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Cloning of the *tyrP* Gene and Further Characterization of the Tyrosine-Specific Transport System in *Escherichia coli* K-12

PETER J. WOOKEY,[1] JAMES PITTARD,[1]* SUSAN M. FORREST,[2]† AND BARRIE E. DAVIDSON[2]

Departments of Microbiology[1] and Biochemistry,[2] University of Melbourne, Parkville, Victoria 3052, Australia

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The *tyrP* gene which codes for a component of the tyrosine-specific transport system of *Escherichia coli* has been cloned on a 2.8-kilobase insert into plasmid pBR322. Transposon mutagenesis, using Tn*1000*, indicates that the *tyrP*$^+$ gene is at least 1.1 kilobase in length. Labeling of the *tyrP* protein in maxicells with [^{35}S]methionine indicates an apparent molecular weight of ca. 24,500. Sedimentation analysis reveals that the *tyrP* protein is associated with the cell membrane and is not free in the cytoplasm or periplasm. Strains with many copies of the *tyrP*$^+$ gene show an enhanced uptake of tyrosine, but the expression of the system is still modulated by tyrosine and phenylalanine in the presence of the *tyrR*$^+$ regulator protein. Accumulated radioactive tyrosine is rapidly effluxed by the addition either of energy uncouplers or of excess nonradioactive tyrosine, indicating that the transport system is energized by the proton motive force and that the internal pool is readily exchangeable. The effect of increasing expression of the *tyrP* gene on the steady-state level of tyrosine accumulated by cells indicates that although the transport system may be dependent on the proton motive force to drive uptake, the system never reaches thermodynamic equilibrium with it.

In *Escherichia coli* it has been demonstrated that two distinct transport systems exist for the accumulation of tyrosine (6, 7): the general aromatic system which also transports phenylalanine and tryptophan and the tyrosine-specific system. Mutations which inactivate the general aromatic system have been located in a single gene *aroP* at min 2 on the chromosome (6), and mutations affecting the tyrosine-specific system have been located in a gene *tyrP* at min 42 (17). The control of the expression of both these genes is affected by the *tyrR* protein combined with phenylalanine, tyrosine, or tryptophan in the case of *aroP* and tyrosine or phenylalanine in the case of *tyrP* (31). In this latter case, however, the actions of tyrosine and phenylalanine are opposed. Whereas tyrosine represses, phenylalanine enhances *tryP* expression (31).

The tyrosine-specific system exhibits a high affinity for tyrosine ($K_m \simeq 2$ μm [6, 7]), and transport has been shown to be an energy-dependent process (M. J. Whipp, Ph.D. thesis, University of Melbourne, Melbourne, Australia, 1977).

This paper describes the cloning and physical mapping of the *tyrP* gene, the characterization of the *tyrP* protein, and the further characterization of the tyrosine-specific transport system.

MATERIALS AND METHODS

Bacterial strains. Strains are listed in Table 1. The strain containing the plasmid with *tyrP* was obtained from a mixed cell population of *E. coli* K-12 containing chimeric plasmids formed by random cloning of the *E. coli* chromosome into pSF2124. This plasmid bank was obtained from I. G. Young (33).

Selection of *tyrP*$^+$ clones. Strains with mutations in both *aroP* and *tyrP* are defective in tyrosine transport. If, in addition, they possess a mutation which blocks endogenous synthesis of the aromatic amino acids (e.g., *aroB351*), they will not grow on medium supplemented with the aromatic end products unless the concentration of tyrosine in the medium is high (10^{-3} M). Selection for *tyrP*$^+$ transformants (19) in an *aroP tyrP aroB* background can be achieved by plating on medium supplemented with the aromatic vitamins, high concentrations (10^{-3} M) of phenylalanine and tryptophan, and low concentrations of tyrosine (5×10^{-5} M).

Although the restoration of *aroP* function will also allow these cells to grow on low levels of tyrosine, the high concentration of phenylalanine both represses *aroP* expression and competes with tyrosine for transport via this system. Therefore, these conditions do not favor growth of *aroP*$^+$ clones.

Tn*1000* insertions. Tn*1000* insertions were introduced into plasmid pMU251 by the method of Guyer (12).

Maxicell preparations. Maxicells were prepared by the method of Sancar et al. (27). Seven plasmids derived from pMU251 with Tn*1000* transposons (six of which were *tyrP*) were transformed into the CSR603 strain necessary to prepare maxicells (27). Samples labeled with [^{35}S]methionine were finally washed twice in 50 mM ammonium acetate and either frozen or used for membrane preparations as described below.

Preparation of the membrane fraction from maxicells. Samples were prepared from four maxicell preparations (27) of 50 ml of a cell suspension grown to Klett 150. These suspensions were washed twice in 50 mM ammonium acetate, passed three times through a French press at 20,000 lb/in^2, and centrifuged at 48,000 × *g* for 5 min to remove whole cells and large fragments. The supernatant was diluted approximately twofold with 50 mM ammonium acetate and 1 mM (final concentration) $MgCl_2$ and centrifuged at 160,000 × *g* for 1.5 h. The pellet was dissolved in sample buffer (20) for analysis on acrylamide gels. The supernatant was dialyzed against 50 mM ammonium acetate, concentrated by pressure filtration (Diaflo XM-10 membrane), lyophilized, and redissolved in sample buffer (20).

