of thymidine metabolism in *Mycoplasma hominis*. The *de novo* pathway to dTMP begins with dCMP; the scavenger pathway begins with DNA in the extracellular environment. Enzymes demonstrated in the present study are marked with an asterisk. Except for CDP, the nucleoside diphosphates have been omitted as well as the nucleosides other than TdR. TdR may also serve as a source of deoxyribose for the synthesis of other deoxyribonucleotides, by means of nucleoside phosphorylase (Razin & Knight, 1960).

the enzymes needed to provide the thymine moiety for DNA synthesis either via the *de novo* pathway from dCMP, or via the scavenger pathway from exogenous TdR. This is in apparent contrast to *M. laidlawii* B which shows an absolute growth requirement for exogenous TdR (Smith & Hanawalt, 1968), although in this case the inability to synthesize TdR *de novo* may reflect a defect in folate metabolism rather than the absence of dTMP synthetase. Which pathway *M. hominis* uses when both substrates are available is not presently known, although Gentry & Stock (1969) have shown that, in L-cell cultures prelabelled with [$2\text{-}^{14}\text{C}$]TdR and doubly infected with *M. hominis* and equine abortion virus, label could subsequently be demonstrated in the *M. hominis* DNA.

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