Transport of Adenine, Hypoxanthine and Uracil into *Escherichia coli*

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Uptake of adenine, hypoxanthine and uracil by an unc strain of *Escherichia coli* is inhibited by uncouplers or when phosphate in the medium is replaced by less than 1 mM-arsenate, indicating a need for both a protonmotive force and phosphorylated metabolites. The rate of uptake of adenine or hypoxanthine was not markedly affected by a genetic deficiency of purine nucleoside phosphorylase. In two mutants with undetected adenine phosphoribosyltransferase, the rate of adenine uptake was about 30% of that in their parent strain, and evidence was obtained to confirm that adenine had then been utilized via purine nucleoside phosphorylase. In a strain deficient in both enzymes adenine uptake was about 1% of that shown by wild-type strains. Uptake of hypoxanthine was similarly limited in a strain lacking purine nucleoside phosphorylase, hypoxanthine phosphoribosyltransferase and guanine phosphoribosyltransferase. Deficiency of uracil phosphoribosyltransferase severely limits uracil uptake, but the defect can be circumvented by addition of inosine, which presumably provides ribose 1-phosphate for reversal of uridine phosphorylase. The results indicate that there are porter systems for adenine, hypoxanthine and uracil dependent on a protonmotive force and facilitated by intracellular metabolism of the free bases.

Nucleic acid bases and their ribosides are readily converted into nucleotides by *Escherichia coli*, only a small proportion being found as free base or nucleoside inside the cells. Hochstadt-Ozer & Stadtman (1971a,b,c) concluded that adenine is taken up by a group translocation involving adenine phosphoribosyltransferase (EC 2.4.2.7). Hochstadt (1974) has reviewed evidence for this and similar group-translocation processes, including reports that uracil is not taken up by mutants lacking uracil phosphoribosyltransferase (EC 2.4.2.9) (Beck & Ingraham, 1971; Pierard et al., 1972). Evidence against a simple group-translocation process was reported by Roy-Burman & Visser (1975), who found $K_m$ values for uptake to be much smaller than those reported for purified phosphoribosyltransferases, and claimed that several purines and pyrimidines, including adenine and uracil, were accumulated as free bases against a concentration gradient. It is, however, difficult to be sure that the small amounts of free intracellular bases were not formed by degradation of nucleotides during the extraction procedure. Kocharian et al. (1975) have given evidence that adenine phosphoribosyltransferase is not needed for adenine utilization.

In the present paper, I aim to clarify the relation between energy coupling and metabolic conversions in the transport of adenine, hypoxanthine and uracil.

**Materials and Methods**

**Bacteria**

In addition to *E. coli* B, several *E. coli* K12 strains were used. Strain A103c (Ymel,ato,fadR*,lacI,metE,-rha,uncA) and its parent strain A1002 (isogenic except for being ltv,uncA+) were both described by Schairer & Haddock (1972); strain PA309 (thr,leu,-thi,his,try,arg,H,ygal,maA,xyl,ara,mtl,tonA,str; cf. Bachman, 1972) was from Professor H. L. Kornberg; strains NE553 and NE571 were mutants of strain PA309 resistant to 2,6-diaminopurine and found to be deficient in adenine phosphoribosyltransferase (apt genotype; Jochimsen et al., 1975). They arose in separate treatments of growing bacteria with 2 μg of N-methyl-N'-nitro-N-nitrosoguanidine/ml as described by Miller (1972). Strain 4K3 (thr,leu,ser,ars,upp; Beacham & Pritchard, 1971) had been derived from *E. coli* C600 via strain 4K (Glover, 1970); strains S0609 (ara,Δpro-gpt-lac,thi,-hpt,pup,purH or purJ,strA), S0199 (metB,strA,purE) and its derivatives S0312 (also pup) and S0446 (also pup,apt) are described by Jochimsen et al. (1975). Relevant phenotypes were routinely checked by appropriate growth tests.