Preparation of plasmid DNA. The method of Birnboim and Doly (2) was used for preparation of plasmid DNA.

Gel electrophoresis. Samples containing cell protein were analyzed by the sodium dodecyl sulfate-polyacrylamide gel electrophoresis single-dimension system with either 8.75 or

* Corresponding author.
† Present address: Repatriation General Hospital, Heidelberg, Victoria 3084, Australia.

TABLE 1. Description of *E. coli* K-12 strains and plasmids

Strain or plasmid designation	Genotype or phenotype[a]	Source or reference
JP2591	F⁻ *thi arg pro aroG365 aroH367 aroP tyrP474*	This laboratory
JP1760	JP2591 *tyrA351 zfh-351*::Tn*10*[a,b]	By P1 *kc* transduction
JP1761	JP1760 *tyrA351*::Δ(*zfh-351*::Tn*10*)*600*[c]	From strain JP1760 by the method of Maloy and Nunn (22)
JP1769	JP1761 *tyrP476*::Tn*10*[b]	By P1 *kc* transduction
JP1774	JP1769 Δ(*tyrP476*::Tn*10*)*601*[c]	From JP1769 by the method of Bochner et al. (3)
JP1775	JP1774 *recA56 zfi-351*::Tn*10*[b]	By P1 *kc* transduction
JP1751	JP2591 *aroB351*	By P1 *kc* transduction
JP1753	JP1751 *tyrP476*::Tn*10*	By P1 *kc* transduction
JP1765	JP1769 *tyrP*⁺	By P1 *kc* transduction
JP1821	JP1775(pMU252)	By transformation with pMU252
JP1776	JP1775(pMU251)	By transformation with pMU251
JP1793	JP1776(Mu 1)	Selection of Mu1 lysogen
JP3211	*thi ilv trp tyrR* (⁺/−) *recA* Δ*lac* Str[r] (pMU309)	pMU309 is an RP4 derivative carrying *tyrR*⁺. This laboratory.
JP1795	JP1793(pMU309)	By conjugation of JP3211 × JP1793
JP3301	F⁺ *purE trp his argG ilv leu metA* or *metB rpsL* Δ*lacU169* (λ p*pheA-lac*) *pheR372 recA56* Δ(*srl-1300*::Tn*10*)*59*	(10, 11) CA7027 *lacU169*, J. Beckwith
JP1773	JP3301(pMU251)	By transformation
CSR603	*thi-1 thr-1 leuB6 proA2 argE3 uvrA6 rpsL31 supE44 gyrA98*	27
JP1799	CSR603(pBR322)	By transformation
JP1798	CSR603(pMU251)	By transformation
JP1812	CSR603(pMU255)	By transformation
JP1813	CSR603(pMU256)	By transformation
JP1814	CSR603(pMU257	By transformation
JP1815	CSR603(pMU258)	By transformation
JP1816	CSR603(pMU259)	By transformation
JP1817	CSR603(pMU260)	By transformation
pBR322	Ap[r] Tc[r]	29
pMU252	pSF2124 *tyrP*⁺	Selection from gene pools (34); this paper
pMU251	pBR322 *tyrP*⁺	This paper
pMU255	pMU251 *tyrP526*::Tn*1000*	By conjugation JP1773 × JP1775 (12)
pMU256	pMU251 *tyrP527*::Tn*1000*	By conjugation JP1773 × JP1775 (12)
pMU257	pMU251 *tyrP528*::Tn*1000*	By conjugation JP1773 × JP1775 (12)
pMU258	pMU251 *tyrP529*::Tn*1000*	By conjugation JP1773 × JP1775 (12)
pMU259	pMU251 *tyrP530*::Tn*1000*	By conjugation JP1773 × JP1775 (12)
pMU260	pMU251::Tn*1000* (*tyrP*⁺)[d]	By conjugation JP1773 × JP1775 (12)
pMU309	RP4::Mu *trkE*⁺ *tyrR*⁺ Mu	This laboratory

[a] The nomenclature for genetic symbols follows that of Bachmann (1) and for Tn transposons follows Kleckner et al. (18).

[b] The *tyrA351 zfh*::Tn*10* and *recA56 zfi*::Tn*10* alleles were 25 and 80% contransducible, respectively.

[c] These putative deletions were generated by selection for tetracycline sensitivity (3, 18, 22).

[d] The position of the Tn*1000* transposon on the 2.8-kb fragment is shown in Fig. 1.

