Characterization of Amino Acid Transport in Membrane Vesicles from the Thermophilic Fermentative Bacterium *Clostridium fervidus*

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Amino acid transport was studied in membrane vesicles of the thermophilic anaerobic bacterium *Clostridium fervidus*. Neutral, acidic, and basic as well as aromatic amino acids were transported at 40°C upon the imposition of an artificial membrane potential (Δφ) and a chemical gradient of sodium ions (ΔμNa⁺). The presence of sodium ions was essential for the uptake of amino acids, and imposition of a chemical gradient of sodium ions alone was sufficient to drive amino acid uptake, indicating that amino acids are symported with sodium ions instead of with protons. Lithium ions, but no other cations tested, could replace sodium ions in serine transport. The transient character of artificial membrane potentials, especially at higher temperatures, severely limits their applicability for more detailed studies of a specific transport system. To obtain a constant proton motive force, the thermostable and thermoactive primary proton pump cytochrome c oxidase from *Bacillus stea rothermophilus* was incorporated into membrane vesicles of *C. fervidus*. Serine transport could be driven by a membrane potential generated by the proton pump. Interconversion of the pH gradient into a sodium gradient by the ionophore monensin stimulated serine uptake. The serine carrier had a high affinity for serine (Kᵣ = 10 μM) and a low affinity for sodium ions (apparent Kᵣ = 2.5 mM). The mechanistic Na⁺-serine stoichiometry was determined to be 1:1 from the steady-state levels of the proton motive force, sodium gradient, and serine uptake. A 1:1 stoichiometry was also found for Na⁺-glutamate transport, and uptake of glutamate appeared to be an electroneutral process.

Thermophilic bacteria are adapted to elevated temperatures in several respects. To elucidate the molecular basis of thermophily, extensive studies have been performed (1, 32). In general, proteins from thermophilic bacteria have an optimum activity at high temperatures and these proteins are more thermostable than those of mesophilic organisms. These properties are often the result of subtle changes in hydrophobic interactions, hydrogen bonds, sulfur-sulfur bonds, and ionic bonds within these proteins (2, 29). At the membrane level, thermophilic bacteria also differ from mesophilic bacteria with respect to fatty acid and polar headgroup composition of the phospholipid bilayer. As a result, the microviscosity of the cytoplasmic membrane and physiological features such as protein-lipid interactions or ion conductivity are adjusted to elevated temperatures (4, 31).

Thermophiles are often applied to biotechnological purposes, since their enzymes can catalyze processes at higher temperatures (3, 33). Of particular interest are fermentative thermophilic bacteria since these organisms can be applied in waste treatment systems or in fermentation processes such as the conversion of polysaccharides to ethanol (3, 33).

Except for some preliminary studies on ATP-linked sugar transport in whole cells (16, 26), an essential aspect of the physiology of fermentative thermophiles, i.e., the import of solutes and export of metabolic (end) products, has received little attention. Since it is difficult to obtain reliable information about the biochemistry of solute transport systems from studies with intact cells, solute transport systems are preferably studied in isolated membrane vesicles (21). It has been realized that in isolated membranes of fermentative organisms the primary proton pump, the membrane-bound H⁺-ATPase, is not accessible to externally added ATP. The generation of a proton motive force by the ATPase is therefore not possible. Artificially, a proton motive force can be generated by imposed diffusion gradients of weak acids (23) or ions or both in the presence of ionophores (19). However, the transient character of these gradients, especially at elevated temperatures, severely limits their applicability for detailed studies of proton motive force-dependent solute transport processes. An elegant and efficient solution to this problem has been found in the incorporation of foreign primary proton pumps by fusion of the membrane vesicles with proteoliposomes containing these pumps. These fused membranes have proved to be very useful for the study of amino acid transport in membranes of fermentative mesophilic organisms such as lactococci and *Clostridium acetobutylicum* (5, 8-10, 12). To study solute transport systems in membranes of a thermophilic fermentative bacterium at or around the growth temperature, a fused membrane system which contains a thermostable and thermoactive primary proton pump is required.

