THE USE OF METABOLIC POOLS OF PURINE COMPOUNDS FOR NUCLEIC ACID SYNTHESIS IN YEAST

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INTRODUCTION

The flow of exogenous carbon through a metabolic pool of amino acids has been shown to be a necessary step in the formation of protein in *Candida utilis*[1]. Similar kinetic investigations were carried out to ascertain whether pool formation is also essential for nucleic acid synthesis. The results reported here show that purines are accumulated into two chemically and functionally distinct metabolic pools prior to nucleic acid incorporation. Some of the characteristics and interrelationships of these two pools are described and their significance for macromolecule synthesis discussed.

PROCEDURES

Previously described methods[1] were used to culture *Candida utilis* (ATCC No. 9950) in C medium*. The ^{14}C- or ^{32}P-labeled compounds used as supplements were prepared from radioactive *Chlorella* or *Escherichia coli*[3]. For the most part the kinetic studies were carried out with the membrane filter technique described by BRITTEN, ROBERTS AND FRENCH[2]. Where further chemical fractionation was necessary this was done by a modification[3] of the SCHNEIDER method[4]. Chromatographic identification of the purine compounds in *C. utilis* was carried out on two-dimensional paper chromatograms. The solvent pairs used for acid hydrolysates of nucleotides and nucleic acids were *tert.*-butyl alcohol/hydrochloric acid and *sec.*-butyl alcohol/water/formic acid (see ref.[3], p. 41) and for components of the cold TCA extracts were ethanol/ammonium acetate[5] (pH 3.8) and ammonium sulfate/*iso*propanol[6].

EXPERIMENTAL RESULTS

Pool formation with ^{14}C-adenine

Candida utilis grown exponentially in C medium which contained nonradioactive fructose incorporated radioactivity from ^{14}C-adenine. Fig. 1 shows the time course of incorporation of ^{14}C-adenine carbon obtained in experiments using the membrane filter technique[2].

Initially the rate of incorporation of ^{14}C into the cold TCA-soluble fraction was more rapid than incorporation into the fraction containing the nucleic acids (TCA-precipitable fraction) which, after a slight lag, took up the adenine carbon at a constant rate until the supply in the medium approached exhaustion. Eventually all of the incorporated ^{14}C appeared in the TCA-precipitable fraction of the cell. These results suggest that the exogenous adenine carbon is first incorporated into a cold TCA-soluble pool prior to incorporation into the nucleic acid fraction of the cells.

Transfer of pool adenine to nucleic acid

Immersion of the yeast for 8 minutes in C medium containing trace amounts of high specific radioactivity ^{14}C-adenine-produced cells with more than 60% of the radioactivity in the cold TCA-soluble fraction. After washing and resuspending such a

* C medium: 2 g NH_4Cl, 6 g Na_2HPO_4, 3 g KH_2PO_4, 3 g NaCl, 0.01 g Mg as $MgCl_2$, 0.026 g S as Na_2SO_4, 100 ml 10% fructose and 900 ml of distilled H_2O.

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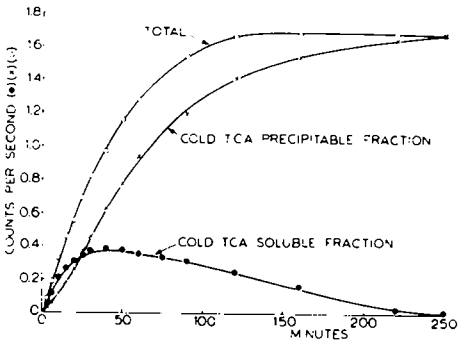

Fig. 1. Time course of incorporation of trace quantities of ^{14}C-adenine by *Candida utilis*. Initial concentration of 0.18 mμmoles adenine per ml medium.

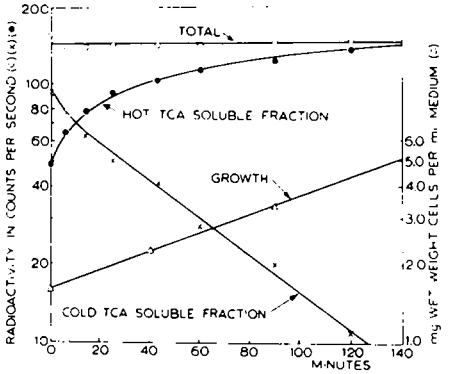

Fig. 2. Kinetics of transfer of pool carbon to nucleic acid during exponential growth.

culture in nonradioactive culture medium it was found that the labeled material rapidly left the cold TCA-soluble fraction, and equally rapidly appeared in the hot TCA-soluble (nucleic acid) fraction (Fig. 2). Little, if any, of the incorporated carbon was lost from the cells during this transformation. Paper chromatography of nucleic acid hydrolysates of the prelabeled cells demonstrated ^{14}C-adenine as the principal labeled component. It may be concluded that the formation of nucleic acid adenine occurs via a metabolic pool of adenine carbon.

