UTILIZATION OF PURINE AND PYRIMIDINE COMPOUNDS IN NUCLEIC ACID SYNTHESIS BY *ESCHERICHIA COLI*

by

ELLIS T. BOLTON AND ALAN M. REYNARD

Department of Terrestrial Magnetism Carnegie Institution of Washington, Washington 15, D.C. (U.S.A.)

In a previous study[1] of nucleic acid synthesis in growing *Escherichia coli*, B, it was shown that the addition of purines or pyrimidines to culture media containing $^{14}CO_2$ caused a suppression of isotope incorporation into the bacterial polynucleotides. Exogenous adenine was used in preference to CO_2 for nucleic acid synthesis and was also converted to guanine. Uracil was similarly used and converted to cytosine. Other studies with different species have revealed variations in the pattern of utilization and interconversion among the purines or pyrimidines as BROWN[2] has pointed out. Relatively wide variety is also found in the patterns of metabolism of nucleosides and nucleotides[2,3]. In view of these differences among species it becomes important to establish the extent to which a given species utilizes purine and pyrimidine compounds in nucleic acid synthesis.

The present report describes the results of a systematic investigation on the utilization and interconversion of purines, pyrimidines, nucleosides, and nucleotides in *E. coli*, B. The study was carried out principally by means of the "isotopic competition method"[4,5,6] in which $^{14}CO_2$ was the labelled compound.

EXPERIMENTAL

E. coli, B, growing in the exponential phase was harvested and resuspended in 20 ml of culture medium* contained in a series of 500 ml polyethylene bottles. The initial optical density of all cultures was 0.075, which is equivalent to 5 mg wet weight of cells in each culture. 500 μM of ^{14}C labelled $NaHCO_3$** and 5 mg glucose were added to control cultures while 1 mg of a purine or pyrimidine compound was in addition supplied to the remaining cultures of the series. Supplements included commercially available adenine, adenosine, yeast adenylic acid (adenosine-3-phosphoric acid,) guanine, guanosine, guanylic acid, cytosine, cytidine, cytidylic acid, uracil, uridine, uridylic acid, thymine, thymidine, and orotic acid. In a series of experiments on adenine compounds, $NaH^{14}CO_3$ having no overtly added carrier, $NaHCO_3$ was used. In these experiments the isotope was contained in approximately 2.4 μM $NaHCO_3$. The bottles were securely stoppered and mechanically shaken for one hour at 37° C. During this period the cells utilized 5 mg of glucose, produced approximately 84 μM of CO_2 and doubled

* The medium contained 6 g Na_2HPO_4, 3 g KH_2PO_4, 1 g NH_4Cl, 5 g NaCl, 0.2 g $MgSO_4 \cdot 7H_2O$ per liter.

** Prepared from $Ba^{14}CO_3$ obtained from the Atomic Energy Commission, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

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in amount. The cells were harvested and chemically fractionated as previously described[7]. Radioactivity of all the fractions was determined on thin samples contained in plastic planchets. Only the "hot trichloracetic acid fraction", which contains the bacterial nucleic acid, differed significantly in ^{14}C content among the cultures of a series. This fraction was hydrolyzed and portions of it chromatographed on paper in two dimensions. The first solvent was sec-butyl alcohol-H_2O-formic acid (70/20/10; V/V) in which the R_F values for the hydrolysis products, guanine, adenine, cytidylic and uridylic acids are 0.36, 0.29, 0.05, and 0.13, respectively. The second solvent was 70% tert-butyl alcohol in 0.8 *N* HCl[8] in which the R_F values for these compounds are 0.21, 0.35, 0.48, and 0.77, respectively. The separated hydrolysis products were located by their ultraviolet absorption, cut out, eluted in 0.1 *N* HCl and quantitatively assayed by u.v. absorption. Specific activities were computed from these assays and from their ^{14}C content determined on aliquots counted with a thin mica end-window Geiger-Müller counter.

RESULTS

Utilization of adenine compounds. Some typical results on the utilization of $^{14}CO_2$ for nucleic acid synthesis in the presence of adenine compounds are summarized in Table I. The data are reported in terms of specific radioactivity, *i.e.* counts/sec/μM, for

TABLE I

EFFECT OF ADENINE COMPOUNDS ON THE UTILIZATION OF $^{14}CO_2$ IN NUCLEIC ACID SYNTHESIS

Supplement	*Specific activity of bacterial nucleic acid component (c/s/μM)**			
	Adenine	*Guanine*	*Cytidylic acid*	*Uridylic acid*
None (control)	110 (240)	110 (230)	— (450)	190 (400)
Adenine	10 (2)	22 (9)	— (340)	— (380)
Adenosine	7 (3)	3 (22)	— (310)	190 (360)
Yeast adenylic acid	90 (120)	90 (120)	— (420)	190 (420)

* Values in parentheses are taken from a series of experiments in which no carrier $NaHCO_3$ was used; *cf.* also reference[1]. All other values are from high carrier experiments.

