UTILIZATION OF RIBOSYLHOMOCYSTEINE BY VARIOUS MICROORGANISMS*

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It has been shown that various microorganisms of the family Enterobacteriaceae possess an enzyme which degrades S-adenosylhomocysteine via the following reaction: (Duerre, 1962a)

S-Adenosyl-L-homocysteine ⟶ Adenine + Ribosyl-L-homocysteine

No further degradation of ribosylhomocysteine by extracts from these organisms was noted during the course of this investigation.

Methods: The microorganisms used were Pseudomonas pavonacea, Candida utilus A.T.C.C. 9950, Rhodotorula glutinis var. rubescens A.T.C.C. 6495, Escherichia coli strain W, Escherichia coli strain B, Salmonella typhimurium M, and a soil fungus. The methionine requiring mutant of S. typhimurium M was obtained from Dr. S. K. Shapiro, Argonne National Laboratory. The soil fungus was isolated on M-9 media (Anderson 1946) in which glucose had been replaced by 0.3% ribosyl-L-homocysteine. All organisms were maintained on M-9 medium except R. glutinis which was cultured on the media of Snell (1948), and C. utilus,

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which was maintained on a basal salts medium previously described (Duerre and Schlenk 1962).

By utilizing the condensing reaction (de la Haba and Cantoni 1959) it was possible to incorporate tritiated L-homocysteine or tritiated adenosine (Wilbach process) into S-adenosyl-L-homocysteine (S-AH). The resultant S-AH was purified (Duerre 1962b) and subsequently degraded with a partially purified S-AH glycosidase. The resultant ribosyl-L-homocysteine (RH) labeled in either the homocysteine or ribose moiety, was purified as previously described (Duerre 1962a).

Results and discussion: The results of the growth response studies with S. typhimurium M are summarized in Table 1. A definite growth

TABLE I

GROWTH RESPONSE OF S. TYPHIMURIUM M

Supplement* (10 mμmoles/ml)	Optical Density (490 mμ)	
	19 hrs.	23 hrs.
None	0.00	0.03
L-Methionine	0.24	0.25
L-Homocysteine	0.02	0.18
Ribosyl-L-homocysteine	0.05	0.25
S-Adenosyl-L-homocysteine	0.02	0.02

*Added to M-9 media.

response was observed with methionine, L-homocysteine and RH although the response to the latter two compounds was somewhat delayed. No growth was observed in tubes containing as much as 1.0 μmole/ml S-AH in 36 hours. In view of the previous finding that S. typhimurium possesses a very active S-AH glycosidase (Duerre 1962a) yielding adenine and RH,

failure of this compound to support growth suggests that the organism is impermeable to S-AH. The ability of homocysteine to support growth of this organism indicates that the organism is not a true methionine mutant but that homocysteine via transmethylation (Shapiro 1958) or direct methylation (Larabee et. al. 1963) can supply the methionine requirement. The ability of RH to support growth could thus be accounted for if this compound was degraded to homocysteine and ribose.

The ability of various organisms to incorporate tritiated RH was investigated. Growing cultures of P. pavonacea and R. glutinis supplemented with tritiated RH were found to incorporate only small amounts of this compound (Table II). The ability of these two organisms to utilize either the ribose or the homocysteine moiety of RH is doubtful. The amount of radioactivity incorporated might well be attributed to trace contaminants in the RH or exchange of tritium with the solvent system.

The amounts of tritium from ribosylhomocysteine-H^3 incorporated by C. utilis, S. typhimurium M and the soil fungus were found to be significantly higher than that observed in P. pavonacea and R. glutinis (Table II). With the exception of the soil fungus they failed to incorporate significant amounts of tritium from ribosyl-H^3-homocysteine. These findings indicate that these organisms can incorporate a limited amount of the homocysteine moiety or part thereof.

E. coli B and E. coli W incorporated significantly higher amounts of tritium from ribosylhomocysteine-H^3 than any of the other organisms (Table II). The results indicate that the amount of tritium incorporated by these organisms from ribosyl-H^3-homocysteine was limited. These findings suggest that RH is degraded by E. coli cells and that the homocysteine moiety or part thereof is incorporated at a much faster rate than the ribose moiety.