Expansion of adenine pool size

When trace amounts of ^{14}C-adenine are used to supplement the medium, there is insufficient exogenous adenine to supply the adenine needs of the growing cell. Consequently, endogenous carbon derived from the ^{12}C-fructose furnishes most of the carbon necessary for nucleic acid adenine synthesis. Studies were therefore made of the incorporation of ^{14}C-adenine carbon supplied at higher exogenous concentrations.

A series of kinetic experiments (*cf.* Fig. 1) yielded data in which the maximum value of adenine pool size could be related to the initial adenine content of the medium. This relationship is shown in Fig. 3 (solid line). Since the cells accumulate adenine avidly, the exogenous concentration continually changes and a steady-state condition cannot be reached over the range of concentration extending to the limit of solubility of adenine in the culture medium. Accordingly, a pool size for a steady-state condition cannot be assigned and no upper limit to the amount of adenine accumulated in the pool could be demonstrated. The maximum observed was 156 μM per g dry cells, calculated as adenine, a value larger than the adenine content of all the nucleic acid in the cell.

Conversion of adenine carbon to guanine

KERR, SERAIDARIAN, AND BROWN[7] have shown that exogenous adenine is converted by *C. utilis* to adenine and guanine of the RNA. In the present kinetic studies chromatograms of nucleic acid hydrolysates of yeast grown in the presence of ^{14}C-adenine also showed radioactivity to be present in both adenine and guanine. However, the

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rate of appearance of radioguanine in the nucleic acids was initially less than for radioadenine. Table I shows the time course of appearance of ^{14}C from ^{14}C-adenine into nucleic acid guanine. The results of chromatographic analysis of hydrolysates of the cold TCA-soluble fraction paralleled those for the nucleic acid fraction. It may be concluded that exogenous adenine is taken into the TCA-soluble pool and in part converted to guanine; both bases are subsequently incorporated into the nucleic acid.

TABLE I

CONVERSION OF ^{14}C-ADENINE* TO ^{14}C-GUANINE BY EXPONENTIALLY GROWING *Candida utilis*

Time in minutes	*mg wet wt cells/ml medium*	*Total radioactivity in hot TCA-soluble fraction (counts per second)*	*Per cent of total radioactivity of hot TCA-soluble fraction found as guanine*
10	1.27	15	26.3
30	1.35	22	34.5
100	1.76	150	39.6
150	2.37	310	44.0
240	3.40	710	45.0

* Initial concentration of ^{14}C-adenine was 0.27 μM per ml medium.

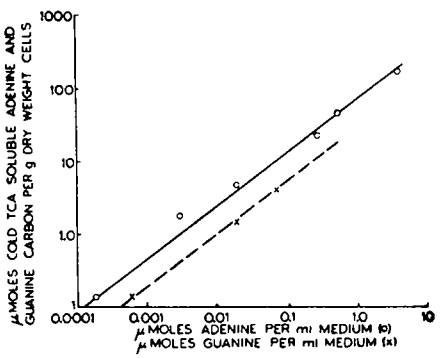

Fig. 3. Expansion of the size of purine pools. The data shown represent the maximum quantity of ^{14}C-adenine carbon incorporated into the cold TCA-soluble fraction as a function of the initial exogenous concentration of ^{14}C-adenine (solid line). Calculations assume all the incorporated radiocarbon remained adenine. Similar data are shown for the expansion of the guanine pool with increasing concentrations of exogenous ^{14}C-guanine (dotted line).

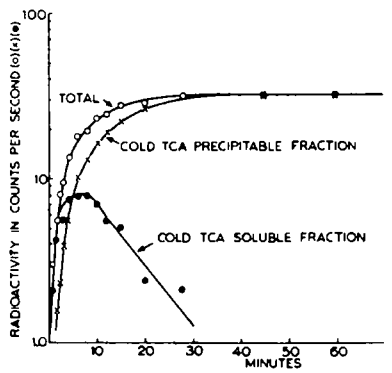

Fig. 4. Time course of incorporation of trace quantities of ^{14}C-guanine during exponential growth. Initial concentration of 0.5 mμmoles guanine per ml medium and 4.0 mg wet weight cells.