**Growth**

Media contained glucose and inorganic salts (Thomas et al., 1970) supplemented with thiamin hydrochloride (1 mg/litre) and trace metal salts (Burton, 1976). Required L-amino acids or purines were added at 10 mg/litre. L-Histidine hydrochloride was also added for all purine-requiring strains. Both L-isoleucine and L-valine were added for both strains A1002 and A103c. Acid-hydrolysed casein (0.1%; Oxoid, London SE1 9HF, U.K.) was also
added for strains NE571 and 4K3, since growth was otherwise poor. Adenosine (50 mg/litre) was used as the purine source for strain SØ609, hypoxanthine for strain SØ446 and adenine for strains SØ199 and 312. Bacteria were grown at 37°C with aeration to 2 × 10^8–4 × 10^8 cells/ml, centrifuged (4500 g for 10 min) at 4°C, suspended in the original volume of phosphate/saline (24 mM-Na_2HPO_4, 11 mM-KH_2PO_4, 0.1 mM-NaCl, 83 μM-MgSO_4, pH 7.3), centrifuged again and suspended in the phosphate/saline at about 2 × 10^9–5 × 10^9 cells/ml. Phosphates were omitted from the washing solution when the effects of arsenate were to be examined. Cell concentrations were calculated turbidimetrically on the basis of a calibration against viable cell count with *E. coli* B (1.9 × 10^9 dry cells weighed 1.0 mg).

**Measurement of uptake by filtration and washing**

Washed bacteria stored for less than 2 h at 0–4°C were preincubated for 15 min with the energy source (usually glucose) and chloramphenicol in 1–2 ml of phosphate/saline shaken at 37°C in a 25 ml conical flask. If further additions were greater than 100 μl, they were dissolved in phosphate/saline. Measured samples (0.25–0.35 ml) were removed directly on to a 21 mm-diameter cellulose acetate filter (Oxoid) lying on a stainless-steel mesh support connected to suction. The filters were washed quickly with 3 × 1 ml of phosphate/saline warmed to 37°C to avoid cold shock and followed by 0.5 ml of ethanol to speed drying. Radioactivity was measured with efficiencies of 12% for ^3H and 66% for ^14C by liquid-scintillation counting (see Burton, 1976).

**Measurement of uptake without filtration**

This procedure was used to eliminate any losses by leakage during washing. Approx. 2 ml of incubation mixture containing 1–2 mg dry wt. of bacteria was filtered as above but by using a filter tower and with no washing: 30 s after filtration appeared to be complete, the moist filter was removed from the suction apparatus and immediately weighed. Evaporation was negligible, and so the volume of water on the filter (about 25 μl) could be found after subtraction of the dry weight of the filter plus bacteria. The filters were dried by air at room temperature. To obviate any colour quenching when carbonyl cyanide m-chlorophenylhydrazone was present, ^14C-labelled precursors were used and the ^14C was measured at 25% efficiency in a solid-sample counter. Comparison of the radioactivity found per mg of water that had been on the moist filter with the concentration of radioactivity in the medium indicated the extent of intracellular concentration of radioactivity.

**Incorporation into acid-insoluble material**

Samples were taken into at least 10 vol. of 0.5 M-HClO_4 at 0°C, filtered on Oxoid filters and washed with 3 × 3 ml of ice-cold 5% trichloroacetic acid, followed by 1 ml of ethanol, before measurement of the radioactivity.

**Intracellular fates of ^14C-labelled compounds**

Washed bacteria (approx. 4 × 10^8 cells) were incubated in 4.4 ml of phosphate/saline containing 52 mM-glucose and 0.36 mM-chloramphenicol. After 15 min, 0.2 μCi of ^14C-labelled substrate was added to give a final concentration of approx. 0.8 μM. After incubation for 0.5–3 min, the bacteria were collected on an Oxoid filter and washed with 3 × 1 ml of phosphate/saline at 37°C. The filter was then immersed in 0.3 ml of 4 M-formic acid at 0°C to extract nucleotides (Bagnara & Finch, 1974) and stored at −70°C until required. The interval was about 30 s between pouring the bacteria on to the filter and immersion in formic acid. For [^14C]adenine or [^14C]hypoxanthine portions (50–100 μl) of the extract were applied after centrifugation (6000 g for 20 min) as a 1.5 cm band on to duplicate papers (0.55 m × 0.4 m) and electrophoresed under ‘white spirit’ for about 40 min with 2 kV applied voltage by using 7% (v/v) formic acid for one paper and 5% (v/v) pyridine/0.5% acetic acid (pH 6.5) for the other. Free adenine was also separated by paper chromatography in ethylacetate/98% (w/w) formic acid/water (7:2:1, by vol.). Descending paper chromatography in propan-2-ol/conc. aq. NH_3 (sp.gr. 0.880)/water (14:3:3 by vol.) was used for [^14C]glycine. Strips were examined in a paper-strip chromatogram scanner and the positions of the ^14C peaks compared with the positions of appropriate markers run on the same sheet of paper. Quantitative comparisons were based on liquid-scintillation counting of the appropriate areas of paper.

