The Utilization of Purines and Their Ribosyl Derivatives for the Formation of Adenosine Triphosphate and Guanosine Triphosphate in the Rabbit Reticulocyte*

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SUMMARY

The rabbit reticulocyte is capable of utilizing purines and their ribosyl derivatives for nucleoside triphosphate formation. Adenine, adenosine, hypoxanthine, and inosine are used extensively for the synthesis of adenosine triphosphate. Xanthine, guanine, and guanosine are utilized primarily for the synthesis of guanosine triphosphate.

The inhibitor 6-diazo-5-oxo-L-norleucine inhibits the utilization of xanthine for guanosine triphosphate formation in rabbit erythrocytes, but does not affect its utilization for the synthesis of adenosine triphosphate. Similarly, the inhibitor interferes with the utilization of xanthine for guanosine triphosphate formation in human erythrocytes, without affecting its utilization for the synthesis of inosine monophosphate.

Earlier studies from this laboratory suggested that metabolic renewal of purine nucleotides may occur in the mammalian erythrocyte (1, 2) and led to investigations of the capacity of this cell to synthesize adenosine and guanosine triphosphate. These investigations showed that mature rabbit erythrocytes, incubated in vitro, do not synthesize the adenine or guanine portion of the acid-soluble purine nucleotides from their simple precursors (3). However, this cell does utilize either 5-amino-4-imidazolecarboxamide or its ribosyl derivative to form the purine portions of both ATP and GTP (3–5). Thus the mature rabbit erythrocyte, incubated in vitro, is capable of carrying out the final steps of purine nucleotide synthesis, but lacks the capacity for their over-all synthesis via the pathway de novo.

In contrast to the fully mature erythrocyte, the rabbit reticulocyte was shown to be capable of carrying out, in vitro, the entire reaction sequence for the biosynthesis de novo of the adenine of ATP and the guanine of GTP (6).

With respect to the utilization of preformed purines and their ribosyl derivatives for purine nucleotide formation, Lowy, Ramot, and London (7) found that in the mature rabbit erythrocyte a number of naturally occurring purines can serve, to a varying degree, as precursors of either ATP, GTP, or both, during incubation in vitro.

The present paper reports a study of the utilization of several purines and purine derivatives for the synthesis of ATP and GTP in the rabbit reticulocyte during incubation in vitro.

EXPERIMENTAL PROCEDURE

Preparation of Reticulocytes—Reticulocytosis was induced in New Zealand white rabbits, as previously described (6), and the animals were exsanguinated by cardiac puncture. The blood, collected in heparin, was centrifuged and the packed cells were washed twice by resuspension in 0.9% sodium chloride solution. The buffy coat was removed, and reticulocyte counts were performed on the pooled washed cells.

Incubation in Vitro—Suspensions of the washed cells were prepared and incubations were carried out as described previously (2). The radiocarbon-labeled purines and ribosyl derivatives were obtained commercially, and were added to the appropriate incubation flasks in concentrations similar to those used by Lowy et al. in their studies of mature erythrocytes (7). The xanthine-8-14C used was shown by paper chromatography to be free of contamination by radioactive hypoxanthine. Dr. Ralph Barclay kindly provided the 6-diazo-5-oxo-L-norleucine.

Isolation and Purification of Purines—ATP, GTP, and IMP were isolated and hydrolyzed as described by Lowy et al. (2, 8). The specific activities of the adenine, guanine, and hypoxanthine were determined. Xanthine, isolated by paper chromatography in the usual solvent system of 1-butanol-butyric acid-acetate acid-NH₄OH (75:37.5:25:2.5) (System I), was further purified by rechromatography in isoamyl alcohol-5% NaH₂PO₄ (1:1) (System II).

RESULTS AND DISCUSSION

The data obtained after the incubation of rabbit reticulocytes with several naturally occurring purines, or with their ribosyl derivatives, are summarized in Table I. The reticulocyte, like the mature rabbit erythrocyte, utilized these compounds to synthesize ATP and GTP during incubation in vitro; however, the
patterns of incorporation differed in some respects from those found with the mature cell (7).