12% acrylamide (20). Heat treatment during sample preparation was avoided since this led to changes in the R_f and impaired the clarity of the *tyrP* protein band. Samples of DNA were analyzed either on 0.8% agarose gels or on 4% acrylamide gels-Tris (borate)-EDTA buffer (pH 8.3), using standard procedures (9).

Transport studies. Strains were grown by shaking at 37°C overnight in half-strength minimal medium (23), referred to here as 56/2 buffer, supplemented with the required growth factors and containing 10^{-3} M tyrosine, 10^{-4} M phenylalanine, 10^{-4} M tryptophan, and the aromatic vitamins 5×10^{-5} M dihydroxybenzoate, 10^{-6} M *para*-aminobenzoate, and 4×10^{-6} M *para*-hydroxybenzoate. Cells were washed twice in sterile 56/2 buffer (23) and resuspended to a Klett of 10 (5×10^7 cells per ml) in fresh medium with the appropriate concentrations of growth factors. To achieve induction or repression of the *tyrP* gene, either 2×10^{-5} M tyrosine and 10^{-4} M phenylalanine or 10^{-4} M tyrosine and phenylalanine were included in the fresh medium, and growth was stopped at approximately Klett 100 (3×10^8 cells per ml). The suspensions were washed twice in 56/2 buffer (23) containing 0.2% glucose and 80 μg of chloramphenicol per ml. The latter was included to inhibit the incorporation of [^{14}C]tyrosine into protein during the uptake assay. Samples of 1 ml of the suspensions were preincubated at 30°C for 5 min and then 0.05 to 10 μM [^{14}C]tyrosine was added. Samples of 150 μl were removed at appropriate times, filtered through membrane filters (HA/0.45 μm; Millipore Corp.), which were then washed twice with 2.5 ml of 56/2 buffer (30°C), dried overnight at room temperature, suspended in PPO(2,5-diphenyloxazole)-POPOP[1,4-bis-(5-phenyloxazolyl)benzene]-toluene scintillation fluid, and counted in a Beckman scintillation counter.

The nonspecific diffusion rate of tyrosine was measured by uptake studies (0 to 3 min) into strain JP1775 (*aroP tyrP*) as described above except that 1 mM [^{14}C]tyrosine was included. In an identical assay system, with the exceptions that chloramphenicol was omitted and suspensions were shaken at 37°C with other growth factors, the cell density and incorporation of radioactive tyrosine were measured over a period of hours. Incorporation was expressed as nanomoles per milligram [dry weight (DW)] per minute.

RESULTS

Cloning the *tyrP*⁺ gene. A population of cells containing chimeric plasmids obtained from the cloning of the entire *E. coli* chromosome into pSF2124 and subsequent transformation into an F⁺ strain was obtained from I. G. Young (33). This mixed population, which is able to transfer its plasmids to recipient cells by conjugation, was crossed with the recipient strain JP1753, and selection was made for *tyrP*⁺ clones as described above. However, because both donor and recipient were *recA*⁺, two additional classes of recombinants were able to grow on the selective media. These comprised *tyrP*⁺ or *aroB*⁺ chromosomal recombinants. Because strain JP1753 has Tn*10* inserted in the *tyrP* gene, clones which retained the chromosomal *tyrP* allele were easily identified because of their tetracycline resistance. Clones which had received *aroB*⁺ could be easily detected by growth in the absence of aromatic end products. One clone obtained from the cross, however, had all of the characteristics expected for the recipient strain possessing additional copies of *tyrP*⁺ on pSF2124.

Plasmid DNA was prepared from this strain and was found by *Eco*RI digestion to contain a 2.8-kilobase (kb) insert. This plasmid is referred to as pMU252. The 2.8-kb insert from pMU252 was recloned into the *Eco*RI site of pBR322 to give

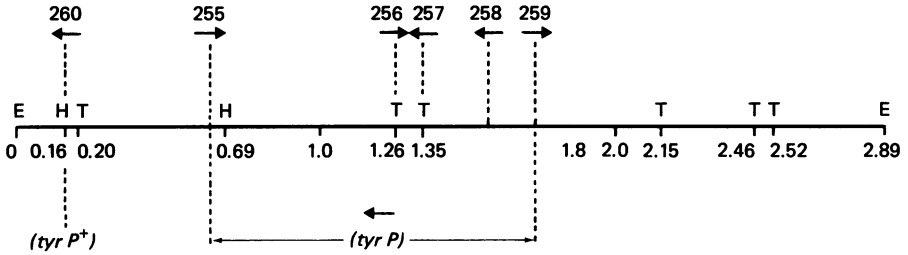

FIG. 1. Restriction endonuclease map of the *Eco*RI chromosomal DNA fragment containing the *tyrP* gene. The restriction enzyme coordinates for *Eco*RI (E), *Hin*dII (H), and *Taq*I (T) are as shown. The Tn*1000* transposon insertion coordinates are as indicated, with the arrows indicating the polarity of the transposon (12). The numbers above each insertion are the pMU designation of each plasmid.

a 7.2-kb plasmid (pMU251) containing the *tyrP*$^+$ gene. A map of the *Hin*dIII and *Taq*I sites of the insert obtained by single and double digestions is shown in Fig. 1. No digestion sites were observed with *Bam*HI, *Hin*dIII, *Pvu*I, *Pvu*II, *Sal*I, *Sac*I, *Bgl*II, *Xho*I, *Sma*I, or *Hpa*I.