Recently, the thermostable and thermoactive cytochrome c oxidase from *Bacillus stea rothermophilus* has been purified and reconstituted in liposomes (36). The use of *B. stea rothermophilus* cytochrome c oxidase as a generator of the proton motive force by incorporating this enzyme into membrane vesicles of the thermophilic fermentative bacterium *Clostridium fervidus* enabled us to study amino acid transport in this latter organism. This is the first report of such a study in a thermophilic fermentative bacterium.

**MATERIALS AND METHODS**

**Abbreviations.** ΔμNa⁺, transmembrane chemical gradient of sodium ions; Δφ, transmembrane electrical potential; Δp, proton motive force; ΔpH, transmembrane proton gradient; Δψ, membrane potential; MES, 2(N-morpholino)ethanesulfonic acid; SF-6847, 3,5-di-tert-butyl-4-hydroxy-benzelidene malonitrite; TMPD, *N,N,N',N'-tetramethyl-p-phenylene-diamine; TPP⁺, tetraphenylphosphonium ion.

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Cell growth and preparation of membrane vesicles. *C. fervidus* ATCC 43204 was grown at 68°C in tryptone-yeast extract-glucose medium as described by Zelkus et al. (36) with the modification that the final concentration of resazurine was 5 × 10⁻⁴ M (wt/vol). A vitamin solution as described by Stams et al. (34) (final concentration, 0.1%) and Na₂S (final concentration, 5 mM) were added to the sterilized medium. The cells were grown anaerobically in serum bottles, and medium was flushed with oxygen-free nitrogen. Exponentially growing cells were harvested at an A₆₅₀ of 0.30 to 0.35.

Membrane vesicles were prepared as described by Konings et al. (22). The lysis temperature was kept at 50°C. Membrane vesicles were suspended in 50 mM potassium phosphate or sodium phosphate (pH 6.0) (5 to 10 mg of membrane protein per ml), rapidly frozen, and stored in liquid nitrogen.

**Purification of* B. stearothermophilus* cytochrome c oxidase.** *B. stearothermophilus* cytochrome c oxidase was purified as described by De Vrij et al. (6) with omission of the final gel filtration step. The oxidase preparation had a heme and L-phosphatidylcholine of acetone-ether-washed membrane protein per ml), rapidly frozen, and stored in liquid nitrogen.

**Reconstitution of* B. stearothermophilus* cytochrome c oxidase.** Proteoliposomes containing *B. stearothermophilus* cytochrome c oxidase were prepared by a modified procedure described by Hinkle et al. (18). *B. stearothermophilus* cytochrome c oxidase was reconstituted into liposomes consisting of acetone-ether-washed *Escherichia coli* phospholipid and L-phosphatidylcholine (3:1, wt/wt) at a ratio of 0.4 mM of heme a per mg of phospholipid. The proteoliposomes were stored in 50 mM potassium phosphate or sodium phosphate (pH 6.0), rapidly frozen, and stored in liquid nitrogen.

**Fusion of oxidase-containing liposomes and *C. fervidus membrane vesicles.** *C. fervidus* membrane vesicles (1 mg of membrane protein) were mixed with *B. stearothermophilus* cytochrome c oxidase-containing liposomes (10 mg of phospholipid) in a total volume of 1 ml. The suspension was rapidly frozen in liquid nitrogen, slowly thawed at room temperature, and sonicated for 8 s with a probe-type sonicator (MSE Scientific Instruments, West Sussex, United Kingdom) at an output of 2 microns. During sonication, the suspension was flushed with nitrogen. If necessary, the fused membranes were concentrated or washed by centrifugation for 25 min at 178,000 × g at room temperature in a Beckman-type airfuge. Fused membranes were prepared in 50 mM potassium phosphate or sodium phosphate (pH 6.0).

**Determination of transmembrane gradients.** The membrane potential (interior negative) was determined from the distribution of the TPP⁺ across the membrane with a TPP⁺-selective electrode (24). The membrane potential was determined by applying a correction for concentration-dependent probe binding as described by Lolkema et al. (24). TPP⁺ uptake in fused membranes was registered at 30 or 40°C in a 2-ml vessel containing fused membranes (30 g of protein), 50 mM sodium phosphate (pH 6.0), 10 mM MgSO₄, and 4 µM TPP⁺. Ascorbate (20 mM) and TMPD (300 µM) were added as electron donors. When a Δψ and serum uptake were measured simultaneously, 400 µg of membrane protein was added. The ionophore monensin (final concentration, 20 nM) and the uncoupler SF-6487 (final concentration, 300 nM) were used.

