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Bioenergetics of dihydrostreptomycin transport by *Escherichia coli*

Stephen R. Goss[†]*, Alan B. Spicer* and Wright W. Nichols[†]

[†]*Public Health Laboratory, Level 6/7, John Radcliffe Hospital, Oxford OX3 9DU* and **Department of Biology, Oxford Polytechnic, Oxford OX3 0BP, England*

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Previous demonstrations of the irreversibility of dihydrostreptomycin transport across the cytoplasmic membrane of *Escherichia coli* were not due to decreases in the magnitude of the cytoplasmic membrane potential ($\Delta\psi$). Irreversibility was probably not due to ATP hydrolysis being coupled to transport, because the rate of energy-dependent dihydrostreptomycin uptake was unaffected by 10-fold reduction in the cellular ATP level.

Dihydrostreptomycin transport; Streptomycin uptake; Transport kinetics; Membrane potential; (*Escherichia coli*)

1. INTRODUCTION

In the rapid, energy-dependent transport of dihydrostreptomycin across the cytoplasmic membrane of *Escherichia coli* (EDP-II phase), the kinetic irreversibility of the process [1,2] is an important factor in the analysis of potential mechanisms [3–5]. Since an electrically gated transport process is feasible [6], it is necessary to study the behaviour of the electric potential difference across the cytoplasmic membrane ($\Delta\psi$) under conditions identical to those used for determining the irreversibility [4]. This paper describes such studies, and confirms that the irreversibility of transport described in [2,7,8] was not due to decreases in the magnitude of $\Delta\psi$.

One potential explanation for irreversibility would be that a chemical reaction is coupled to the transport process [2,5]. We also show here that the rate of dihydrostreptomycin transport is not influenced by the cellular level of ATP, one candidate for being the substrate of such a coupled reaction.

Correspondence address: W. Nichols, Public Health Laboratory, Level 6/7, John Radcliffe Hospital, Oxford OX3 9DU, England

2. EXPERIMENTAL

2.1. *Bacterial strains*

E. coli K10 strains 7 [9] and NR71 (*unc*; a partial revertant of the *uncA* strain NR70 that was itself derived from strain 7 as a neomycin-resistant mutant) [10] were obtained from I.R. Booth; *E. coli* K12 was NCTC number 10538.

Strains 7 and NR71 were grown with shaking at 37°C in the glucose minimal medium described by Davis and Mingioli [11]. *E. coli* K12 was grown in the same medium except that disodium succinate (10 g/l) replaced glucose.

2.2. *Uptake of [^{3}H]dihydrostreptomycin*

Uptake was assayed at 37°C in an oxygenated medium with oxygen monitoring as before [2] except that for strains 7 and NR71, the reaction mixture contained 40 mM glucose as well as 40 mM succinate at the start of the experiment, and the concentration of dihydrostreptomycin was 50 μg of base·ml^{-1}.

The radiochemical purity of [^{3}H]dihydrostreptomycin was monitored by descending paper chromatography, using the developer propan-1-ol:acetic acid:water (9:1:10). An unlabelled dihydrostreptomycin standard was a kind gift from A. Thomas.

2.3. *ATP determinations*

ATP was extracted from cells as described by Cole et al. [12], and stored frozen at −70°C. Five replicate measurements of

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each ATP concentration were made using purified, separate firefly luciferin and luciferase [13].

