Short Communication

Is HCO₃⁻ Transport in Anabaena a Na⁺ Symport?¹

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ABSTRACT

Na⁺ strongly promoted HCO₃⁻ transport in Anabaena variabilis. The effect was highly specific to this cation. Kinetic analysis indicated a progressive decrease in the $K_a$ (HCO₃⁻) of the transport system with increasing Na⁺ concentration. $V_{max}$ was also affected. We raise the possibility that the transport is a Na⁺-HCO₃⁻ symport; alternatively, that a Na⁺-H⁺ antiport (or Na⁺-OH⁻ symport) system mediates the efflux of the OH⁻ ions derived from the entering HCO₃⁻ ions, and that this antiport can rate-limit HCO₃⁻ influx.

Many aqueous photosynthesizing organisms have been shown to possess the capacity for active membrane transport of HCO₃⁻. The molecular mechanism of this transport process is currently attracting considerable attention (see 5) but is at present poorly understood. We have recently brought evidence (2) for the participation of an electrogenic pump in the transport process in Anabaena. We now wish to report findings indicating a crucial role for Na⁺ ions.

MATERIALS AND METHODS

Cells of Anabaena variabilis M-3 (from the Tokyo University collection) were grown as described elsewhere (7), at a CO₂ level equal to that in air. After harvesting, the cells were resuspended in 25 mm bis-tris(hydroxymethyl)methylammonopropane (BTP) brought to the desired pH with Hepes. Oxygen evolution was measured by O₂ electrode (Rank Brothers, Cambridge, U.K.). Uptake of inorganic carbon (C) was determined radioisotopically following the supply of H⁺-HCO₃⁻, using the filtering centrifugation technique (7).

RESULTS AND DISCUSSION

We have observed that, in the presence of Na⁺, HCO₃⁻ transport in Anabaena is strongly promoted. Figure 1 shows O₂ electrode tracings from an experiment where Anabaena cells had been allowed to approach the O₂ compensation point. Under these conditions photosynthetic O₂ evolution is limited by the HCO₃⁻ transport process (1). Figure 1a shows that additions of KCl and MgCl₂ (at the first and second arrows, respectively) were without effect on the rate of O₂ evolution. The addition of NaCl, however (at the 3rd arrow), produced a dramatic increase in rate. The effect was not dependent on the presence of Cl⁻, since it was also produced by Na₂SO₄ (Fig. 1b).

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![Figure 1. O₂ electrode tracings for photosynthetic O₂ evolution by A. variabilis cells. When the cells had nearly reached O₂ compensation point, KCl was added (first arrow in tracing a) followed by MgCl₂ (second arrow) and NaCl (third arrow), each to a concentration of 10 mm. In b, Na₂SO₄ was added at arrow to a concentration of 5 mm. Experimental conditions: 2 ml cell suspension in the O₂ electrode, corresponding to 11 µg Chl/ml; 40 mm 1,3-bis-tris(hydroxymethyl)methylammonopropane (BTP) buffer, brought to pH 9.0 with Hepes; 30°C; light intensity, 35 mE·cm⁻²·s⁻¹ (400–700 nm).](image)
slope was strikingly increased. The size of this effect (at a given pH) increased with decrease in HCO₃⁻ concentration. In a report which has appeared since this manuscript was submitted, a similar effect of NaCl has been described for Synechococcus (6).

That the Na⁺ effect was indeed exerted on HCO₃⁻ transport, and not directly on photosynthesis, is established by the following finding: The effect was clearly seen when rate of photosynthesis was plotted against external inorganic C concentration, but was not detectable when photosynthesis was plotted against internal inorganic C concentration (3). Moreover, the presence of Na⁺ markedly affected the amount of C₅ taken up within 5 s of supply of H⁺CO₃⁻ (see Fig. 2). After this brief uptake period by far the greater part of the C detected in the cells is still in inorganic form (Volokitkia et al., unpublished).

The experiment shown in Figure 2 investigated the kinetics (rate versus concentration) of HCO₃⁻ uptake in the absence and presence of various concentrations of Na⁺. The progressive decrease in the slope of the line relating 1/v to 1/s with increasing concentration of Na⁺ indicates a progressive decrease in apparent Kₙ (i.e. a progressive increase in the apparent affinity of the transport system for HCO₃⁻) as the Na⁺ concentration was increased. Vₘₐₓ was also affected by Na⁺ but to a lesser extent than was Kₙ.

The highly specific effect of Na⁺ on the apparent Kₙ and Vₘₐₓ for HCO₃⁻ transport demonstrated here could be taken to indicate that binding of Na⁺ to the carrier (or to some component of the carrier system) at the outer membrane surface brings about a change in the transport parameters (model I). There is no need to postulate that the Na⁺ which is bound is also transported into the cell. However, in view of the numerous well documented cases of Na⁺-substrate symport (for the most part in animal and microbial cells), this possibility, in our opinion, merits consideration (model II). In cases of Na⁺ symport, a Na⁺ extrusion pump transfers Na⁺ out of the cell against its electrochemical potential gradient, thus establishing a transmembrane ΔΨNa⁺. The downhill return flux of Na⁺ ions is coupled to inward flux of the symported substrate, in this case HCO₃⁻. Evidence for the operation of a Na⁺ extrusion pump exists for a number of types of plant cell, since it is commonly observed that the internal Na⁺ concentration is below that predicted by the Nernst equation. It is unnecessary to speculate at this stage as to whether the extrusion is 'primary active', that is, directly energized by ATP, or 'secondary active'. The model requires, however, that HCO₃⁻ accumulation must not proceed beyond the point at which μHCO₃ becomes equal to n ΔΨNa⁺, where n indicates the Na⁺-to-HCO₃⁻ stoichiometry (see 9).

A third possibility also deserves serious consideration. According to this model (model III), the Na⁺ involvement is not directly concerned with HCO₃⁻ influx, but with the efflux of the OH⁻ ions which are liberated within the cell when CO₂ derived from the transported HCO₃⁻ is fixed. An Na⁺-H+ antiport system (or Na⁺-OH⁻ symport) is currently thought to play an important role in pH maintenance in bacteria (4, 8) and such a system may also regulate intracellular pH during HCO₃⁻ uptake in photosynthesizing cells. Na⁺ in this case would be the 'driving', not the 'driving' ion (see 9); the latter would be H⁺ (or OH⁻), which moves downhill along its electrochemical potential gradient. The accumulation of OH⁻ ions in the absence of Na⁺ may retard the unloading of the HCO₃⁻ carrier at the inner membrane surface and hence inhibit HCO₃⁻ uptake.

The essential feature distinguishing models II and III from model I above is the linkage of Na⁺ and HCO₃⁻ fluxes in the two former cases. An essential distinguishing feature between models II and III, on the other hand, would be the role of ΔΨNa⁺. In the Na⁺ symport models (model II), ΔΨNa⁺ is the driving force for HCO₃⁻ uptake, and the maximum accumulation ratio will be such that, as pointed out above, ΔΨHCO₃⁻ will not exceed n ΔΨNa⁺. In the Na⁺-H⁺ antiport model, on the other hand, the upper limit for accumulation of HCO₃⁻ will not be set by ΔΨNa⁺.

LITERATURE CITED