Adenine-8-14C was utilized primarily for the formation of adenine nucleotide in the reticulocyte, as had previously been found in the mature rabbit erythrocyte. However, the nucleoside, adenosine-8-14C, was utilized for the synthesis of both ATP and GTP, with a pattern of incorporation into the purine portions of the two nucleotides similar to that observed with hypoxanthine-8-14C and with inosine-8-14C. This finding suggests that direct phosphorylation to adenylic acid, or preliminary conversion to adenine, was relatively unimportant in the utilization of adenosine-8-14C, and that the major route for utilization of this nucleoside was via a deamination product. This interpretation is consistent with the wide distribution of adenosine deaminase in rabbit tissues. In the mature cell, following incubation with (a) formate-2-14C and either 5-amino-4-imidazolecarboxamide or its ribosyl derivative, (b) hypoxanthine-8-14C, or (c) inosine-8-14C, the relative specific activity of the guanine of GTP was greater than that of the adenine of ATP. In the reticulocyte, following incubation with these compounds or with sodium formate-2-14C and the other small molecule precursors of the purine ring, the specific activity of the guanine of GTP was much less than that of the adenine of ATP (6). These differences in labeling pattern may result from an alteration in the relative rates of conversion of IMP to AMP and GMP, or in the relative sizes of the metabolic pools of the purine nucleotides.

Guanine-8-14C and guanosine-8-14C were utilized extensively for the synthesis of ATP by the reticulocyte, but to a very limited extent for the synthesis of GTP. This pattern of utilization is similar to that reported by Lowy, Williams, and London (7) for the mature rabbit erythrocyte. They suggested that the small amount of labeling which they found in the adenine of ATP after incubation with guanine-8-14C or guanosine-8-14C could have occurred through a mechanism of reductive deamination. This suggestion is supported by recent evidence of guanylic acid reductase activity in rabbit erythrocytes that resembles, in certain respects, the activity of the GMP reductase isolated from colon bacilli (8-11).

Xanthine-8-14C was utilized by the rabbit reticulocyte for the synthesis of GTP and ATP. The relative specific activity of the adenine of ATP was about half that of the guanine of GTP. In mature rabbit erythrocytes, on the contrary, the relative specific activity of the adenine of ATP was a small fraction of that found for the guanine of GTP (7). This difference between the two cell types may be a reflection of metabolic differences in the utilization of xanthine, or of differences in the pool sizes of the purine nucleotides.

The utilization of xanthine-8-14C is presumably via XMP, the intermediate nucleotide in the conversion of IMP to GMP. The irreversibility of the dehydrogenase reaction, whereby IMP is converted to XMP, precludes the direct conversion of XMP to IMP by that route as a mechanism in the utilization of xanthine for the synthesis of adenine nucleotides. However, the conver-

\begin{table}[ht]
\centering
\caption{Utilization of purines and purine derivatives for formation of purine nucleotides in rabbit reticulocytes*}
\begin{tabular}{|c|c|c|c|c|}
\hline
\textbf{Compound} & \textbf{Incubated} & \textbf{Isolated} & \textbf{Specific activity} & \textbf{Relative specific activity} \\
\hline
 & \text{pmoles/ml} & & \text{cpm/pmol} & \text{Adenine of ATP} & \text{Guanine of GTP} \\
\hline
Hypoxanthine-8-14C & 1.1 & 7.8 \times 10^4 & 10.7 & 5 \\
Adenine-8-14C & 1.2 & 7.6 \times 10^4 & 46.3 & 1 \\
Guanine-8-14C & 1.1 & 5.2 \times 10^4 & 5.1 & 67 \\
Insosine-8-14C & 2.1 & 4.7 \times 10^4 & 30 & 7.9 \\
Adenosine-8-14C & 2.1 & 4.7 \times 10^4 & 55.8 & 12.2 \\
Guanosine-8-14C & 2.1 & 4.7 \times 10^4 & 8.9 & 66.4 \\
\hline
\end{tabular}
\end{table}

* Erythrocyte preparations contained 80% reticulocytes and 0.7% leukocytes.
† Washed cells, 20 ml, were incubated in 20 ml of isotonic sodium phosphate buffer (pH 7.2) with glucose (2 amoles per ml of erythrocytes per hour) for 3 hours at 37°C.
‡ Relative specific activity was calculated as follows.
\[ \text{Cpm per \( \mu \)mole of isolated purine} = \frac{\text{Cpm per \( \mu \)mole of incubated labeled compound}}{100} \]

\begin{table}[ht]
\centering
\caption{Effect of 6-diazo-5-oxo-L-norleucine on utilization of xanthine for formation of purine nucleotides in rabbit reticulocytes*}
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Compound} & \textbf{Incubated} & \textbf{Isolated} & \textbf{Relative specific activity} \\
\hline
 & \text{pmoles/ml} & & \text{cpm/pmol} & \text{Adenine of ATP} & \text{Guanine of GTP} & \text{Xanthine-8-14C} \\
\hline
 & & & & \text{Adenine of ATP} & \text{Guanine of GTP} & \text{Xanthine-8-14C} \\
\hline
Xanthine-8-14C & 1.8 & 8.5 \times 10^4 & 1.67 & 3.8 \\
Xanthine-8-14C & 1.8 & 8.5 \times 10^4 & 1.72 & 0.639 \\
With 6-diazo-5-oxo-L-norleucine & 1.2 & & & & & \\
\hline
\end{tabular}
\end{table}

