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Na^+/Glutamine (Asparagine) Cotransport by *Staphylococcus lugdunensis* and *Corynebacterium amycolatum*

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***Staphylococcus lugdunensis* and *Corynebacterium amycolatum* each have a Na^+/glutamine cotransporter that displays an ordered reaction sequence at the extracellular surface, with sodium binding (K_m of 6.5 mM) before glutamine (K_m of 50 μM). Asparagine is low-affinity substrate ($K_m \approx 1$ mM) for each system.**

Staphylococci and corynebacteria are normal residents on human skin (17). In an effort to describe the physiology of bacteria in this ecosystem, we have studied membrane transport by *Staphylococcus lugdunensis* and *Corynebacterium amycolatum*. We chose glutamine as the test substrate, both because of its ready availability in human sweat (13) and because as the most abundant amino acid in serum (22), glutamine might serve as a source of carbon and nitrogen during infection (7, 20). Here, we show that kinetically similar Na^+/glutamine cotransporters are found in *S. lugdunensis* and *C. amycolatum*.

S. lugdunensis (12) and *C. amycolatum* (9) were grown aerobically at 37°C on Trypticase soy broth with (*C. amycolatum*) or without (*S. lugdunensis*) soy lecithin (0.7 g/liter) and Tween 80 (5 g/liter). Cells were harvested, washed, and resuspended in buffer A (50 mM MOPS [morpholinepropanesulfonic acid]-K, 100 mM potassium sulfate [pH 7]) at 3 mg of cell protein/ml. In routine assays, cells (0.3 mg of protein/ml) were suspended at 23°C in buffer A containing 50 mM sodium sulfate and given 50 μM [^{14}C]glutamine (NEN/Life Science, Inc.) or 100 μM [^{14}C]asparagine (NEN/Life Science, Inc.). Samples were filtered through 0.45-μm-pore-diameter Millipore filters and washed twice with cold buffer A.

Starved cells reenergized by glucose or lactate were used to study the effects of metabolic inhibitors. For this purpose, cells were incubated in buffer A with 5 mM 2,4-dinitrophenol, as described previously (3, 15). After removing dinitrophenol, inhibitors were added ($t = -8$ min), followed by 10 mM glucose or 20 mM lactate ($t = -3$ min) and labeled substrate ($t = 0$ min). Starved cells were also used when ion motive gradients were imposed. In this case, cells were suspended in LowNa buffer (buffer A plus 50 mM *N*-methylglucamine sulfate) or HighNa buffer (buffer A plus 50 mM sodium sulfate). Such cells were starved as described above, along with 10 μM valinomycin to facilitate equilibration of internal and external Na^+ and K^+ contents. Cells were then washed in the original buffers, resuspended to 1.5 mg of protein/ml, and diluted 100-fold into the following solutions, each containing 50 μM labeled glutamine and 10 μM valinomycin. To generate a sodium chemical gradient (ΔpNa), LowNa cells were diluted into buffer A containing 50 mM sodium sulfate. To generate a sodium electrochemical gradient (ΔpNa plus ΔΨ), LowNa cells were diluted into buffer B (buffer A in with 100 mM MOPS-Tris replacing 100 mM potassium sulfate) plus 50 mM sodium sulfate. To establish a membrane potential (ΔΨ), HighNa cells were diluted into buffer B plus 50 mM sodium sulfate. As a control (no gradient), HighNa cells were diluted 100-fold without changing buffer composition. The imposed ion motive gradients would have had initial values of −120 mV (ΔpNa or ΔΨ) or −240 mV (ΔpNa plus ΔΨ).

Glutamine transport by *S. lugdunensis* or *C. amycolatum* was greatly stimulated by sodium (Fig. 1A). Preliminary study indicated the two cells had similar kinetic behavior, and detailed analysis of *S. lugdunensis* showed that the apparent Michaelis constant (K_{mapp}), but not the maximal velocity (10 to 12 nmol/min/mg of protein), depended on sodium concentration (Fig. 1B). This was interpreted as reflecting an ordered bireactant system at the extracellular surface, with Na^+ binding before glutamine (23). With this assumption, a replot of such data gave the true K_m values for Na^+ (6.5 mM) and glutamine (47 μM) (Fig. 1C).