Insertions of Tn*1000* (γδ) into *tyrP*. The location of the *tyrP* gene in the 2.8-kb insert of pMU251 was established by transpositional inactivation. The method used was that described by Guyer (12) in which mobilization of plasmids derived from pBR322 by the sex factor F results in the insertion of the Tn*1000* (γδ) transposon into the transferred plasmid. Plasmid pMU251 was transformed into the F$^+$ strain JP3301, and the resulting strain JP1773 was used to transfer pMU251 into the recipient strain JP1775. Strain JP1775 is resistant to tetracycline (*recA56 zfi*::Tn*10*) and requires tyrosine for growth. Because of mutations in *tyrP* and *aroP*, it is only to grow on medium supplemented with high levels of tyrosine (10^{-3} M). Clones that had received the plasmid from strain JP1773 were selected on minimal medium containing ampicillin, tetracycline, and tyrosine (10^{-3} M). These were purified and screened for growth on medium containing low levels of tyrosine. Approximately one quarter of these clones failed to grow on this medium and were presumed to have Tn*1000* inserted within the *tyrP* gene on the plasmid. Five of these strains and one with a *tyrP*$^+$ phenotype were selected for further investigation.

Each of these strains were found to contain a plasmid with a molecular weight corresponding to that expected for pMU251 (7.2 kb) containing an additional 5.7 kb of Tn*1000* DNA. Plasmids pMU255 to pMU259 have Tn*1000* inserted within *tyrP*, and pMU260 has Tn*1000* inserted elsewhere on the pMU251 plasmid. Transport studies confirmed the TyrP$^+$ or TyrP$^-$ phenotypes inferred from the growth tests (data not shown).

The Tn*1000* insertion sequence has been shown to contain one *Bam*HI towards one end and two *Eco*RI restriction sites toward the opposite end of the molecule (12). This facilitated the mapping of these insertions in pMU251 by *Eco*RI, *Bam*HI, *Hin*dIII, and *Taq*I digestions (Fig. 1). As can be seen, Tn*1000* has inserted at a different site in each of the five plasmids in which *tyrP* function had been lost. These sites span a region of 1.1 kb, which therefore represents a minimum size for the *tyrP* gene.

[^{35}S]methionine labeling of plasmid-coded proteins and identification of the *tyrP* protein. Strain CSR603 was transformed with plasmid pBR322 (JP1799), pMU251 (JP1798), and pMU255-260 (JP1812 through JP1817). Transformants were purified, and in each case, plasmid-coded proteins were selectively labeled with [^{35}S]methionine as described by Sancar et al. (27) (Fig. 2). It can be seen that an intense protein band which runs just ahead of 27,000-dalton β-lactamase protein is present in strain JP1798, which contains pMU251 (track 3), but absent from strains JP1812 through JP1816 (tracks 4 to 8) (Fig. 2).

The protein which is presumed to be the *tyrP* protein has an apparent molecular weight of ca. 24,500, calculated from the relative mobilities of the tetracycline (34,000) and β-lactamase (27,000) proteins. As might be expected, the *tyrP*$^+$ strain JP1817 also produces this band (data not shown).

Intracellular location of the *tyrP* protein. The intracellular location of the *tyrP* protein was investigated again, using maxicells and separating membrane and soluble fractions as described above. The gel electrophoresis of [^{35}S]methionine-labeled proteins present in membrane or soluble fractions is shown in Fig. 3. It can be seen that the protein band, identified previously as the *tyrP* protein, is present in the membrane fraction along with the proteins that confer tetracycline resistance (Fig. 3, track 6). This band is absent from the soluble fraction which contains the β-lactamase protein (Fig. 3, track 5) (24).

Transport studies. Cells for use in transport studies were grown under conditions which derepress the expression of the *tyrP*$^+$ gene (see above). The observed tyrosine transport was shown not to be affected by a 20-fold excess of phenylalanine and tryptophan in the uptake assay (data not shown), thereby confirming that the uptake was due to the TyrP system and not the AroP system.