The membrane potential in the fused membranes was measured qualitatively by using the membrane potential indicator 3,3'-dipropylthiocarbocyanine iodide by measuring membrane potential-dependent absorbance changes (ΔA₆₃₀/Δt) as described by De Vrij et al. (6).

**Uptake of amino acids in membrane vesicles.** Uptake of amino acids driven by artificial gradients was measured at 40°C in membrane vesicles (5 to 10 mg of membrane protein per ml) suspended in 20 mM potassium phosphate (pH 6.0) supplemented with 100 mM potassium acetate and 10 mM MgSO₄, unless stated otherwise. Valinomycin was added to the membrane vesicles at a final concentration of 2 nmol/mg of protein. A combination of a membrane potential (Δψ) and a Δψₕₐₙ was generated by dilution into 20 mM sodium phosphate (pH 6.0)–100 mM sodium acetate–10 mM MgSO₄. A membrane potential (Δψ) only was generated by dilution of the membrane vesicles into 20 mM choline phosphate (pH 6.0)–100 mM choline acetate–10 mM MgSO₄. A chemical gradient of sodium ions was applied by diluting the membrane vesicles 100-fold into 20 mM sodium phosphate (pH 6.0)–100 mM sodium acetate–10 mM MgSO₄ without the prior incubation with valinomycin. Dilution of the membrane vesicles suspended in 20 mM potassium phosphate–50 mM NaOH–50 mM KOH–100 mM acetic acid–10 mM MgSO₄ (pH 6.0) into 20 mM potassium phosphate–50 mM NaOH–50 mM KOH–100 mM MES–10 mM MgSO₄ (pH 6.0) resulted in the generation of a transmembrane pH gradient (ΔpH) only. In all cases, the dilution buffer (200 µl) was supplemented with L-[U-¹⁴C]-labeled amino acids at final concentrations of 2.9 µM for serine, arginine, phenylalanine, or lysine; 2.6 µM for glutamate; 4.4 µM for glycine; 3.3 µM for alanine, leucine, or threonine; 3.5 µM for glutamine or aspartate; and 4.8 µM for asparagine.

At given time intervals, 2 ml of ice-cold 0.1 M KCl was added and the samples were filtered immediately on cellulose-nitrate filters (0.45-µm pore size; Millipore Corp., Bedford, Mass.). Filters were washed once with 2 ml of 0.1 M KCl. Dried filters were transferred to scintillation vials, and 3 ml of scintillation fluid was added. The radioactivity was measured with a liquid scintillation counter (Tri-Carb 460 CD; Packard Instrument Co., Inc., Rockville, Md.).

**Uptake of amino acids in fused membranes.** The uptake of serine and glutamate in fused membranes driven by a Δψ generated by *B. stearothermophilus* cytochrome c oxidase was measured at 40°C, unless stated otherwise, by the following procedure. Fused membranes (0.2 ml; 0.15 mg of membrane protein) were added to 0.8 ml of oxygen-saturated 50 mM potassium phosphate or sodium phosphate (pH 6.0)–10 mM MgSO₄. After 1.5 to 5 min of preincubation with the electron donor system ascorbate (20 mM) plus TMPD (300 µM), [1⁴C]serine (final concentration, 5.8 µM) or [1⁴C]glutamate (final concentration, 3.5 µM) was added and at given time intervals samples of 100 µl were taken and rapidly filtered as described above. During the assay, the reaction mixture was flushed with water-saturated oxygen. Uptake was assayed as described above.

**Protein determination.** Protein was determined by the method of Lowry et al. (25) with bovine serum albumin as a standard.

**Internal volume.** The internal volume of the fused membranes was determined from the relative fluorescence quenching by cobalt of membrane-entrapped calcein as described by Oko et al. (27). An internal volume of 8 µl/mg of membrane protein was determined for the fused membranes. An internal volume of 3 µl/mg of membrane protein was used for *C. fervidus* membrane vesicles.