To determine the specificity of the glutamine transport system, the other 19 amino acids were added in 100-fold molar excess (3 mM versus 30 μM [^{14}C]glutamine) during assays with each cell type; only asparagine gave significant inhibition (≈50%) of glutamine transport. Further tests showed that each cell displayed Na^+-dependent asparagine transport ($K_m \approx 1$ mM; V_{max} of 7 to 10 nmol/min/mg of protein) that was inhibitable (>90%) by a 100-fold excess of glutamine. These data are best explained by the presence of a single Na^+-dependent system with a 20-fold higher affinity for glutamine than for asparagine.

To study the energetics of glutamine transport, we first monitored the responses to metabolic inhibitors by starved cells reenergized with glucose or lactate (Table 1). In both instances, a proton conductor (carbonyl cyanide *m*-chlorophenylhydrazone [CCCP]) gave full inhibition. Iodoacetate was also an effective inhibitor when cells used glucose, but this position was occupied by KCN during lactate oxidation. Moreover, when the proton motive force was increased by blocking the F_1-ATPase reentry pathway with *N*,*N*′-dicyclohexylcarbodiimide (DCCD) (16), we observed stimulation of glutamine transport, especially for lactate-energized cells. Considered together with the Na^+ dependence of glutamine transport, these responses are most easily explained if glutamine transport oc-

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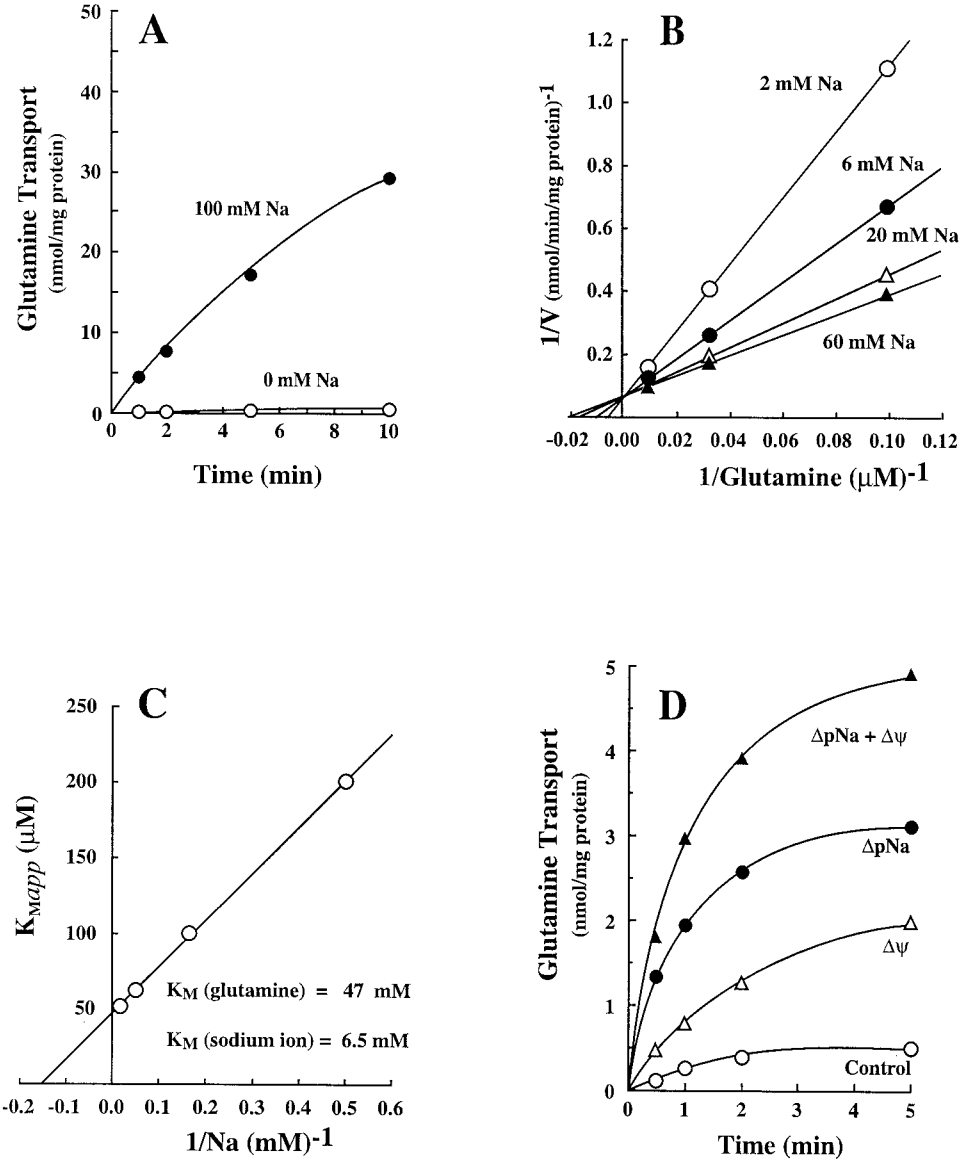

