EFFECTIVE UTILIZATION OF EXOGENOUS DEOXYTHYMIDINE-5'-MONOPHOSPHATE FOR DNA SYNTHESIS IN ENTEROBACTERIA

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Received 14 July 1975

1. Introduction

Wild type strains of *Escherichia coli, Salmonella typhimurium* and related enterobacteria scarcely utilize exogenous thymine and dThd for DNA synthesis, although in these organisms the enzymes catalyzing the interconversion of thymine to dThd and dTMP are present [1]. As shown by Jensen et al. [2] these enzymes mainly fulfill catabolic functions. Thymineless mutants in which the de novo synthesis of dThd nucleotides is blocked, are dependent on the supply of exogenous thymine or dThd. Therefore, in these strains the total thymine in DNA must derive from exogenous precursors. In contrast to wild type strains in these mutants a very effective labelling of DNA with radioactive dThd is possible. From some strains however thymineless mutants are not always obtainable and in other cases experimental work is severely impaired by the phenomenon of thymineless death [3].

It is possible to eliminate these difficulties by use of dTMP instead of thymine or dThd. This study gives an account of the effective utilization of the dThd moiety of dTMP for DNA-synthesis in wild type strains of *Escherichia coli* and *Salmonella typhimurium*. Some properties of the uptake mechanism for dThd are described.

2. Material and methods

2.1. *Bacterial strains and their growth*

In most experiments *E. coli K12* (ATCC 25 257) and *S. typhimurium* (ATCC 15 277) were used. Thymineless mutants from these strains were obtained by the method of Okada [4]. *E. coli K12 KY 688* (deficient in dThd kinase) and *E. coli K12 3110* [5] were kindly supplied by Dr S. Hiraga, Kyoto/Japan.

Cells were grown under shaking at 37°C on synthetic medium M 63 [6] supplemented with 1% glycerol as carbon source. Further additions to these cultures were made as mentioned below. Cell free extracts were prepared by sonication. Osmotic shock treatment was performed according to [7]. DNA was isolated by the method of Miura [8]. The rate of incorporation was measured by transfering samples of 0.2 ml into 5.0 ml ice-cold 10% trichloracetic acid, filtrating the mixture after 30 min through a Millipore filter, and counting the filter in a Packard Liquid Scintillator.

3. Results

3.1. *Incorporation of thymine, dThd and dTMP*

In fig.1 the incorporation of the radioactivity from ^{3}H-labelled thymine, dThd and dTMP into macromolecules of *S. typhimurium LT 2* is compared. In agreement with earlier studies [9] there is only a limited incorporation of thymine and a small incorporation of dThd in the first 8–10 min. The incorporation of the radioactivity from dTMP however proceeds at a linear rate over a period of at least 1 hr.

3.2. *Incorporation of [^{3}H]dThd from double-labelled [^{3}H]dTMP*

The effective incorporation of ^{3}H-dTMP raises the question whether the nucleotide is taken up or incorporated as a whole. In experiments with double-labelled dTMP (either methyl-^{3}H and α-^{32}P or deoxyribose-^{14}C and α-^{32}P) a high incorporation of the tritium labelled

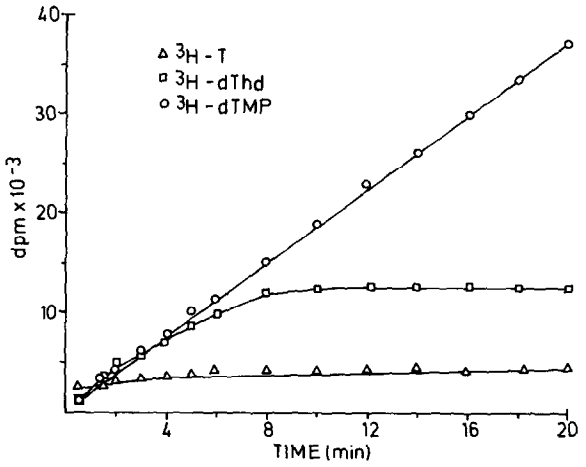

Fig.1. Incorporation of thymine, dThd and dTMP in wild type cells of *S. typhimurium* LT_2. Concentration of the thymine derivates 10^{-4} M; radioactivity in all cases 1.25 mCi/mmol.