**Materials.** The following uniformly labeled [¹⁴C]-labeled amino acids were used: L-serine, L-alanine (6.4 TBq/mol); L-glutamate, L-glutamine (10.5 TBq/mol); L-leucine, L-arginine.
TABLE 1. Uptake of amino acids by membrane vesicles of *C. fervidus* upon imposition of a membrane potential and $\Delta \psi_{Na^+}$ at 40°C

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Initial rate of uptake (nmol/min per mg of protein)</th>
</tr>
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<tbody>
<tr>
<td>Serine</td>
<td>8.5</td>
</tr>
<tr>
<td>Threonine</td>
<td>7.6</td>
</tr>
<tr>
<td>Leucine</td>
<td>7.6</td>
</tr>
<tr>
<td>Glycine</td>
<td>6.2</td>
</tr>
<tr>
<td>Glutamate</td>
<td>4.8</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>4.7</td>
</tr>
<tr>
<td>Arginine</td>
<td>3.9</td>
</tr>
<tr>
<td>Glutamine</td>
<td>2.6</td>
</tr>
<tr>
<td>Alanine</td>
<td>2.4</td>
</tr>
<tr>
<td>Lysine</td>
<td>2.4</td>
</tr>
<tr>
<td>Asparagine</td>
<td>2.2</td>
</tr>
<tr>
<td>Aspartate</td>
<td>2.1</td>
</tr>
</tbody>
</table>

*Valinomycin-treated membrane vesicles (2 nmol/mg of protein) prepared in 20 mM potassium phosphate (pH 6.0)–100 mM potassium acetate were diluted 100-fold into 20 mM sodium phosphate (pH 6.0)–100 mM sodium acetate–10 mM MgSO$_4$ and the uptake of amino acids was observed. Initial rates of uptake were determined during the first 5 s.*

L-lysine (12.6 TBq/mol); L-glycine (4.2 TBq/mol); L-threonine (8.4 TBq/mol); L-asparagine (3.9 TBq/mol); L-aspartate (8.0 TBq/mol); and L-phenylalanine (19.3 TBq/mol).

All radioactive chemicals were obtained from the Radiochemical Centre, Amersham, United Kingdom. All other chemicals were reagent grade and were purchased from commercial sources.

RESULTS

Uptake of amino acids in membrane vesicles. Membrane vesicles were isolated from *C. fervidus*. Uptake of several neutral, basic, and acidic as well as aromatic amino acids in these membrane vesicles was observed over time at pH 6.0 and 40°C. This temperature was used because the membrane permeability and the evaporation rate allowed uptake for several minutes. Upon imposition of an artificial membrane potential and a chemical gradient of sodium ions, all these amino acids were taken up by the membrane vesicles at relatively high rates (Table 1). These results suggest that the $\Delta \psi$ or the chemical gradient of sodium ions or both function as the driving force for uptake of these amino acids.

To discriminate among these possibilities, we studied uptake of the neutral amino acid serine under conditions such that only a $\Delta \psi$, a $\Delta \phi$, or a chemical gradient of sodium ions was imposed (Fig. 1). Serine was not taken up when a $\Delta \psi$ or a $\Delta \phi$ only was applied in the absence of sodium ions nor was it taken up with a $\Delta \phi$ only in the presence of sodium ions ($[Na^+]_i = [Na^+]_o$). Uptake of serine, however, was observed upon imposition of a chemical gradient of sodium ions (Fig. 1). When both a chemical gradient of sodium ions and a $\Delta \psi$ were applied, the uptake of serine was even further stimulated (Fig. 1). These observations indicate that the uptake of serine is electrogenic and sodium dependent. The uptake of the other amino acids tested appeared to depend similarly on the presence of sodium ions (data not shown), showing that the amino acid transport systems from *C. fervidus* are most likely Na$^+$-solute symport systems.