Pool formation with ^{14}C-guanine

The results of kinetic experiments with ^{14}C-guanine are generally similar to those described above for the ^{14}C-adenine. Figs. 4 and 5 show the course of uptake of radiocarbon from ^{14}C-guanine. The uptake of ^{14}C-guanine is rapid, ^{14}C appearing first in the TCA-soluble fraction. The TCA-soluble fraction gains in radioactivity until the exogenous supply of ^{14}C-guanine approaches exhaustion. This fraction then loses its radioactivity to the TCA-precipitable fraction, which continues to gain ^{14}C until the TCA-soluble pool is depleted.

The maximum size of the guanine pool is proportional to the amount of guanine initially present in the medium, as observed for the case of adenine supplementation.

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The data describing this observation are given in Fig. 3. The largest value observed was 4 μM per g dry cells. Guanine is much less soluble than adenine and supplements exceeding its solubility in the medium were not used.

Chromatographic analysis of hydrolysates of the cold TCA-soluble and -precipitable fractions showed guanine as the sole radioactive constituent regardless of the external guanine concentration used. Guanine is not readily converted to adenine by exponentially growing *C. utilis*.

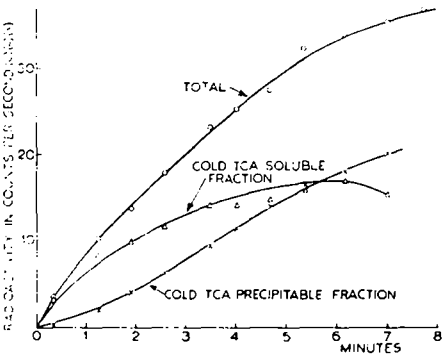

Fig. 5. Short-term experiment showing early kinetics of incorporation of trace quantities of ^{14}C-guanine. Initial guanine concentration of 0.9 mμmoles per ml medium and 3.5 mg wet weight cells.

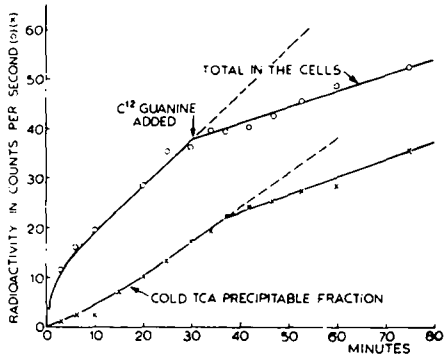

Fig. 6. Kinetics of ^{14}C-adenine incorporation in the presence of ^{12}C-guanine. At $t = 30$ minutes the exogenous concentration of ^{14}C-adenine and ^{12}C-guanine was 0.016 and 0.018 μmoles per ml medium respectively. Initial cell concentration was 0.5 mg wet weight cells per ml medium.

Isotopic competition results

Additional support for the conclusion that pool adenine is converted in part to pool guanine and that guanine is not readily converted to adenine is given by the isotopic competition results shown in Fig. 6. Fig. 6 shows the results of adding ^{12}C-guanine to a yeast culture growing in a medium containing ^{14}C-adenine. At the time of addition of the guanine, the concentration of the exogenous ^{14}C-adenine was approximately equal to the added guanine. Guanine immediately suppresses ^{14}C-adenine uptake by the cell. Initially the effect is largely upon the uptake into the TCA-soluble fraction. After about 10 minutes the rate of incorporation of ^{14}C into the TCA-precipitable fraction also decreases. On the other hand, there is no effect of ^{12}C-adenine upon the uptake and utilization of ^{14}C-guanine.

The relationship between the phosphorus pool and the purine pool

The kinetics of formation of a pool of phosphorus compounds in *C. utilis* and its utilization for nucleic acid synthesis has been previously described[1]. This pool contains one-third of the phosphorus of the cell. Chromatograms (ethanol/acetate: sulfate/*iso*-propanol system) of the cold TCA-soluble fraction from yeast cells grown in C medium and radiophosphorus were very similar to those from *E. coli*[8]. In each case 50% of the phosphorus is found as orthophosphate and most of the remainder is distributed among compounds having R_F's corresponding to those observed for the nucleoside mono-, di-, and triphosphates of adenine, guanine, cytosine and uracil.