* Erythrocyte preparations contained 80% reticulocytes and 0.4% leukocytes.
† Washed cells, 45 ml, were incubated in 45 ml of isotonic sodium phosphate buffer (pH 7.2) with glucose (2 amoles per ml of erythrocytes per hour) for 3 hours at 37°C.

\begin{table}[ht]
\centering
\caption{Effect of 6-diazo-5-oxo-L-norleucine on utilization of xanthine for formation of purine nucleotides in human erythrocytes*}
\begin{tabular}{|c|c|c|c|c|}
\hline
\textbf{Compound} & \textbf{Incubated} & \textbf{Isolated} & \textbf{Relative specific activity} \\
\hline
 & \text{pmoles/ml} & & \text{cpm/pmol} & \text{Adenine of ATP} & \text{Guanine of GTP} & \text{Xanthine-8-14C} \\
\hline
 & & & & \text{Adenine of ATP} & \text{Guanine of GTP} & \text{Xanthine-8-14C} \\
\hline
Xanthine-8-14C & 0.5 & 5.3 \times 10^4 & 0 & 23.2 & 0.3 \\
Xanthine-8-14C & 0.5 & 5.3 \times 10^4 & 0 & 1.0 & 0.3 \\
With 6-diazo-5-oxo-L-norleucine & 1.2 & & & & & \\
\hline
\end{tabular}
\end{table}

* Normal human erythrocytes, luffys coat removed.
† Washed cells (three washes in NaCl), 100 ml, were incubated in 100 ml of isotonic sodium phosphate buffer (pH 7.2) with glucose (2 amoles per ml of erythrocytes per hour) for 3 hours at 37°C.
sion of XMP to GMP and reductive deamination of the GMP to IMP, as suggested by Lowy et al. (7), could account for the observed utilization of xanthine for the synthesis of adenine nucleotides.

The conversion of XMP to GMP, which requires glutamine, is inhibited in the presence of the glutamine analogue, 6-diazo-5-oxo-L-norleucine. The relative specific activity of the adenine of ATP in rabbit reticulocytes after incubation with xanthine-$^{14}$C was unchanged in the presence of an inhibitor concentration (1.2 mM) that produced a 100-fold decrease in the relative specific activity of the guanine of GTP (Table II). This observation is interpreted to indicate that the principal metabolic route whereby xanthine is utilized for the synthesis of ATP does not include the reductive deamination of GMP.

The observation that guanine-$^{14}$C and guanosine-$^{14}$C were utilized to a much lesser extent than xanthine-$^{14}$C for the synthesis of ATP is also consistent with the existence of an undefined pathway, not proceeding through GMP, by which xanthine may be utilized for the synthesis of ATP.

The mature human erythrocyte has been shown to be deficient in the capacity to convert IMP to AMP (8). The finding of considerable radioactivity in the hypoxanthine of IMP after the incubation of human erythrocytes with guanine or guanosine may be interpreted as evidence for a reductive deamination of GMP in the human erythrocyte similar to that suggested for the erythrocytes of other species, including the rabbit (8-11).

In the present study, human erythrocytes were incubated with xanthine-$^{14}$C, after which ATP, GTP, and IMP were isolated and their relative specific activities were determined (Table III). After paper chromatography in System I and identification by spectrophotometry, the hypoxanthine samples were twice re-chromatographed on paper in System II to ensure freedom of the final sample any from contamination with small amounts of highly radioactive xanthine-$^{14}$C. In the absence of 6-diazo-5-oxo-L-norleucine, extensive incorporation of radioactivity into the guanine of GTP was found, only slight incorporation into the hypoxanthine of IMP, and none into the adenine of ATP. In the presence of 6-diazo-5-oxo-L-norleucine, the incorporation of radioactivity from xanthine-$^{14}$C into the guanine of GTP was markedly inhibited, but the relative specific activity of the isolated hypoxanthine of IMP was the same as that from IMP isolated after incubation without 6-diazo-5-oxo-L-norleucine. These results are interpreted to indicate that the mechanism of conversion of xanthine to IMP is not via GMP.

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REFERENCES
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