the compound isolated from hydrolysates of the hot-trichloracetic acid-soluble fraction of the bacteria. These data reveal a number of facts. The bacterial purines of a given culture have similar specific activities, as do the pyrimidines. Addition of adenine or adenosine to the culture medium causes a marked suppression in $^{14}CO_2$ utilization for purine synthesis. Thus, exogenous adenine and adenosine are effectively utilized for nucleic acid synthesis, and are efficiently converted to cellular guanine. The yeast adenylic acid (Schwarz, adenosine-3-phosphoric acid) supplied is presumably the natural isomer[9]. It had a much smaller effect upon the utilization of $^{14}CO_2$ than did adenine or adenosine. Nevertheless, it was converted to polynucleotide guanine and served as a source of adenine. Little, if any, effect upon the bacterial pyrimidines is to be noted. Qualitatively similar results are evident whether data from cells cultured in media rich or relatively poor in CO_2 are considered. In the experiments with high concentrations of CO_2 (500 μM per culture) the specific radioactivity of the carbon dioxide decreases by only 15% at the end of one hour as a result of CO_2 production from glucose. Under the conditions of these experiments the mean specific activity amounted to *ca.* 230 c/s/μM. Since the cells doubled in mass during the incorporation

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of ^{14}C, the specific activity actually determined for any compound utilizing the carbon of one mole of CO_2 per mole of compound formed is expected to be 115 c/s/μM. It is seen from Table I that adenine and guanine of the control cells had 110 c/s/μM. This result demonstrates that little or no turn-over of the cell nucleic acid has taken place. Since it is known that guanine is labelled by $^{14}CO_2$ largely at carbon-6[10], it is evident that the bacterial purines derive this carbon atom from the CO_2 pool without appreciable isotopic dilution. Where no carrier CO_2 was supplied, the specific radioactivity of the $^{14}CO_2$ was initially about 40-fold higher than for the "high carrier" (500 μM/culture) case. Nevertheless, the radioactivity of the purines was determined to be *ca.* 240 c/s/μM (Table I, values in parentheses). Most of this radioactivity is taken up in the first 10 minutes of growth. This determination corresponds to a $^{14}CO_2$ pool of average specific activity *ca.* 480 c/s/μM. These values reflect the marked isotopic dilution resulting from the production of CO_2 by the bacterial oxidation of glucose. Where adenine or adenosine were added to these cultures only 2–22 c/s/μM could be found in the bacterial purines. Such small amounts of radioactivity could have been incorporated in a matter of seconds in the absence of supplementation. Consequently, it is concluded that the capacity to utilize exogenous adenine and adenosine was present in the cells at the start of the experiment and adaptation to the supplement did not take place.

Utilization of guanine compounds. Exogenous guanine, guanosine, and guanylic acid suppress incorporation of $^{14}CO_2$ into bacterial guanine as Table II demonstrates.

TABLE II

EFFECT OF GUANINE COMPOUNDS ON THE UTILIZATION OF $^{14}CO_2$ IN NUCLEIC ACID SYNTHESIS

Supplement	*Specific activity of bacterial nucleic acid component (c/s μM)*			
	Adenine	*Guanine*	*Cytidylic acid*	*Uridylic acid*
None (control)	110	110	—	190
Guanine	110	2	—	150
Guanosine	100	1	—	220
Guanylic acid	70	3	—	230

In contrast to the results of adenine supplementation, the polynucleotide adenine of cells grown in the presence of the guanine compounds contains appreciable ^{14}C derived from $^{14}CO_2$. Thus, there is relatively little conversion of guanine-containing supplements to bacterial adenine. These compounds are poorly, if at all, converted to bacterial pyrimidines. KOCH, PUTNAM AND EVANS[10], on the other hand, have shown some conversion of guanine to adenine where cells were adapted to lactate.

Utilization of pyrimidine compounds. When cytosine, cytidine or cytidylic acid are supplied to growing bacteria they strongly influence the utilization of $^{14}CO_2$ for pyrimidine synthesis but not for purine synthesis. Table III indicates that both bacterial cytidylic and uridylic acids are found to have much lower specific activities when grown in the presence of one of the pyrimidine supplements than when grown in a medium containing $^{14}CO_2$ and glucose as the only carbon sources. The exogenous pyrimidines have no effect upon the radioactivity of the bacterial purines. Table IV demonstrates a similar set of results for uracil, uridine and uridylic acid supplementation. Comparison of the data of Tables III and IV shows that the pyrimidine compounds are quite freely interconvertible: each supplement is readily utilized as a source for both bacterial cytosine and uracil.

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Neither thymine, thymidine, nor orotic acid (4-carboxyuracil) could be demonstrated to influence the utilization of $^{14}CO_2$ for the synthesis of bacterial adenine, guanine, cytidylic acid or uridylic acid. This result parallels that in rats where ^{14}C-labelled thymine compounds apparently do not contribute to the ribonucleic acid components[11]. ^{14}C-labelled orotic acid contributes only relatively small amounts of radioactivity to *E. coli*, B, nucleic acid even after a 100-fold growth[12]. Thus, suppression of $^{14}CO_2$ incorporation in the presence of unlabelled orotic acid is expected to be low. The method employed in the present work would overlook minor contributions of carbon from orotic acid and, indeed, from all sources except the labelled substrate.