**Phosphoribosyltransferases**

The adenine enzyme was assayed as described by Hochstadt-Ozer & Stadtman (1971a), except that AMP and adenine were separated by descending paper chromatography in 1 M-ammonium acetate. The uracil enzyme was assayed similarly except that 10 mM-GTP was added (Molloy & Finch, 1969) and the chromatography was on DEAE-paper developed by 0.01 M-Tris/HCl, pH 8.0. For testing the inhibition of the adenine enzyme by hypoxanthine, the concentrations of adenine, 5-phosphoribosyl 1-pyrophosphate and extract protein were lowered to 0.18 μM, 0.4 mM and 11 μg/ml respectively.

**Other materials**

Radiochemicals were from The Radiochemical Centre, Amersham, Bucks., U.K. 3,5,3',4'-Tetra-
chlorosalicylanilide was very kindly given by Dr. W. A. Hamilton, University of Aberdeen.

Results

Concentration-dependence for adenine uptake

Initially, adenine uptake was studied because of the extensive work of Hochstadt-Ozer & Stadtman (1971a,b,c) with this base. They reported $K_m$ values of 15–20$\mu$M for adenine uptake by non-growing $E. coli$ or by membrane vesicles and stressed that these $K_m$ values agreed with those found for the purified adenine phosphoribosyltransferase. However, Roy-Burman & Visser (1975) reported much lower $K_m$ values for the uptake by growing $E. coli$ (e.g. 0.12$\mu$M for strain B), and I have approximately confirmed this particular value. Since regulatory controls in growing bacteria (cf. Ames, 1964) might cause erroneously low $K_m$ values, growth was prevented in further experiments by the presence of chloramphenicol and the absence of a readily utilizable nitrogen source. As shown in Fig. 1, the total uptake process is virtually saturated even at the lowest concentration of adenine used (0.78$\mu$M). Quite similar results were obtained with bacteria that had been washed and stored for 48 h at 4°C. The high $K_m$ values of Hochstadt-Ozer & Stadtman (1971c) are evidently not due to the lack of growth or to the aging of the bacteria. Because the rate of uptake may fall rapidly after the first 2 min, the duration of the uptake measurements was kept below 3 min by changing the bacterial concentration in parallel with the adenine concentration. The rates of uptake observed in Fig. 1 are at least as great as those in growing cultures. Approx. 87% of the radioactivity in the washed cells was soluble in 0.5M HClO$_4$ at 0°C, at least 75% being acid-soluble nucleotides.

Energy coupling in the uptake of adenine, hypoxanthine and uracil

Adenine uptake occurs normally in an unc$A$ strain, A103c (Schairer & Haddock, 1972), which has no adenosine triphosphatase to interconvert ATP energy and the proton motive force. The uptake is, however, more sensitive to carbonyl cyanide $m$-chlorophenylhydrazone than it is in the isogenic unc$A^+$ strain A1002 (Fig. 2). The sensitivity to uncouplers is not appreciably affected by increasing the concentration of adenine 100-fold (Fig. 3). Anaerobiosis also inhibits uptake in both strains. Carbonyl cyanide $m$-chlorophenylhydrazone and other agents with uncoupling activity have quite similar effects on the uptake of adenine, hypoxanthine, uracil and glycine in strain A103c (Table 1), but they have negligible effects on the rate of O$_2$ consumption. The effects of carbonyl cyanide $m$-chlorophenylhydrazone were not reversed by dithiothreitol or mercaptoethanol, showing that it was not inhibiting by virtue of its reported action on thiol groups (Kaback et al., 1974). Since glycine transport is linked to proton movements (Collins et al., 1976), the findings imply that uptake of adenine, hypoxanthine or uracil needs a protonmotive force independently of any need for ATP or other energy-rich metabolites.