A comparison of tyrosine uptake by strains JP1775 (*tyrP aroP*), JP1765 (haploid for *tyrP*$^+$), and JP1821 and JP1776 (carrying the *tyrP*$^+$ gene on medium- and high-copy-number plasmids, respectively) showed that increasing the copy number of *tyrP*$^+$ in the cells results in a corresponding

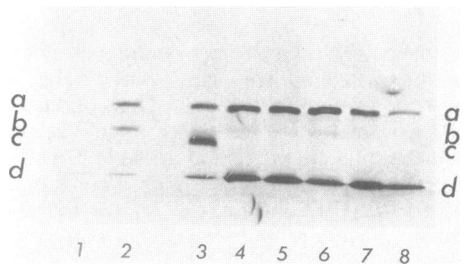

FIG. 2. Autoradiograph of proteins labeled with [^{35}S]methionine from maxicell preparations of: strain CSR603 (track 1), strain JP1799 (track 2), strain JP1798 (tract 3), and strains in which the *tyrP* gene is inactivated by Tn*1000* (JP1812 to JP1816 [tracks 4 to 8]). Band a is the tetracycline 34,000-dalton protein (29), band b is the 27,000-dalton β-lactamase protein (28), band c is the *tyrP* protein, and band d is a mixture of unresolved proteins which probably include the low-molecular-weight proteins associated with tetracycline resistance (29).

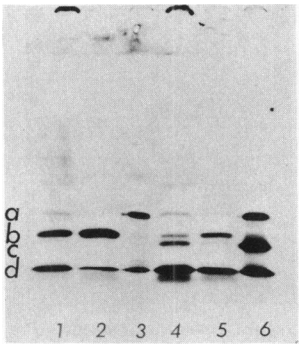

FIG. 3. Autoradiograph of proteins from maxicell preparations labeled with [^{35}S]methionine. The multicopy strains JP1799 (tracks 1 to 3) and JP1798 (tracks 4 to 6) were processed to derive total cell protein (tracks 1 and 4), soluble protein (tracks 2 and 5), and membrane-associated protein (tracks 3 and 6).

increase both in the initial rate of uptake and in the steady-state levels of tyrosine in the cell (Fig. 4A). As expected, the expression of *tyrP*$^+$ on the multicopy plasmid pMU251 could be shown to be controlled by the *tyrR*$^+$ protein with tyrosine and phenylalanine (Fig. 4A) as has previously been demonstrated for haploid strains (31).

It is evident that when the multicopy strain JP1776 was grown in repressing concentration of tyrosine, the steady-state level was reduced from 70 to 40 nmol/mg (DW) (Fig. 4A). That any uptake occurred at all in the multicopy *tyrP*$^+$ strain was thought to result from escape from repression due to the limiting dosage of the *tyrR*$^+$ gene. This was substantiated by introducing extra copies of the *tyrR*$^+$ gene on the compatible plasmid pMU309 into the multicopy *tyrP*$^+$ strain JP1793. Uptake in the resulting strain JP1795 was measured after growth in repressing levels of tyrosine. The steady-state level of uptake was reduced from 40 to 6 nmol/mg (DW) in strain JP1795 (Fig. 4A).

The rate at which accumulated tyrosine effluxed from a multicopy strain was examined by promoting efflux either with an eightfold excess of nonradioactive tyrosine or with 100 μM of the uncoupler, carbonyl-cyanide-chlorophenyl hydrazone (13). The results for the strain containing pMU251 are shown in Fig. 4B. Both treatments resulted in rapid efflux of tyrosine from the cell and in the case of 100 μM carbonyl-cyanide-*m*-chlorphenyl hydrazone at a rate comparable to uptake. These two observations are consistent with a model in which the tyrosine-specific transport system is dependent on the proton motive force for its energization.

The K_m and V_{max} of the transport system were determined under conditions which allowed significant differences in the level of the *tyrP* protein in the cell. The conditions under which these parameters were determined are described above, and the results are presented in Table 2. The maximal velocity of uptake clearly increases with increasing levels of expression of *tyrP*. By contrast, the K_m (or the affinity) of the system for tyrosine remains relatively constant at ca. 3 μM.

Estimation of the nonspecific diffusion rate across the cytoplasmic membrane. When cell suspensions of strain JP1775 (*aroP tyrP*) were incubated under normal conditions for measuring transport except that the external concentration of [^{14}C]tyrosine was 1 mM, no uptake could be detected during the first 3 min of incubation. However, when chloramphenicol is omitted and other growth factors are included, these cells grow at 37°C with a doubling time of 1.4 h. Since strain JP1775 is a tyrosine auxotroph, their growth must depend on the utilization of exogenously supplied tyrosine. The finding that the doubling time of strain JP1775 under these conditions is twice that of the *tyrP*$^+$ related strain JP1765 supports the hypothesis that tyrosine diffusion is limiting the growth rate in the case of strain JP1775. The rate of incorporation of [^{14}C]tyrosine into strain JP1775 can thus