2.4. *Transmembrane electric potential difference ($\Delta\psi$)*

The uptake of the lipophilic cation, [^{14}C]tetraphenylphosphonium ion (TPP: 0.4 mM; 1 Ci·mol^{-1}), was assayed under identical conditions to the [^{3}H]dihydrostreptomycin uptake experiments except that the dihydrostreptomycin present was not radiolabelled, and that EDTA (10 mM final concentration) was added just prior to the TPP^+. Five replicate samples of 100 μl were filtered but without dilution or washing. [^{14}C]TPP^+ dissolved in the periplasmic and interstitial water, and that bound to the cells, was estimated by adding carbonylcyanide *m*-chlorophenylhydrazone (CCCP) to the cells (20 μM final concentration) followed 10 min later by re-sampling. Filters were dried and radioactivity counted as in [2]. The [^{14}C]TPP^+ retained on filtering CCCP-treated cells was subtracted from that obtained on filtering the untreated cells to give the amount of TPP^+ dissolved in the cytoplasmic water. The value of $\Delta\psi$ was calculated using the Nernst equation and a cytoplasmic water volume of 1.4 μl·mg dry wt^{-1} [14]. Despite the possibility that *E. coli* pumps TPP^+ out of the cell [15], estimates such as the above have agreed well with direct microelectrode measurements [16].

3. RESULTS

3.1. *Measurements of $\Delta\psi$ in* E. coli *K12 respiring on succinate in the presence of unlabelled dihydrostreptomycin*

When cells oxidizing succinate in the presence of 10 μg dihydrostreptomycin·ml^{-1} were made anaerobic, $\Delta\psi$ before anaerobiosis was -102 mV (SE = 9.4; n = 3); 5 min following anaerobiosis it was -104 mV (SE = 8.9; n = 3) and 15 min following anaerobiosis it was -108 mV (SE = 7.9; n = 3).

Table 1 shows the values of $\Delta\psi$ observed in cells exposed to 1 mg of dihydrostreptomycin·ml^{-1} for up to 2 h, when exchange diffusion between cytoplasmic and exogenous antibiotic could not be demonstrated [2].

3.2. *Effect of depleting the ATP pool on the uptake of dihydrostreptomycin by* E. coli

Fig.1 shows the effect of adding iodoacetate to cells of *E. coli* NR71 on ATP levels and on dihydrostreptomycin uptake. The results of an identical experiment using the energetically competent parent of NR71, strain 7, are shown in fig.2.

Iodoacetate addition did not affect the magnitude of $\Delta\psi$ in either strain 7 or strain NR71 incubated in the reaction mixture but without dihydrostreptomycin. In strain 7, $\Delta\psi$ was -129 mV (SE = 4.3 mV; n = 3) before and -132 mV (n = 1) following iodoacetate addition. In strain NR71, $\Delta\psi$ was -118 mV (SE = 12 mV; n = 3) before and -134 mV (n = 1) following iodoacetate addition.

Table 1

Measurement of $\Delta\psi$ in *E. coli* K12 respiring on succinate and exposed to dihydrostreptomycin at 1 mg of base·ml^{-1}

Time of adding EDTA and TPP^+ (min)	Presence/absence of dihydrostreptomycin (DHS)	Membrane potential ($\Delta\psi$, mV)		
		Mean	SE	Number of determinations
3	+ DHS	−107	–	1
3	− DHS	−129	3.9	4
120	+ DHS	−113	11	5
120	− DHS	−106	10	3

The conditions were as described by Nichols and Young [2] except that the dihydrostreptomycin was not radiolabelled

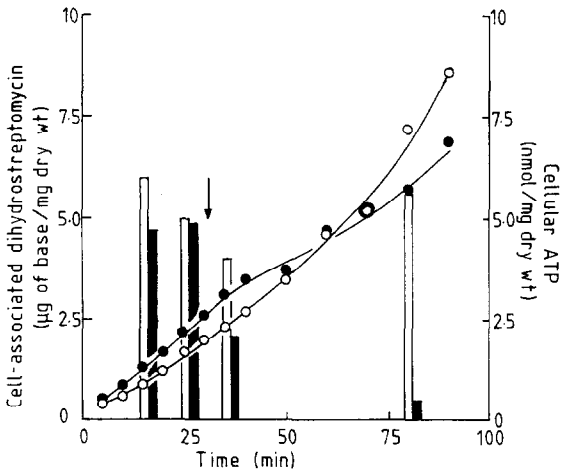

Fig.1. The effect of iodoacetate on ATP levels and dihydrostreptomycin uptake in *E. coli* NR71. The circles represent cell-associated dihydrostreptomycin: (○) control, 13 μl water and (●) experiment, 13 μl iodoacetate solution (0.44 mM final concentration) added at 31 min (arrow). Samples for ATP assay were taken from the same uptake reaction mixture at 16, 26, 36 and 81 min. The empty bars show ATP levels in the control cells, the filled bars show ATP levels in the cells to which iodoacetate was added.