Replacement of phosphate in the incubation medium by arsenate severely inhibits the uptake of adenine, hypoxanthine and uracil, but not the uptake of glycine (Fig. 4), originally shown by Berger & Heppel (1974) to be insensitive to arsenate. As might be expected in the presence of chloramphenicol, the $[^{14}C]$glycine was found to remain as the free amino acid, in contrast with the nucleic acid bases, which were found predominantly as nucleotides or nucleic acids.
Fig. 2. Effects of carbonyl cyanide m-chlorophenylhydrazone and requirement for O₂ on adenine uptake in uncA and uncA⁺ strains

Conditions were as for Fig. 1, but 4.7 mM-glucose, 0.32 mM-chloramphenicol, 5 × 10⁷ cells/ml and 0.78 μM-[2-³H]adenine were used, with: O, no further addition; ▲, 42 μM-carbonyl cyanide m-chlorophenylhydrazone at -15 min; ●, 10.5 μM-carbonyl cyanide m-chlorophenylhydrazone at -15 min; ●, 42 μM-carbonyl cyanide m-chlorophenylhydrazone at zero time; □, at -5 min flask contents were transferred to a test tube at 37°C and bubbled with O₂-free N₂. (a) Strain A103c (uncA). (b) Strain A1002 (uncA⁺).

Table 1. Effects of agents with uncoupling activity on uptake of adenine, hypoxanthine, uracil and glycine in an uncA strain

Conditions were as for Fig. 4. Values are uptake measured 2 min after addition of the transported substrate expressed as percentages of that without uncoupler. Uncouplers were added to give the final concentrations shown 1 min before the substrate: 1 mM-1,4-DL-dithiothreitol or 1 mM-mercaptoethanol was added 0.5 min before substrate where indicated. The thiol compounds had no significant effect by themselves.

<table>
<thead>
<tr>
<th>Uncoupler</th>
<th>Uncoupler concn. (mM)</th>
<th>Adenine</th>
<th>Hypoxanthine</th>
<th>Uracil</th>
<th>Glycine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbonyl cyanide m-chlorophenylhydrazone</td>
<td>0.009</td>
<td>50</td>
<td>18</td>
<td>19</td>
<td>14</td>
</tr>
<tr>
<td>Carbonyl cyanide m-chlorophenylhydrazone</td>
<td>0.044</td>
<td>24</td>
<td>12</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Carbonyl cyanide m-chlorophenylhydrazone</td>
<td>0.044</td>
<td>24</td>
<td>8</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Carbonyl cyanide m-chlorophenylhydrazone and</td>
<td>0.044</td>
<td>24</td>
<td>10</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>mercaptoethanol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetrachlorosalicylanilide</td>
<td>0.018</td>
<td>—</td>
<td>25</td>
<td>51</td>
<td>31</td>
</tr>
<tr>
<td>Tetrachlorosalicylanilide</td>
<td>0.036</td>
<td>15</td>
<td>10</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>2,4-Dinitrophenol</td>
<td>0.88</td>
<td>66</td>
<td>85</td>
<td>73</td>
<td>73</td>
</tr>
<tr>
<td>2,4-Dinitrophenol</td>
<td>1.76</td>
<td>52</td>
<td>70</td>
<td>48</td>
<td>51</td>
</tr>
</tbody>
</table>

Since arsenate inhibits the formation of phosphate esters by phosphorolysis and other substrate-linked phosphorylation reactions, there may be a coupling between transport and intracellular metabolism of the bases by reaction with ribose 1-phosphate or 5-phosphoribosyl 1-pyrophosphate. This suggestion
is consistent with the ability of uridine to support adenine uptake better than lactate does in the absence of glucose (Fig. 5). Uridine and lactate give similar rates of O₂ consumption (38% of that given by glucose) and are thus expected to generate similar protonmotive forces.

**Uptake of adenine and hypoxanthine in a pup mutant**

Both purines are taken up by the pup strain S0312 at rates closely similar to those shown by the pup⁺ parent, strain S0199. For this comparison, the same medium, which contained adenine, hypoxanthine and histidine was used to grow both strains, and uptake was measured as for Fig. 2. Most, if not all, of the transport of adenine and hypoxanthine does not therefore depend on purine nucleotide phosphorylase.