FIG. 1. (A) Glutamine transport was measured for washed cells of *S. lugdunensis* in buffer A with (solid symbols) or without (open symbols) 50 mM sodium sulfate. Equivalent findings were made with *C. amycolatum* (not shown). (B) Initial rates (1 min) of glutamine transport for *S. lugdunensis* were used to estimate the K_{mapp} of glutamine for the indicated Na^+ concentrations. (C) Data from panel B were used to derive true K_m values for sodium and glutamine. (D) Ion motive gradients were imposed as described in the text, with starved cells of *S. lugdunensis*. Equivalent findings were made with *C. amycolatum* (not shown).

curs via an Na^+/glutamine cotransporter. This idea was strongly supported by the finding that an inwardly directed sodium motive gradient can drive transport in starved cells in the absence of ongoing metabolism (Fig. 1D). Thus, background levels of transport were found when no ion motive gradients were imposed on starved cells, while increasing levels of glutamine accumulation were elicited by imposition of a membrane potential, a sodium chemical gradient, or both. In the last case, the initial rate of glutamine transport (3 to 4 nmol/min/mg of protein) was comparable to that found for unstarved cells (Fig. 1A).

We conclude that both *S. lugdunensis* and *C. amycolatum* possess kinetically similar Na^+/glutamine (asparagine) cotransporters whose properties are well adapted to exploit the composition of adult eccrine sweat with respect to Na^+ (35 to 60 mM), glutamine (20 μM), and asparagine (160 μM) (13, 14, 22). Because serum has a great excess of glutamine (0.4 mM) (22), we also assume the reaction will operate in situations associated with infection. We note that transport of glutamine is most often attributed to an ATP-dependent system, either as the result of direct experimentation (4–6, 10, 26, 29) or by inference from the genomic sequence (1, 8, 11, 18, 19, 21, 27). Ion-coupled transporters have been less frequently described (2, 24, 25), but of those reported, the account of Siewe et al. (25) is the most relevant. In that instance, study of *C. glutamicum* revealed a Na^+/glutamine cotransporter (reaction order

TABLE 1. Effect of metabolic inhibitors on glutamine transport by starved cells of *S. lugdunensis*[a]

Addition	Glutamine transport (nmol/mg of protein/10 min)
None	4.4
Glucose	41
+1 mM iodoacetate	8.0
+5 mM KCN	20
+10 μM CCCP	2.0
+50 μM DCCD	54.0
Lactate	22
+1 mM iodoacetate	23
+5 mM KCN	4.4
+10 μM CCCP	2.3
+50 μM DCCD	66

[a] *C. amycolatum* could not be starved of metabolizable reserves by using 2,4-dinitrophenol alone. In contrast, the starvation protocol was equally effective for *S. lugdunensis* and *C. amycolatum* when 2,4-dinitrophenol was used in combination with valinomycin, as in Fig. 1D.

unknown) resembling the systems described here, except for a somewhat higher affinity for Na^+ (1.4 mM) than we observed (6.5 mM) (Fig. 1C). It is also worth comment that the finding of similar systems in *S. lugdunensis* and *C. amycolatum* is unexpected, since among gram-positive bacteria, *S. lugdunensis* belongs to the group whose DNA has high G+C content, while *C. amycolatum* is assigned to the low G+C group (28). The presence of similar systems in such divergent cells could be explained by either convergent evolution, driven by the shared ecosystem, or horizontal gene transfer. The availability of genomic sequences should help address this issue.

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