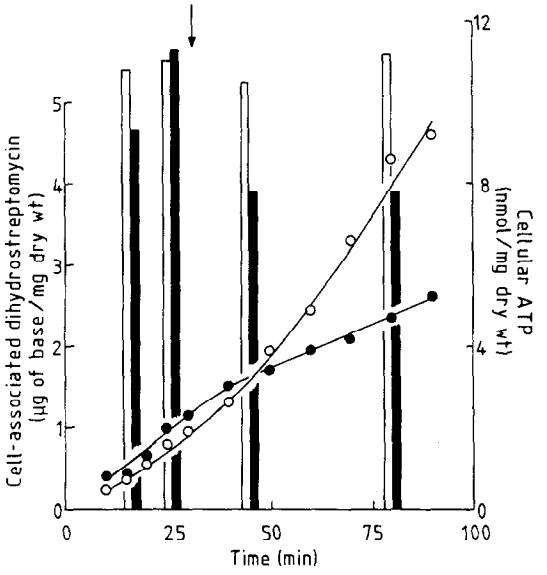

Fig.2. The effect of iodoacetate on ATP levels and dihydrostreptomycin uptake in *E. coli* 7. The symbols are identical to those used in fig.1. In this experiment the final concentration of iodoacetate was 0.47 mM.

4. DISCUSSION

4.1. *Lack of a role of $\Delta\psi$ in irreversibility*

In two critical experiments, falls in the magnitude of $\Delta\psi$ were shown not to be responsible for demonstrated irreversibility of dihydrostreptomycin transport. When cells respiring on succinate were made anaerobic, the driving force for uptake was abolished but no efflux of radiolabelled dihydrostreptomycin occurred [2,8]. However, mean $\Delta\psi$ values estimated before and after such anaerobiosis were similar (-102 and -104 mV, respectively). Secondly, when dihydrostreptomycin at an external concentration of 1 mg of base $\cdot$ ml^{-1} was taken up to a plateau level over a 2 h period, exchange diffusion between cytoplasmic and exogenous dihydrostreptomycin could not be demonstrated [2]. Nevertheless the membrane potential remained fairly constant over this time period (-107 mV at the start and -113 mV after 2 h; table 1).

We conclude that the transport process is itself kinetically irreversible and that the irreversibility described in [1,7,8] cannot be ascribed to electrical gating of hypothesized uniporters [17] or non-specific channels [18].

4.2. *The rate of dihydrostreptomycin uptake was independent of the cellular ATP level*

Stewart et al. [19] showed that the rate of K^+-uptake mediated by the Kdp system fell sharply over 2 min following iodoacetate addition to strain NR71. Over the same period in a parallel experiment the level of intracellular ATP fell by about 50%. Since the Kdp system appears to be a K^+-translocating ATPase [20], the results of Stewart et al. [19] imply that functioning of other transport systems driven directly by ATP hydrolysis could respond rapidly to changes in cellular ATP level. The functioning of the dihydrostreptomycin transport system was independent of the size of the ATP pool (figs 1 and 2), so we suggest that hydrolysis of ATP is not coupled to this transport process. Furthermore we also suggest that the inhibition by iodoacetate of dihydrostreptomycin uptake in *E. coli* that was reported by Andry and Bockrath [1] was not mediated by a fall in the cellular ATP pool. Possibly it was due to a direct sensitivity of the transport process to thiol group reagents [7].

In the work reported here we have examined two potential explanations of why dihydrostreptomycin transport should be irreversible. Our working hypothesis as a result of these studies is that transport is coupled to a chemical reaction that is not the hydrolysis of ATP.

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