**Role of adenine phosphoribosyltransferase in adenine uptake**

By using the method of Kalle & Gots (1961), who obtained apt mutants of *Salmonella typhimurium* by selection for resistance to 2,6-diaminopurine, similar mutants of *E. coli* resistant to 2,6-diaminopurine at 500 µg/ml were isolated. No adenine phosphoribosyltransferase activity was found in strains NE553 and NE571, and adenine was taken up much more slowly than by the parent strain, PA309 (Fig. 6). Also, in the apt strains less than 7% of the ³H taken up was found in acid-insoluble material, compared with 20–50% in strain PA309. The large fraction of macromolecular synthesis in the parent strain is a consequence of the richer growth medium that was used for this particular experiment because of the poor growth of strain NE571 on simpler media. The smaller incorporation of ³H into macromolecules by the apt strains suggested that the [³H]adenine might not have been converted into adenine nucleotides in the conditions of the uptake measurements.

In further experiments, strains PA309 and NE553 were grown on minimal medium supplemented with the required amino acids, washed and supplied with [8-¹⁴C]adenine under the usual conditions for uptake measurement as in Fig. 1, except that L-histidine was
Fig. 4. Inhibition of uptake of adenine, hypoxanthine and uracil by arsenate

Strain A103c was washed and re-suspended in Tris/saline (0.1 m-NaCl, 0.058 m-Tris and 0.65 mm-MgSO₄ adjusted to pH 7.3 by adding HCl) at 1.7 × 10⁸ cells/ml. Chloramphenicol and glucose were added as for Fig. 1. Additions were made as follows, 0.5 min before radioactive substrate: ○, none; ●, 4.6 mm-Na₂HPO₄ (final concentration) ▲, 4.6 mm-Na₂HAsO₄; △, 0.92 mm-Na₂HAsO₄; ▽, 0.23 mm-Na₂HAsO₄. (a) [2-³H]Adenine (0.77 μM, 115 μCi/litre). (b) [2-¹⁴C]Uracil (0.85 μM, 12 μCi/litre). (c) [8-¹⁴C]Hypoxanthine (0.91 μM, 47 μCi/litre). (d) [1-¹⁴C]Glycine (0.91 μM, 51 μCi/litre).

added to prevent histidine biosynthesis, which could conceivably generate ¹⁴C-labelled 5-amino-4-imidazolecarboxamide ribonucleotide and hence [¹⁴C]-IMP via [¹⁴C]ATP. After uptake of [¹⁴C]adenine for 1 or 2 min, the mixtures were rapidly filtered, the bacteria washed and nucleotides extracted with 4 M-formic acid. Strain NE553 gave essentially one radioactive component, which migrated on electrophoresis as 5'IMP. The parent, strain PA309, gave little [¹⁴C]IMP, most ¹⁴C being in the areas occupied by AMP, NAD and ADP. The deamination of adenine in strain NE553 could only have occurred at the nucleoside level (Koch & Vallee, 1959; Jochimsen et al., 1975), and thus the adenine must have been first converted into adenosine, presumably by purine nucleoside phosphorylase (EC 2.4.2.1). The uptake could not have been linked to a hypothetical latent adenine phosphoribosyltransferase, since the ¹⁴C would then have been found mainly in adenine nucleotides.
Fig. 5. Abilities of certain carbon sources to support adenine uptake by the uncA strain, A103c
Details were as for Fig. 2, except that $3.2 \times 10^7$ cells/ml were used, and the carbon sources added at -15 min were: ○, none; ●, 4.9 mM glucose; ■, 22 mM DL-lactate; △, 8.8 mM uridine; ▲, 22 mM DL-lactate plus 8.8 mM uridine.

Uptake of adenine and hypoxanthine in strains lacking purine nucleoside phosphorylase and phosphoribosyltransferase enzymes

Uptake and metabolism of the two purines are very much diminished in pup strains that are also deficient in the appropriate phosphoribosyltransferase activity. Because of their deficiency in the metabolism of adenine or hypoxanthine, uptake was measured without any washing in case the free purines were leached out of the cells.