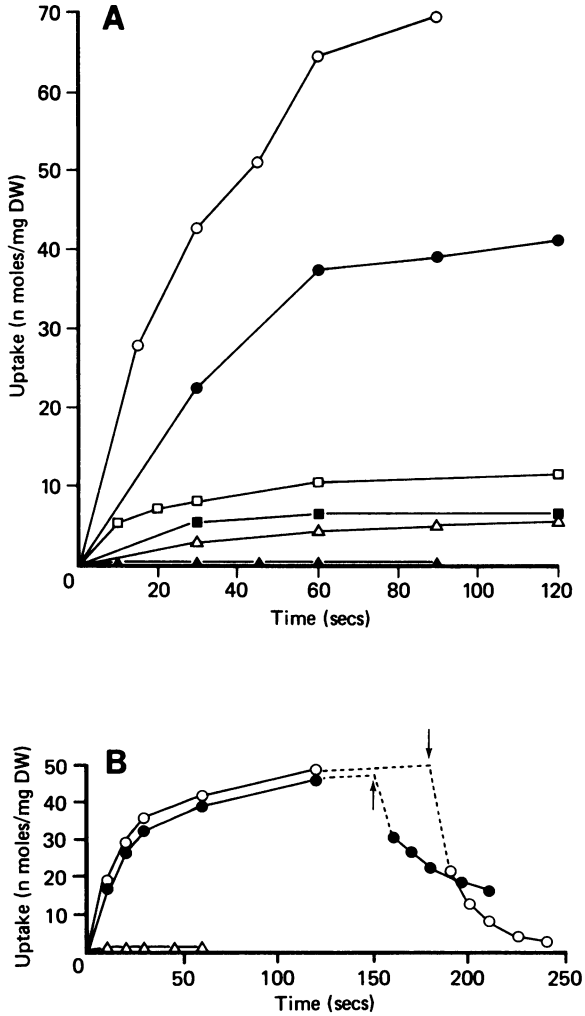

FIG. 4. (A) Accumulation of [^{14}C]tyrosine (10 μM) into strains which contain differing states of the expression of *tyrP*. Symbols: ▲, strain JP1775 which lacks both the *TyrP* and *AroP* systems; △, JP1765, a haploid *tyrP*$^+$ strain; □, strain JP1821 which contains the medium-copy-number (4) plasmid pMU252; ○, strain JP1776 which contains the high-copy-number (27) plasmid pMU251. The last three represent induced levels of *tyrP*$^+$ expression as the cells used for uptake assays were grown in excess phenylalanine and starved of tyrosine. Symbols: ●, strain JP1776 repressed by growth in excess tyrosine; ■, strain JP1795 in which the expression of *tyrP*$^+$ in the multicopy plasmid pMU251 was further repressed by growth in excess tyrosine and the presence of extra copies of the *tyrR*$^+$ gene. (B) Uptake and efflux of [^{14}C]tyrosine (10 μM) in strain JP1776. Efflux was initiated by the addition of 80 μM nonradioactive tyrosine (●) and 100 μM carbonyl-cyanide-*m*-chlorophenylhydrazone (○) at the times indicated by the arrows. The complete inhibition of [^{14}C]tyrosine accumulation into strain JP1776 by the prior (−30 s) addition of 100 μM carbonyl-cyanide-*m*-chlorophenylhydrazone is indicated (△).

be taken as a measure of the diffusion rate for these organisms. This rate was 0.14 nmol/mg (DW) per min, which is only 1% of the V_{max} of tyrosine transport observed in the *tyrP*$^+$ strain JP1765 (Table 2) and explains why no diffusion could be detected in strain JP1765 during 3 min of incubation. The concentration of 1 mM was chosen as the appropriate level of exogenous tyrosine for this experiment as we had calculated that the internal concentration of tyrosine at the steady state in a *tyrP*$^+$ haploid strain (JP1765) was ca. 1 mM when tyrosine was saturating the transport system from the outside. This estimate is based on the steady-state level accumulated [5 nmol/mg (DW); Fig. 4A] and the estimate that 1 mg (DW) of cells contains ca. 2.7 μl of cell water (32).

DISCUSSION

The *tyrP* gene has been cloned in a 2.8-kb insert in pBR322, and the gene has been mapped in the insert by the use of Tn*1000* insertions. Confirmation that the elevated tyrosine transport system in cells carrying these multicopy plasmids is, in fact, the system previously characterized as tyrosine specific comes from studies on both its control and its specificity. It is worth noting that the extent of repression of this system by tyrosine in the multicopy strains is less marked than in haploid strains and probably reflects some escape from repression under these circumstances. This was overcome when additional copies of the *tyrR*$^+$ gene were introduced in the cell.

The minimum length of the *tyrP* gene is ca. 1.1 kb, and the molecular weight of the *tyrP* protein was observed on gels to be 24,500. Assuming that the mass of an average amino acid residue is 110 daltons, a protein of this size could be encoded on a gene of 0.67 kb. This means either that the *tyrP* protein undergoes significant posttranslational proteolysis or that *tyrP* has as unusually large regulatory region of ca. 0.43 kb, or that the estimation of either the gene or protein size is anomalous. We think that the last alternative is the most likely in view of similar results having been reported for two other membrane-bound proteins, namely the *lacY* (8, 16) and *hisP* (15) proteins. Anomalous mobility in sodium dodecyl sulphate-polyacrylamide gel electrophoresis has been established unequivocally in the case of the *hisJ5625* protein (26).