The cation specificity of the serine transport system was examined in more detail by measuring the effect of different cations on the uptake of serine (Fig. 2). Potassium- and acetate-loaded membrane vesicles preincubated with valinomycin were diluted 100-fold into an MES buffer (pH 6.0).

containing 100 mM choline acetate in the presence of the different chloride salts (50 mM final concentration). Upon imposition of a $\Delta \psi$, the uptake of serine was low in the presence of Rb$^+$, NH$_4^+$, Cs$^+$, or choline$^+$, and only in the presence of Na$^+$ or Li$^+$ were high rates of serine uptake observed.
membrane proteins of C. fervidus were fused with liposomes containing cytochrome c oxidase from *B. stearothermophilus* (0.4 nmol of heme a per mg of phospholipid) in a ratio of 0.1 mg of membrane protein per mg of phospholipid. At pH 6.0 and 40°C, oxidation of reduced TMPD in the fused membrane system led to the generation of a Δp, consisting of a high Δφ (−130 mV) and ZΔpH (35 mV). Under these conditions, a high accumulation of serine was observed in the fused membrane system, while no uptake of serine was observed in the presence of reduced TMPD in the membrane vesicles of *C. fervidus* or in the proteoliposomes containing the oxidase (Fig. 3). These results show that fusion of both membrane preparations had occurred and confirm that the Δp or one of its components can act as a driving force for uptake of serine.

To obtain more support for the crucial role of Na⁺, we studied the uptake of serine in fused membranes upon 10-fold dilution in potassium phosphate or sodium phosphate buffer at pH 6.0. Dilution (10-fold) of the fused membranes into potassium phosphate buffer resulted in a very low uptake even in the presence of the electron donor system ascorbate plus TMPD. However, dilution of the fused membranes into sodium phosphate buffer led to accumulation of serine even in the absence of the electron donor (Fig. 4). Higher uptake rates and steady-state levels of accumulation of serine were observed upon dilution of the membranes into sodium phosphate buffer in the presence of a Δp generated by the cytochrome c oxidase (Fig. 4). The protonophore SF-6847 (300 nM) had no effect on sodium ion gradient-driven serine uptake, indicating that no significant reversed Δp was generated.

The uptake of serine by the fused membranes in the presence of sodium ions ([Na⁺]_o = [Na⁺]_i) and a Δp could be inhibited in several ways (Fig. 5A). The addition of the protonophore SF-6847 (300 nM) before the radioactively labeled amino acid was added completely abolished the generation of a Δp and also the uptake of serine. Preincubation of the membranes with the cytochrome c oxidase inhibitor cyanide (2 mM) also led to complete inhibition of serine uptake. Specific collapse of the membrane potential by the addition of the ionophore nonactin (4.5 μM) resulted in an increase of ΔpH (data not shown) but completely reduced serine uptake, supplying additional evidence that serine is not symported with protons (Fig. 5A).

Strong support that sodium ions instead of protons are symported with serine was supplied by the observation that the sodium/proton exchanger monensin (20 nM) at low external pH strongly enhanced the rate and the steady-state level of serine uptake (Fig. 5B). In the presence of monensin, the pH gradient is completely interconverted into a ΔpNa⁺.
with some increase of the membrane potential (data not shown).

Mechanistic stoichiometry of serine transport system. The results presented above demonstrate that a pH 6.0 serine transport is electrogenic and that serine is symported with at least one Na⁺ ion. To determine the stoichiometry, we determined the relation between the membrane potential and the chemical gradient of serine in the fused membranes in the absence of a chemical gradient of sodium ions \(([\text{Na}^+]_\text{in} = [\text{Na}^+]_\text{out})\). \(\Delta\psi\) was varied by manipulating the concentration of reduced TMPD. \(\Delta\psi\) and serine uptake were measured simultaneously. At thermodynamic equilibrium between the driving force for serine uptake and the chemical serine gradient, the mechanistic stoichiometry \((n)\) can be derived from the equation 
\[-Z\Delta\bar{\mu}_\text{ser} = n\Delta\psi - nZ\Delta\bar{\mu}_\text{Na}^+\].

The absence of a chemical gradient of sodium ions simplifies this equation to 
\[-Z\Delta\bar{\mu}_\text{ser} = n\Delta\psi\]. The steady-state level of accumulation of serine depended linearly on the \(\Delta\psi\), indicating that the Na⁺-serine stoichiometry was constant at different \(\Delta\psi\) values. A stoichiometry \((n)\) of 0.9 can be calculated from the slope of this linear relationship (Fig. 6).