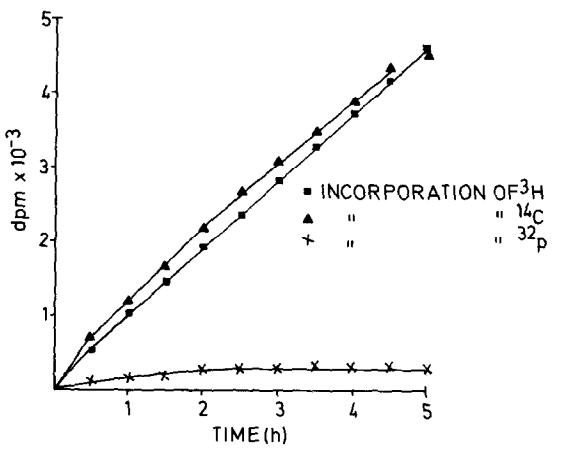

Fig.2. Incorporation of radioactivity from double labelled (methyl-^{3}H; α-^{32}P)dTMP or (deoxyribose-^{14}C; α-^{32}P)dTMP by *S. typhimurium*. Concentration of dTMP 10^{-5} M; radioactivity of each isotope 1.0 mCi/mmol.

base moiety or of the ^{14}C-labelled deoxyribose moiety is observed, yet only a negligible ^{32}P-incorporation (fig.2). The data indicate a dephosphorylation of the nucleotide in the course of uptake. The almost equal rates of the uptake of the tritium and of the ^{14}C-label support an uptake of the remaining dThd without cleavage of the *N*-glycosidic linkage.

3.3. *Relative amount of DNA-thymine derived from exogenous dTMP*

Growth of cells in the presence of increasing concentrations of dTMP in the medium leads to an increased incorporation of the dThd moiety into the DNA of the cells (table 1). Finally, at high concentrations of exogenous dTMP a saturation occurs. At this point about 40% of the dThd in the DNA is derived from exogenous dTMP, while the other 60% is formed by the cells via de novo synthesis.

3.4. *The specificity of the incorporation of dThd from dTMP*

A comparison between the incorporation of the base moieties of several other purin- and pyrimidine ribo- or deoxyribonucleotides shows a remarkable

Table 1
Labelling of DNA in the presence of increasing concentrations of exogenous dTMP

Concentration of dTMP in the medium	Specific activity of [^{3}H]dTMP		Exogenous dTMP in the DNA of the cells
	in the medium	in isolated DNA of the cells	
(mM)	(μC/μmol)	(μC/μmol)	(%)
1	10	1.7×10^{-3}	0.1
5	2	2.3×10^{-3}	0.11
10	1	2.1×10^{-2}	2.1
20	0.5	9.4×10^{-2}	18.8
40	0.25	8.7×10^{-1}	38.8
80	0.13	5.3×10^{-1}	41.0

Table 2
Specificity of the utilization of free nucleosides and of the nucleoside moiety from the corresponding 5'-mononucleotides. Concentration of nucleosides resp. nucleotides in the medium 10^{-4} M; in all cases ^{3}H-labelled compounds (1 mCi/mmol) were used

Nucleoside or Nucleotide	Incorporation nmol/ml cell suspension	
	after 60 min incubation	after 120 min incubation
dTMP	0.74	1.46
dThd	0.19	0.19
dCMP	0.86	1.40
dCR	2.08	8.10
dAMP	4.90	6.71
dAR	5.53	13.82
AMP	0.87	2.17
AR	2.05	6.29
UMP	0.46	0.90
UR	2.60	8.25

specificity of incorporation of the nucleoside moiety of dTMP (table 2). In all cases with the exception of dThd resp. dTMP the incorporation of the free nucleosides occurs at a higher rate than the incorporation of the nucleosides derived from the corresponding nucleotides. dThd from dTMP however is incorporated at a 4 to 8 times higher rate than free dThd.