With strain S0446 (apt,pup), [8-14C]adenine was incorporated into HClO$_4$-insoluble material at about 0.2% of the rate for wild-type bacteria. The total uptake in strain S0446 was considerably greater than that into acid-insoluble material, especially in the first 90s, but little $^{14}$C was lost on washing. Carbonyl cyanide $m$-chlorophenylhydrazone inhibited the uptake strongly (Fig. 7). Between 15 and 20% of the $^{14}$C extracted from washed bacteria by 4M-formic acid was found as free adenine after uptake for 1 or 3 min. Adenine is readily taken up by

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spontaneous revertants of strain SØ446, which have recovered adenine phosphoribosyltransferase activity.

The analogous uptake of [8-14C]hypoxanthine by strain SØ609 (hpt,gpt,pup) was also inhibited by carbonyl cyanide m-chlorophenylhydrazone, but most of the acid-soluble 14C was lost from the bacteria by washing on the filters (Fig. 8). The residual acid-soluble 14C appeared to be present both as hypoxanthine and as nucleotides, but reliable measurements were not obtained.

Strain SØ609 has adenine phosphoribosyltransferase and can use adenine readily. Likewise strain SØ446 will use hypoxanthine normally.

Concentration gradient of free adenine

The intracellular concentrations of free [14C]-adenine detected in strain SØ446 represent an accumulation of 30-fold or less over the external concentration, assuming 2.7 µl of cell water/mg dry wt. of bacteria (Winkler & Wilson, 1966). This contrasts with a 900-fold accumulation of glycine seen in Fig. 4(d), or similar accumulations of cytidine and thymidine found by Munch-Petersen & Mygind (1976). However, the distribution of free [14C]-adenine in strain SØ446 might underestimate the actual concentration gradient, because there can be an endogenous production of free adenine during the growth of similar strains (Kocharian et al., 1975).

Roy-Burman & Visser (1975) reported a 144-fold concentration of free adenine in E. coli B, but this could possibly be an overestimate, owing to enzymic degradation of adenine nucleotides during the washing and extraction, or an underestimate, owing to metabolism of free adenine during washing. Since the major route for producing free adenine must be purine nucleoside phosphorylase, it is desirable to use other extraction methods and to study a pup strain. When the fate of [14C]adenine was examined in strain SØ609, up to 9% of the 14C extracted by 4M-formic acid was found as free adenine, representing an internal/external concentration ratio of 90 at 10–15 s.

Concentration gradient of free hypoxanthine

Although the concentrations of hypoxanthine accumulated in strain SØ609 could not be measured, the internal concentration could not have been greater than 25 times the external concentration even if all the material that leaked out on washing had been free hypoxanthine.

Uptake of uracil in a strain without uracil phosphoribosyltransferase

Mutants in the upp gene lack uracil phosphoribosyltransferase and have been reported to take up negligible amounts of uracil (Beck & Ingraham, 1971; Pierard et al., 1972). Uracil is, however, readily taken up by E. coli B, which has also been reported to lack the same enzyme (von Dippe et al., 1975), but I can detect considerable activity in extracts of this strain in the presence of 10mm-GTP. Molloy & Finch (1969) originally showed that GTP is a strong activator of uracil phosphoribosyltransferase in E. coli.

To investigate the possible role of this enzyme in uracil transport a upp strain, 4K3, known to be resistant to uracil analogues (Beacham & Pritchard, 1971), has been examined further. No activity of the uracil phosphoribosyltransferase was detected in the presence of 10mm-GTP, and little [14C]uracil was taken up even when measured without washing the bacteria. However, addition of inosine or adenosine facilitated rapid uptake of uracil, whereas deoxyinosine had virtually no effect (Fig. 9). Since the 14C could be incorporated into nucleic acids if ammonium salts were supplied, the uracil was presumably metabolized by uridine phosphorylase (EC 2.4.2.3) and uridine kinase (EC 2.7.1.48), ribose 1-phosphate being generated by purine nucleoside phosphorylase, for which inosine is a better substrate than adenosine (Robertson & Höffee, 1973). These results indicate that the uracil-transport system requires removal of intracellular uracil by either the phosphoribosyltransferase or uridine phosphorylase.

Cross-inhibition effects

In the apt,pup strain SØ446, 2.2mm-adenine inhibited the uptake of 0.8 µM-hypoxanthine by 53%.