Kinetic analysis of serine transport in hybrid membrane system. The kinetic constants of the Na⁺-serine symporter for Na⁺ and serine were determined in the fused membranes at 40°C and at an external pH of 6.0 by using the incorporated cytochrome c oxidase as a generator of \(\Delta\psi\) by adding the electron donor system ascorbate plus TMPD. The initial rates of serine uptake were determined in the serine concentration range of 1.5 to 35 μM and in the presence of 50 mM Na⁺ and 20 mM monensin. Eady-Hofstee analysis indicated a high affinity for serine \((K_s = 10 \mu M)\) with a \(V_{\text{max}} = 5.3\) nmol/min per mg of protein (data not shown).

FIG. 5. Effect of the cytochrome c oxidase inhibitor KCN and ionophores on the uptake of serine in membrane vesicles of *C. fervidus* fused with cytochrome c oxidase-containing liposomes. The fusion product was prepared in 50 mM sodium phosphate (pH 6.0), and the uptake was measured at 40°C in 50 mM sodium phosphate (pH 6.0) supplemented with 10 mM MgSO₄. (A) Serine transport in the absence of the electron donor system ascorbate plus TMPD (○); in the presence of ascorbate (20 mM) and TMPD (300 μM) (●); or with the addition of KCN (2 mM) (□), SF-6847 (300 mM) (■), or monensin (4.5 μM) (▲). (B) Serine transport in the absence of ascorbate plus TMPD (○), or in the presence of ascorbate (20 mM) plus TMPD (300 μM) without (●) or with (■) monensin (20 nM).

FIG. 6. Relationship between the steady-state accumulation level of serine \((Z\Delta\bar{\mu}_{\text{ser}})\) and the membrane potential \((\Delta\psi)\) in *C. fervidus* membrane vesicles fused with cytochrome c oxidase-containing liposomes. The experiments were performed at 30°C as described in Materials and Methods. Serine uptake and the membrane potential were measured simultaneously. The membrane potential was varied by manipulating the TMPD concentration. The dashed line represents \(-Z\Delta\bar{\mu}_{\text{ser}}/\Delta\psi = 1\).
The affinity of the serine transport system for sodium ions appeared to be rather low, since the sodium ion contamination from glassware (which can account for up to 100 μM) was not sufficient to allow a significant rate of uptake of serine. Eady-Hofstee plots of the initial rates of serine uptake as a function of the Na⁺ concentration at a saturating concentration of serine (44 μM) indicated a Kᵢ of 2.5 mM.

**1. Glutamate uptake in hybrid membrane system.** The uptake of the acidic amino acid L-glutamate was also studied in more detail in the fused membranes (Fig. 7). The generation of a Δp by the incorporated cytochrome c oxidase did not lead to significant accumulation of L-glutamate, irrespective of the absence or presence of sodium ions ([Na⁺]ᵢ = [Na⁺]ₐ₀). However, the addition of monensin (20 nM) in the presence of Na⁺ resulted in the interconversion of the ΔpH into a chemical gradient of sodium ions and led to a high rate of L-glutamate accumulation (Fig. 7A). These data indicate that the symport of Na⁺ and L-glutamate is an electroneutral process and occurs most likely in a 1:1 stoichiometry. This was confirmed by applying an artificial chemical gradient of sodium ions, which led to high accumulation of L-glutamate (Fig. 7B). Additional Δp generation by the cytochrome c oxidase did not improve the uptake rate or the steady-state level of L-glutamate accumulation (Fig. 7B).

**DISCUSSION**

*C. fervidus* was found to possess several Na⁺-amino acid symport systems. No precautions were taken against possible inactivation by oxygen of the amino acid carriers from *C. fervidus*. However, since no higher amino acid transport activity was observed in membrane vesicles of *C. fervidus* after an artificial membrane potential and sodium gradient under anaerobic conditions were imposed (unpublished data), the carrier proteins of this obligate anaerobic bacterium appear to be rather insensitive to oxygen inactivation.