The nature of the internal tyrosine pool generated in the presence of the multicopy plasmid pMU251 was investigated by measuring the efflux of [^{14}C]tyrosine with the uncoupler carbonyl-cyanide-*m*-chlorophenyl hydrazone and in a chase experiment with an eightfold excess of nonradioactive tyrosine. Clearly, like the wild-type system, the internal pool is in rapid equilibrium with the external pool (Fig. 4B), as has been previously described (6). Furthermore, the gradient of tyrosine formed at the steady state is subject to rapid depletion by uncouplers, suggesting that the system is energized by the proton motive force. The further observation that the *tyrP* protein is membrane bound suggests a similarity with the LacY (25), AraE (21), or GalP (14) systems, in which a single polypeptide alone is necessary in each case to promote the transport of a specific moiety. This polypeptide is also clearly necessary, although perhaps not sufficient, for the coupling of energy from the proton motive force. It will, however, be necessary to carry out reconsitution experiments with the purified *tyrP* protein and proteoliposomes, as have been reported for the *lacY* (25), *mel*A (30), and *galP* (14) proteins to determine the validity of these statements.

The determination of the apparent K_m, V_{max}, and steady state levels of tyrosine indicates that the last two parameters are dependent on the number of copies of *tyrP*$^+$ gene and hence the amount of the *tyrP* protein expressed (Table 2).

TABLE 2. K_m and V_{max} of the tyrosine-specific transport system in haploid and multicopy strains

Strain no.	Plasmid present	Approx gene dosage of tyrP$^+$	K_m[a] (μM)	V_{max}[a] (nmol/mg (DW)[b] per min)	Maximum steady-state level[a] (nmol/mg (DW)[b]
JP1765	None	1	3.9	14	4.9
JP1821	pMU252	10[c]	3.3	50	11.6
JP1776	pMU251	60[c]	2.5	111	72

[a] These figures are derived from double-reciprocal plots of uptake data, and the initial rates were determined from the linear portion of the curves, the first 20 s of uptake. Points were determined at 10, 20, 30, 45, and 60 s (data not shown). The maximum steady-state level was taken as the level of [^{14}C]tyrosine accumulated when uptake had leveled off. These data were derived from strains induced for *tyrP* expression by growth in excess phenylalanine and starved of tyrosine.

[b] 1 mg (DW) = 2.5×10^9 cells per ml (31).

[c] Data are from references 4 and 28.

However, according to the chemiosmotic hypothesis, the steady-state level should be determined rather by the magnitude of the proton motive force than by the amount of porter present (P. Mitchell, personal communication). Our result indicates that at steady state, the tyrosine-specific transport system is not in thermodynamic equilibrium with the proton motive force, although we have shown that it is dependent on the proton motive force since the transport system is profoundly influenced by the presence of uncouplers. This conclusion has also been reached for the lactose system (5) by a different experimental approach. As described above, we estimated the nonspecific diffusion rate across the cell membrane (nonspecific efflux) at the steady state to be ca. 1% of the initial rate of influx. According to current understanding of steady-state velocities (5), the nonspecific diffusion rate should be a significant proportion (ca. >50%) of the V_{max} to invoke the kinetic argument (5) necessary to account for the observation that the transport system is not in thermodynamic equilibrium with the proton motive force.

A suitable solution to the conundrum, that the magnitude of the steady-state level of tyrosine is dependent on the amount of the *tyrP* gene product rather than the magnitude of the proton motive force, is now being sought.

LITERATURE CITED

1. **Bachmann, B. J.** 1983. Linkage map of *Escherichia coli* K-12, edition 7. Microbiol. Rev. **47**:180–230.
2. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.
3. **Bochner, B. R., H.-C. Huang, G. L. Schieven, and B. N. Ames.** 1980. Positive selection for loss of tetracycline resistance. J. Bacteriol. **143**:926–933.
4. **Bolivar, F., R. L. Rodriguez, P. J. Greene, M. C. Betlach, H. L. Heyneker, H. W. Boyer, J. Crosa, and S. Falkow.** 1977. A new multipurpose cloning system. Construction and characterization. Gene **2**:95–113.
5. **Booth, I. R., W. J. Mitchell, and W. A. Hamilton.** 1979. Quantitative analysis of proton-linked transport systems. The lactose permease of *Escherichia coli*. Biochem. J. **182**:687–696.
6. **Brown, K. D.** 1970. Formation of aromatic amino acid pools in *Escherichia coli* K-12. J. Bacteriol. **104**:177–188.
7. **Brown, K. D.** 1971. Maintenance and exchange of the aromatic amino acid pool in *Escherichia coli*. J. Bacteriol. **106**:70–81.
8. **Büchel, D. E., B. Gronenborn, and B. Müller-Hill.** 1980. Sequence of the lactose permease gene. Nature (London) **283**:541–545.
9. **Davis, R. W., D. Botstein, and J. R. Roth.** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