Since the purine pool of the yeast could be enormously expanded upon the addition of exogenous adenine or guanine (Fig. 3), experiments were performed to

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ascertain whether a corresponding increase in the phosphorus of the pool also occurred. Fig. 7 shows that the rate of incorporation of radiophosphorus was not altered by the addition of large amounts of adenine. Chromatograms (ethanol/acetate: sulfate/*iso*propanol system) of the cold TCA-soluble fraction obtained from cells removed just prior to and also 30 minutes after the addition of the adenine, showed no significant differences in the distribution of the radiophosphorus. No free adenine or guanine was observed on the chromatogram of the early sample; the later sample however contained a large quantity of free adenine which was readily detected by ultraviolet light absorption. Free guanine could not be detected. No increase in the amount of purine nucleotide was found. It is concluded that the adenine in the expanded pool was present as the purine base, and not as the nucleotide.

Thus, the yeast cell can contain two kinds of purine pools: a purine nucleotide pool and a pool of purine bases.

The nucleotide pool may be specifically labeled by adding trace quantities of ^{14}C-adenine to the culture medium. Under this condition the ^{14}C-adenine becomes a part of the nucleoside phosphates of adenine and guanine and no free base can be detected. The kinetics of transfer of ^{14}C to nucleic acid from such a labeled nucleotide pool in the presence and absence of nonradioactive exogenous adenine or guanine is shown in Fig. 8. Even though the exogenous purine is accumulated within the cell (*cf.* Figs. 3 and 6), during the course of the experiment its presence as the free base has no effect upon the rate of transfer from the nucleotide pool to the nucleic acid fraction. Thus, rapid mixing of the two pools does not occur. On the other hand the purine base can compete with fructose carbon providing material for the formation

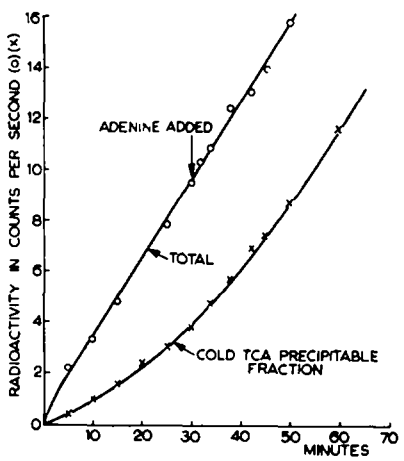

Fig. 7. Kinetics of incorporation of radiophosphorus (^{32}P-orthophosphate) during expansion of adenine pool. ^{12}C-adenine added at a concentration of 1 μmole per ml medium at time indicated and cell concentration was 1.0 mg wet weight cells per ml medium.

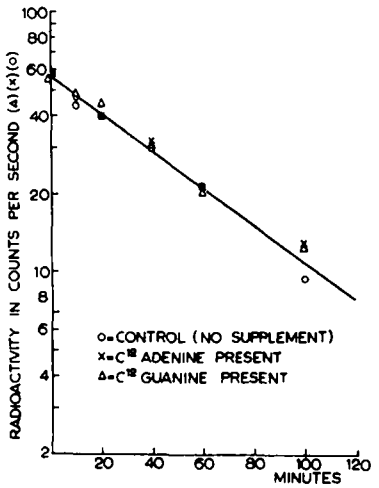

Fig. 8. Loss of ^{14}C-adenine carbon from the cold TCA-soluble fraction. Data obtained from cells briefly immersed in medium containing ^{14}C-adenine (carrier-free) and transferred after washing to nonradioactive medium containing supplements (×) of ^{12}C-adenine (0.74 μmole per ml medium); (△) ^{12}C-guanine (0.005 mg per ml medium); and (○) control (unsupplemented C medium). At t = 0, 80% of the incorporated radiocarbon was in the cold TCA-soluble fraction and at the end of the experiment the majority of the radiocarbon was contained in the hot TCA-soluble fraction. There was little loss of pool radiocarbon to the medium.

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of the nucleotide; carbon of either source must flow through the nucleotide pool in order to be used for nucleic acid synthesis. Thus, the nucleotide pool serves as a preferred source of carbon for nucleic acid synthesis. From these considerations it is evident that at least two distinct processes are involved in the incorporation of purines into the nucleic acid of yeast. One involves the accumulation of purines within the cell and the other involves alteration and selection for furnishing the proper building blocks for the nucleic acids.