3.5. *Localisation of the uptake system*

The treatment of cells from *S. typhimurium LT 2* by an osmotic shock procedure [7] leads to a decrease in the incorporation of dThd from dTMP (fig.3). The rate of incorporation is almost completely restored by the addition of the shock fluid to the treated cells. This indicates at least a participation of components of the periplasmatic space in the process of uptake.

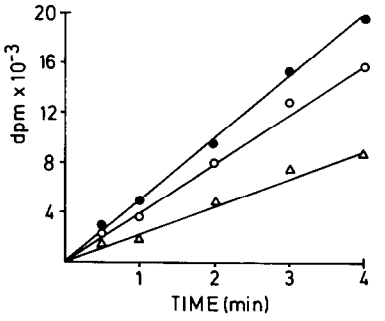

Fig.3. The effect of osmotic shock treatment of the incorporation of dThd from dTMP by cells of *S. typhimurium* LT_2. Concentration of dTMP 10^{-5} M; radioactivity 12.5 mCi/mmol; •, untreated cells; △, treated cells; ○, treated cells supplemented with their own shock fluid after 15 min preincubation.

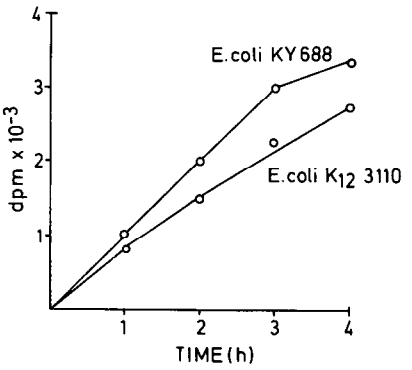

Fig.4. Incorporation of free dThd and dThd from dTMP by a strain of *E. coli K12 (KY 688)* lacking soluble dThd kinase and a corresponding wild type strain. Concentration of dThd resp. dTMP 10^{-4} M; radioactivity 1.0 mCi/mmol.

Soluble dThd kinase (fig.4) is not required for the uptake of dThd from dTMP. The measurement of the incorporation of dThd from dTMP in a mutant of *E. coli K12* which lacks the soluble dThd kinase [5] shows an even slightly higher incorporation in comparison with the corresponding wild type strain.

4. Discussion

Wild type strains of enterobacteria are able to incorporate the nucleoside moiety of dTMP much more effectively than free dThd. In most experiments with different strains of *E. coli* or *S. typhimurium* no significant differences are observed. During the process of uptake the nucleotide is dephosphorylated as shown in experiments with double labelled nucleotides. The rephosphorylation of dThd to dTMP is a prerequisite for the synthesis of DNA. Soluble dThd kinase does not catalyze this reaction as shown by a comparison between the incorporation of dThd into the DNA of wild type cells and mutant cells which lack soluble dThd kinase. The similarity of the uptake of base- or sugar-labelled dThd from dTMP renders

a split of the *N*-glycoisidic linkage unlikely. This is in contrast to recent studies of Yagil and Beacham [10], who described a similar uptake of AMP by *E. coli K12*.

Attempts to characterize a further, possibly membrane-bound enzyme for the conversion of dThd, derived from exogenous dTMP, to intracellular dTMP have failed so far. The system for the utilization of dThd from dTMP shows a remarkable specificity when compared with other nucleosides. Ribo- or deoxyribonucleosides from other nucleotides are generally incorporated at a lower rate than the corresponding free nucleosides.

Treatment of the cells by osmotic shock procedure results in a remarkable decrease in the incorporation of dThd from dTMP. This indicates at least a participation of components of the periplasmatic space during the process of nucleotide splitting and nucleoside uptake.

The use of labelled dTMP instead of dThd allows an effective labelling of the DNA of enterobacteria by [^{3}H]- or [^{14}C] thymidine.

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