Fig. 8. Uptake of hypoxanthine by strain SØ609 (hpt,gpt,pup)

Conditions were as for Fig. 7, but [8-14C]hypoxanthine (0.87 µM, 52 Ci/mol) was used and 1.53 × 10^6 cells. Uptake with washing; uptake with no washing; uptake with no washing; uptake into nucleic acids.
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but 4 mM-uracil had no effect. Likewise 0.73 mM-hypoxanthine inhibited the uptake of 0.8 \( \mu \)M-adenine by 61% in strain S0609 (hpt, gpt, pup), 4 mM-uracil again having no effect. Because of the strains used, these inhibitions could not be due to competition for ribose phosphates or to feedback inhibition by nucleotides formed from the inhibitory purine. Uracil is apparently transported by a different system from those involved in hypoxanthine or adenine uptake.

The effect of hypoxanthine on adenine uptake is unlikely to be due to an action on adenine phosphoribosyltransferase, because 3 mM-hypoxanthine inhibited this reaction by only about 15% when the concentration of adenine was only 10.18 \( \mu \)M. Thus hypoxanthine appears to inhibit the adenine-transport system directly. A reciprocal situation arises in the action of adenine on hypoxanthine uptake by strain S0446. It is not yet possible to say if the two purines are transported by the same or by different porter systems.

Discussion

The findings in the present paper can be explained if adenine, hypoxanthine and uracil are taken up by transporter systems that are coupled to a proton-motive force, but are facilitated if the intracellular base is removed by a phosphoribosyltransferase. In the absence of such an enzyme, its function can be partly replaced by an appropriate nucleoside phosphorylase acting in the direction of nucleoside synthesis. Studies of spheroplasts have shown that the nucleoside phosphorylases are located intracellularly and not in the periplasmic space (Taketo & Kuno, 1972), despite the partial release of those enzymes by osmotic shock. This view has been elegantly confirmed by Munch-Petersen & Mygind (1976), who found that mutants that cannot transport nucleosides into the cell are unable to phosphorolysse nucleosides.

Although the factors that limit the intracellular accumulation of free bases are as yet poorly understood, appreciable concentration gradients of adenine are built up. There is therefore much evidence pointing to transport systems for the free bases and none to implicate a group-translocation process directly. On the contrary, the latter mechanism would not explain why the uncA strain, A103c, is more sensitive to uncouplers than is the nearly isogenic uncA\( ^*\) strain. At the concentrations used, the various uncouplers do not decrease the rate of \( O_2 \) consumption in strain A103c metabolizing glucose. Consequently they are not expected to inhibit the uptake of nucleic acid bases by any indirect effects on the concentrations of ATP or glycolytic intermediates. Glycine uptake occurs against a much greater concentration gradient of the transported solute, so that on thermodynamic grounds a higher protonmotive force is likely to be needed with perhaps a greater sensitivity to uncouplers. Yet there are general similarities in the sensitivities of the adenine, hypoxanthine, uracil or glycine uptake processes to uncouplers, though precise comparisons are difficult, since the rates of uptake, especially those for adenine in the presence of carbonyl cyanide \( m \)-chlorophenylhydrazone, can fall markedly during the first 2 min of uptake.

Inhibition of certain transport processes by arsenate in an uncA strain has been taken to indicate a dependence on ATP (Berger & Heppel, 1974). The nature of this dependence is obscure and may even be regulatory (Lieberman & Hong, 1976). Arsenate could cause a deficiency of ribose phosphates either by arslenolysis of nucleosides or by inhibiting the synthesis of energy-rich phosphates. This would inhibit the removal of intracellular bases by nucleoside or nucleotide formation. It is not necessary to invoke a direct need for ATP in the translocation process.

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Fig. 9. Effects of purine nucleosides on uracil uptake in a upp strain

Conditions were as for Fig. 1, but 0.25 \( \mu \)M-[\( ^3 \)H]-uracil (89 \( \mu \)Ci/litre) and \( 1.3 \times 10^8 \) cells/ml were used. Other additions at zero time were: ○, none; ●, 4 mM-inosine; □, 4 mM-adenosine; △, 4 mM-deoxyinosine; ■, 4 mM-adenosine plus 30 \( \mu \)M-carbonyl cyanide \( m \)-chlorophenylhydrazone.

[Graph showing the effects of purine nucleosides on uracil uptake in a upp strain]
References

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