TABLE II

INCORPORATION OF TRITIATED RIBOSYLHOMOCYSTEINE BY VARIOUS MICROORGANISMS

Organisms[1]	Media (10 ml)	Supplement	Temp of Incubation	Time	Radioactivity Incorporated by the Cells	
				hr.	cpm/ml	%
P. pavonacea	M-9	R* H[2]	24°	48	4200	2.5
		R H*[3]			1600	2.0
R. glutinis	Snells	R* H	24°	48	5900	3.5
		R H*			1600	2.0
C. utilis	C. utilus	R* H	30°	48	4750	2.8
		R H*			4000	5.0
S. typhimurium M	M-9	R* H	37°	48	4200	2.5
		R H*			3200	4.0
Soil Fungus	M-9	R* H	24°	96	10000	6.0
		R H*			4400	5.5
E. coli W	M-9	R* H	37°	24	6800	4.0
		R H*			15200	19.0
		S-A H*[4]			3500	0.7
E. coli B	M-9	R* H	37°	24	7300	4.3
		R H*			7900	10.0

[1]Organisms were harvested by centrifugation; washed twice (4°) and resuspended in 1.0ml water.
[2]Ribosyl-H^3-L-homocysteine; 0.6 μmole/ml; 28,500 counts/min/μmole.
[3]Ribosyl-L-homocysteine-H^3; 0.6 μmole/ml; 13200 counts/min/μmole.
[4]S-Adenosyl-L-homocysteine-H^3; 1.0 μmole/ml; 44,500 counts/min/μmole.

E. coli cells failed to incorporate significant amounts of tritium from S-adenosylhomocysteine-H^3. Inasmuch as this organism possesses an active S-AH glycosidase (Duerre 1962a) failure to utilize this compound might well result from lack of an S-AH permease. Failure to utilize S-AH could also result if the S-AH glycosidase is bound in an inactive state within the cell.

The distribution of tritium from ribosylhomocysteine within various fractions of E. coli W cells was then investigated. Tritiated RH was used as a supplement as described. The culture volume was increased to 50ml, and the cells were harvested as outlined under Table II. The cells were then disrupted with a cell fractionator (Duerre & Ribi 1963). Proteins and nucleic acids were precipitated with 5% trichloroacetic acid and this precipitate washed twice with cold TCA. The precipitate was then digested with 5% perchloric acid for 30 min at 80° to solubilize the nucleic acids. Phospholipids and fatty acids were extracted with hot ethanol-ether and the distribution of tritium measured in the various fractions. When the cells were cultured in the presence of Ribosyl-H^3-homocysteine the bulk of the isotope was found in the hot perchloric acid extract (nucleic acids) with lesser amounts associated with the other fractions (Table III). When the cells were cultured in the presence of ribosylhomocysteine-H^3 the bulk of the isotope was found associated with the proteins. These findings indicate that RH is degraded by E. coli cells and that the ribose moiety is metabolized independently and at a substantially slower rate than the homocysteine moiety. Inasmuch as the bulk of tritium from ribosyl-homocysteine-H^3 appeared in the proteins, it is suggested that the homocysteine is converted to methionine via transmethylation or direct methylation.

TABLE III

DISTRIBUTION OF RADIOACTIVITY IN VARIOUS FRACTIONS FROM E. COLI W

	Supplement	Radioactivity Incorporated	
		Total cpm	%
Cells	R* H[1]	24000	
	R H*[2]	75000	
Cold TCA extract	R* H	4000	16.6
	R H*	5000	6.7
Hot PCA extract	R* H	14000	58.0
	R H*	7500	10.0
Ethanol-ether	R* H	1200	5.0
	R H*	2500	3.3
Protein residue	R* H	1600	6.6
	R H*	50000	67.0

[1]Ribosyl-H^3-L-homocysteine; 0.6 μmole/ml; 28500 counts/min/μmole.
[2]Ribosyl-L-homocysteine-H^3; 0.6 μmole/ml; 13200 counts/min/μmole.

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