10. **Gowrishankar, J., and A. J. Pittard.** 1982. Construction from Mu d1 (*lac* Ap^r) lysogens of lambda bacteriophage bearing promoter-*lac* fusions: isolation of λ p*pheA-lac*. J. Bacteriol. **150:**1122–1129.
11. **Gowrishankar, J., and J. Pittard.** 1982. Molecular cloning of *pheR* in *Escherichia coli* K-12. J. Bacteriol. **152:**1–6.
12. **Guyer, M. S.** 1978. The γδ sequence of F is an insertion sequence. J. Mol. Biol. **126:**347–365.
13. **Harold, F. M.** 1972. Conservation and transformation of energy by bacterial membranes. Bacteriol. Rev. **36:**172–230.
14. **Henderson, P. J. F.** 1982. Sugar transport proteins of *Escherichia coli*. 12th International Congress of Biochemistry, Perth, Australia. Alfred Ruskin Publications, Melbourne, Australia.
15. **Higgins, C. F., P. D. Haag, K. Nikaido, F. Ardeshir, G. Garcia, and G. Ferro-Luzzi Ames.** 1982. Complete nucleotide sequence and identification of membrane components of the histidine transport operon of *S. typhimurium*. Nature (London) **298:**723–727.
16. **Jones, T. H. D., and E. P. Kennedy.** 1969. Characterization of the membrane protein component of the lactose transport system of *Escherichia coli*. J. Biol. Chem. **244:**5981–5987.
17. **Kasian, P., and A. J. Pittard.** 1984. Construction of a *tyrP-lac* operon fusion strain and its use in the isolation and analysis of mutants depressed for *tyrP* expression. J. Bacteriol. **160:**175–183.
18. **Kleckner, N., J. Roth, and D. Botstein.** 1977. Genetic engineering *in vivo* using translocatable drug-resistance elements. New methods in bacterial genetics. J. Mol. Biol. **116:**125–159.
19. **Kushner, S. R.** 1978. An improved method for transformation of *Escherichia coli* with ColE1 derived plasmids, p. 17–23. *In* H. W. Boyer and S. Nicosia (ed.), Genetic engineering. Elsevier/North-Holland Biomedical Press, Amsterdam.
20. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
21. **Macpherson, A. J. S., M. C. Jones-Mortimer, and P. J. F. Henderson.** 1981. Identification of the AraE transport protein of *Escherichia coli*. Biochem. J. **196:**269–283.
22. **Maloy, S. R., and W. D. Nunn.** 1981. Selection for loss of tetracycline resistance by *Escherichia coli*. J. Bacteriol. **145:**1110–1112.
23. **Monod, J., G. Cohen-Bazive, and M. Cohn.** 1951. Sur la biosynthese de la β-galactosidase (lactase) chez *Escherichia coli*. La specificite de l'induction. Biochim. Biophys. Acta **7:**585–599.
24. **Neu, H. C.** 1968. The surface location of penicillinases in *Escherichia coli* and *Salmonella typhimurium*. Biochem. Biophys. Res. Commun. **32:**258–263.
25. **Newman, M. J., D. L. Foster, T. Hastings Wilson, and H. R. Kaback.** 1981. Purification and reconstitution of functional lactose carrier from *Escherichia coli*. J. Biol. Chem. **256:**11804–11808.
26. **Noel, D., K. Nikaido, and G. F.-L. Ames.** 1979. A single amino acid substitution in a histidine-transport protein drastically alters its mobility in sodium dodecyl sulphate polyacrylamide gel electrophoresis. Biochemistry **18:**4159–4165.
27. **Sancar, A., A. M. Hack, and W. D. Rupp.** 1979. Simple method for identification of plasmid-coded proteins. J. Bacteriol. **137:**692–693.
28. **So, M., R. Gill, and S. Falkow.** 1976. The generation of ColE1-Ap^R cloning vehicle which allows the detection of inserted DNA. Mol. Gen. Genet. **142:**239–249.
29. **Sutcliffe, J. G.** 1978. pBR322 restriction map derived from the DNA sequence: accurate DNA size markers up to 4361 nucleotide pairs long. Nucleic Acids Res. **5:**2721–2728.
30. **Tsuchiya, T., K. Ottina, Y. Moriyama, M. J. Newman, and T. H. Wilson.** 1982. Solubilization and reconstitution of the melibiose carrier from a plasmid-carrying strain of *Escherichia coli*. J. Biol. Chem. **257:**5125–5128.
31. **Whipp, M. J., and A. J. Pittard.** 1977. Regulation of aromatic amino acid transport systems in *Escherichia coli* K-12. J. Bacteriol. **132:**453–461.
32. **Winkler, H. H., and T. H. Wilson.** 1966. The role of energy coupling in the transport of β-galactosides by *Escherichia coli*. J. Biol. Chem. **241:**2200–2211.
33. **Young, I. G., A. Jaworowski, and M. Poulis.** 1978. Amplification of the respiratory NADH dehydrogenase of *Escherichia coli* by gene cloning. Gene **4:**25–36.