TABLE III

EFFECT OF CYTOSINE COMPOUNDS ON THE UTILIZATION OF $^{14}CO_2$ IN NUCLEIC ACID SYNTHESIS

Supplement	*Specific activity of bacterial nucleic acid component (c/s/μM)*			
	Adenine	*Guanine*	*Cytidylic acid*	*Uridylic acid*
None (control)	140	110	180	210
Cytosine	120	110	66	68
Cytidine	120	110	23	10
Cytidylic acid	120	110	28	50

TABLE IV

EFFECT OF URACIL COMPOUNDS ON THE UTILIZATION OF $^{14}CO_2$ IN NUCLEIC ACID SYNTHESIS

Supplement	*Specific activity of bacterial nucleic acid component (c/s/μM)*			
	Adenine	*Guanine*	*Cytidylic acid*	*Uridylic acid*
None (control)	140	110	180	210
Uracil	110	120	38	37
Uridine	150	120	16	5
Uridylic acid	130	115	43	100

DISCUSSION

The data presented in Tables I–IV demonstrate that *E. coli*, B, utilizes exogenous compounds similar to those which comprise its nucleic acid in preference to synthesizing these constituents *de novo* from carbon dioxide. The interconversions which occur are relatively specific. Thus, adenine is converted to guanine and cytosine to uracil. Uracil in turn gives rise to cellular cytosine. The purines are not converted to pyrimidines nor the pyrimidines to purines. The nucleosides studied are utilized with equal or greater effectiveness for nucleic acid synthesis than are the free bases while the relative effectiveness of the nucleotides is variable.

These observations are indicative of the versatile synthetic abilities of these cells. The cultures showed neither growth inhibition, lag nor other adaptive response to the presence of purine or pyrimidine compounds. Furthermore, even though CO_2 was supplied at a level 50–100 times that of the purine or pyrimidine compound, in many of the cultures its function as a carbon source for purine or pyrimidine synthesis was all but eliminated. It may be inferred then that the biochemical activities shown by the data of Tables I–IV are *normally* in operation. It may also be inferred that a series of compounds such as guanine: guanosine: guanylic acid is involved in, or in equilibrium

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with intermediates on, the synthetic pathway normally giving rise to nucleic acid. Similar inferences may be drawn for the other series of compounds tested. In addition the effective conversion of adenine to guanine and the near lack of conversion of guanine to adenine imply that in *E. coli*, B, the pathway which leads first to adenine and thence to guanine predominates (*cf.*[10]). The fact that the pyrimidine ribosides are used more effectively than the free bases suggests that these nucleosides are synthesized by the cell prior to their incorporation into the polynucleotide structure.

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SUMMARY

Utilization of purine and pyrimidine compounds by growing *Escherichia coli*, B, was studied with the aid of $^{14}CO_2$. It was found that adenine and adenosine were efficiently utilized for nucleic acid synthesis in preference to $^{14}CO_2$ and were converted to bacterial guanine. Yeast adenylic acid was relatively poorly utilized. Guanine, guanosine, and guanylic acid were used as a source of bacterial guanine but were poorly converted to adenine. Cytosine, cytidine, cytidylic acid, uracil, uridine, and uridylic acid were utilized as sources for both bacterial cytosine and uracil. No utilization of thymine, thymidine acid or orotic acid could be demonstrated by the method used.

RÉSUMÉ

L'utilisation des composés puriques et pyrimidiques par *E. coli*, B, en voie de croissance, a été étudiée à l'aide de $^{14}CO_2$. L'adénine et l'adénosine sont utilisés efficacement pour la synthèse des acides nucléiques, de préférence à $^{14}CO_2$ et sont transformés en guanine bactérienne. L'acide adénylique de la levure est relativement peu utilisé. La guanine, la guanosine et l'acide guanylique, sont employés comme source de guanine bactérienne mais sont faiblement transformées en adénine. La cytosine, la cytidine, l'acide cytidilique, l'uracile, l'uridine et l'acide uridylique sont utilisés comme source de cytosine et d'uracile bactériennes. L'utilisation de la thymine, de la thymidine et de l'acide orotique n'a pu être démontrée à l'aide des méthodes mises en oeuvre.

ZUSAMMENFASSUNG

Der Verbrauch von Purin- und Pyrimidinverbindungen von wachsenden *Escherichia coli*, B, wurde mit Hilfe von $^{14}CO_2$ untersucht. Es wurde gefunden, dass Adenin und Adenosin zur Nucleinsäuresynthese dem $^{14}CO_2$ vorgezogen wurde und in Bakterienguanin umgewandelt wurde. Hefeadenylsäure wurde in relativ geringen Mengen verbraucht. Guanin, Guanosin und Guanylsäure wurden als Quelle für Bakterienguanin verwendet, wurden jedoch nur in geringem Ausmass in Adenin umgewandelt. Cytosin, Cytidin, Cytidylsäure, Uracil, Uridin und Uridylsäure wurden als Quellen für Bakteriencytosin und -uracil verwendet. Bei Benutzung dieser Methode konnte kein Verbrauch von Thymin, Thymidin oder Orotsäure festgestellt werden.

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