The fused membrane system, composed of proteoliposomes containing the thermostable and thermoactive Δp generator cytochrome c oxidase from *B. stearothermophilus* and membrane vesicles of the thermophilic fermentative bacterium *C. fervidus*, proved to be very convenient for the study of Δp-dependent processes in *C. fervidus*. Only the membrane potential generated by the incorporated oxidase could function as a driving force for uptake of a neutral amino acid, such as serine. Evidence that serine transport in the fused membrane system is driven by one of the components of the Δp generated by the cytochrome c oxidase was obtained by the inhibitory effects on serine uptake of the protonophore SF-6847 and of the cytochrome c oxidase inhibitor cyanide. Direct evidence that this component is the membrane potential was obtained by the inhibitory effect of the ionophore nonactin. The Δp gradient generated by the cytochrome c oxidase did not drive serine uptake. Only upon interconversion by monensin of the ΔpH into a chemical gradient of sodium ions was serine uptake observed.

Although the hybrid membrane system was applied at a temperature of 40°C, temperatures up to 50°C can be used (unpublished data). The enhanced activity of the *B. stearothermophilus* cytochrome c oxidase compensates for the high proton permeability of the membranes at higher temperatures up to 50°C. Above 50°C, the extremely high proton
permeability leads to a decreased $\Delta p$. More rigid phospholipids isolated from thermophilic bacteria should be used for transport studies in fused membranes at extremely high temperatures. The applicability at elevated temperatures of this fused membrane system can also be restricted by the oxygen supply and the high evaporation rate of the reaction mixture. The introduction of reaction centers from thermophilic phototrophic bacteria as $\Delta p$ generators in these fused membrane systems can be used to avoid these problems and allow uptake in closed vessels under strictly anaerobic conditions with light as an energy source (5, 10).

The fused membrane system made it possible to characterize some properties of the serine transport system in more detail. Serine was shown to be transported by a high-affinity transport system in this organism ($K_0 = 10 \mu M$). A linear relationship was found between the steady-state level of accumulation of serine and the $\Delta \psi$. The slope of this linear relationship depended on the number of cations which were symported with serine and on the rate of non-carrier-mediated serine efflux (passive diffusion) across the lipid membrane (5, 11). A $\Delta \mu_{sou}/\Delta \psi$ ratio of 0.9 was measured. The mechanistic Na$^+$/serine stoichiometry is thus most likely 1:1. Sodium ion or lithium ion concentration gradients could drive serine uptake in membranes of C. fervidus, while a $\Delta pH$ could not. This indicated that the amino acids are symported with Na$^+$ or Li$^+$ ions rather than with protons. Na$^+$-solute symport is rather common in bacteria living in Na$^+$-rich environments and even in freshwater organisms (7). High-affinity Na$^+$-solute transport systems have been reported frequently. For example, in the enterobacterium E. coli, high-affinity Na$^+$-solute symporters are found for serine, proline, and glutamate (7, 14), and in the thermophilic Bacillus strain PS-3, such a symport exists for alanine (20). Na$^+$ is the predominant ion for solute transport in marine, halophilic, and obligately alkalophilic bacteria (7). In these organisms, Na$^+$-solute symporters with a low affinity for Na$^+$ are found. A low-affinity Na$^+$-serine-threonine transporter ($K_m$ of Na$^+$ = 30 mM) has been observed in the ruminal bacterium Streptococcus bovis JB, (30). C. fervidus, which has been described recently (28), is a neutrophilic organism. The hot pool RT4 from which this organism was isolated contained 16 mM Na$^+$ (B. K. C. Patel, personal communication).

Because Na$^+$ gradients appear to play an important role in energy transduction in C. fervidus, the question arises how these gradients are generated. In fermentative bacteria, primary sodium pumps have been reported, such as membrane-bound decarboxylases (7) and membrane-bound Na$^+$/K$^+$-ATPases (2, 15, 17). The most general mechanism, however, to create a chemical gradient of sodium ions is by the action of Na$^+$/H$^+$ antiporters, which convert a $\Delta p$ into a $\Delta \mu_{sou}$. Na$^+$/H$^+$ antiporters have been described in thermophilic bacteria (13, 35). The low steady-state level of reaction of L-glutamate in the presence of Na$^+$ and $\Delta p$ and the drastic stimulatory effect of monensin on the uptake of serine and glutamate suggests that a $\Delta \mu_{sou}$ is not effectively generated by Na$^+$/H$^+$ exchange under the experimental conditions employed.

LITERATURE CITED