Tests with other nucleic acid compounds

When mixtures of the 3′- and 5′-isomers of ^{14}C-cytidylic or ^{32}P- or ^{14}C-uridylic acids were used as supplements no radioactivity was incorporated by the cells. No diminution was observed in the exponential growth rate upon the addition of the supplements. The specific radioactivity of these compounds was sufficiently high so that trace levels of incorporation should have been observed were these compounds usable or degraded to usable products. In each case there was no detectable radioactivity in the cells even after several generations of cellular growth in the radioactive medium. Labeled nucleosides were not tested.

DISCUSSION

Fig. 9 summarizes the findings presented above in terms of the flow of purine carbon for synthesis of nucleic acid. Two metabolic pools of purine compounds serve as precursor material for nucleic acid synthesis.

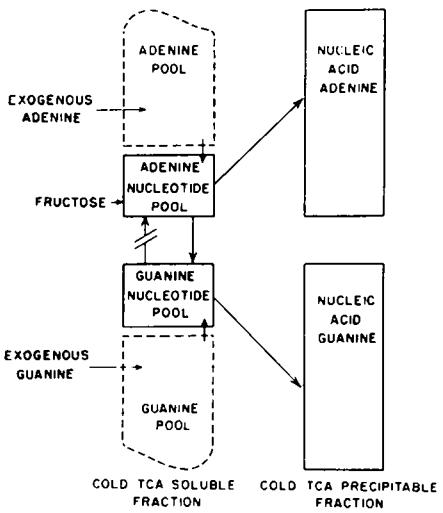

Fig. 9. Flow of purine carbon through metabolic pools in the synthesis of nucleic acid.

Pool of purine nucleotides

In the absence of purine supplementation a pool of purine nucleotides is formed deriving its carbon solely from the metabolism of fructose. In steady-state cells this pool remains fixed in size and comprises one or two per cent of the total carbon of the cell. It is in this pool that the conversion of one nucleotide to another occurs providing the proper materials for nucleic acid formation. The nucleotide pool is in the direct line of nucleic acid synthesis. The exponential rate of loss of ^{14}C from this pool (Fig. 8) and its subsequent appearance in nucleic acid indicates that there is complete mixing of the radioactive and non-radioactive nucleotide pool material, whether the latter is derived solely from fructose or also from exogenous purines.

Pool of purine bases

When the synthetic medium is supplemented by high concentrations of purine bases these materials are found concentrated within the cell at levels exceeding their external concentration. The quantity concentrated is dependent upon the exogenous concentration (Fig. 3). In the case of adenine supplementation the largest pool size

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observed was 57 μM per ml wet cells (156 μM per g dry weight) which is 7.5 times the reported solubility of adenine in water at 25° C. In the case of guanine supplementation the largest pool size found was 1 μM per ml of cells or about 30 times the solubility of guanine in water.

The expandable pool may compete with fructose in providing carbon for the nucleotide pool. At the highest internal concentrations almost all of the nucleotide carbon, and consequently nucleic acid purine carbon is derived from this pool rather than the *de novo* synthesis from fructose. On the other hand trace levels of exogenous purines are quickly incorporated into the nucleotide pool without much alteration of the flow of fructose carbon.

The material concentrated in the expandable pool is not converted to other related metabolic products. For example, adenine is concentrated as adenine and remains so until assimilated into the nucleotide pool. Once incorporated into the latter pool, conversion to the guanine nucleotide is possible.

Both pools are extractable with cold TCA, alcohol, or boiling water. Energy is required for pool formation and for transfer of carbon from the expandable pool to the nucleotide pool as well as for ultimate incorporation into nucleic acid. Little or no exchange is observed in the nucleotide pool with exogenous purines. The material concentrated in the expandable pool however has been observed to be less tightly bound. Some exchange with exogenous purines occurs when the pool is greatly expanded.

The mechanism for concentrating purines in the expandable pool is not known nor is the process of maintaining these high concentrations within the cell understood. The fact that purines are held within the cell at levels exceeding their solubility in water suggests that some association of the purine with other cytoplasmic molecules exists.

It is concluded that at least two processes are involved in the incorporation of exogenous purine into nucleic acid. One is the incorporating mechanism that builds up materials to concentrations exceeding that of the external environment. The other is the process in which alteration and selection occur, furnishing the proper compounds for the macromolecules.

SUMMARY

Exogenous purines are incorporated first into metabolic pools and thence into nucleic acids by exponentially growing *Candida utilis*. Kinetic studies show that a purine pool, whose size is determined by the amount of purine in the medium, forms first. This pool contributes material to a nucleotide pool whose pool size is independent of the purine concentration in the medium. Conversion of adenine to guanine occurs in the nucleotide pool which serves as a preferred source of purine for nucleic acid synthesis.

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