Hypothesis

BIOLOGICAL ENERGY PRODUCTION IN THE APPARENT ABSENCE OF ELECTRON TRANSPORT AND SUBSTRATE LEVEL PHOSPHORYLATION

Alexander J. B. ZEHNDER and Thomas D. BROCK
Department of Bacteriology, University of Wisconsin, Madison, WI 53706, USA

Received 8 July 1979

1. Introduction and basic considerations

The energy required for the synthesis of ATP is generally provided by exergonic redox processes. Energy is generated either by donating electrons (formally, hydrogen formation) or by accepting electrons (formally, hydrogen consumption). Two general mechanisms have been recognized for conservation of chemical energy in the high-energy phosphate bonds of ATP: substrate-level phosphorylation and electron-transport phosphorylation. In the former process, part of the energy released in a redox reaction is conserved in an 'energy-rich' bond of one of the products, and a transfer to ATP occurs by a kinase reaction. In electron-transport phosphorylation, energy is used to drive protons through a membrane, thus establishing a pH gradient and a membrane potential. According to the chemiosmotic theory [1] the resulting protonmotive force can be used to synthesize ATP. A few substrate-level phosphorylation reactions are known which do not involve redox reactions: several anaerobes have been shown to metabolize substrates by lysis rather than by redox [2].

The methanogenic bacteria are a unique group of organisms which have been the subject of considerable evolutionary interest [3]. Most of the methanogenic bacteria obtain energy from redox reactions involving the oxidation of hydrogen gas or formate, with carbon dioxide serving as the terminal electron acceptor. ATP is apparently synthesized by conventional electron-transport phosphorylation [2]. A redox carrier has been identified, factor 420, and a specific coenzyme has been shown to be the methyl carrier in the terminal reaction of methane formation, coenzyme M [4].

Another process of methanogenesis that has been recognized for many years but only recently demonstrated in pure culture is the anaerobic conversion of acetate to equal amounts of methane and carbon dioxide [5]. Chemically, acetate cleavage is a decarboxylation reaction. Studies with deuterium-labeled compounds have shown that the methyl group of acetate goes intact into methane, and the fourth proton is supplied by water [6].

$$CH_3COO^- + H_2O = CH_4 + HCO_3^-$$

$$\Delta G^{o'} = -6.7 \text{ kcal .mol}^{-1}(-28 \text{ kJ .mol}^{-1}) \qquad (1)$$

Such a decarboxylation, however, is not a process by which electrons are transferred from one molecule to another, and therefore an electron transport mechanism for energy conservation is not obvious. ATP formation by substrate-level phosphorylation is also unlikely to occur, since an enzymic mechanism for substrate-level phosphorylation is not obvious and the energy released from acetate cleavage is too low to form an 'energy-rich' compound. Nevertheless, two methane bacteria, *Methanosarcina barkeri* [7] and the 'acetate organism' [8] grow with acetate as exclusive energy source. The overall stoichiometry for growth and methane formation for the 'acetate organism' is as follows:

Abbreviations: Δp, protonmotive force, it is the amount of work done by a single proton going once around the circuit; $\Delta\psi$, membrane potential; ΔpH, pH gradient. These are related by: $\Delta p = \Delta\psi - (2.3\,RT \cdot F^{-1})\,\Delta pH$. Units: millivolts, mV. R,T,F have their usual meaning

$$1137.5\ CH_3COOH + 10\ CO_2 + 14.25\ H_2O =$$

$$1100\ CH_4 + 1136.4\ CO_2 + 12.15\ (\text{biomass: } C_4H_7O_3) \quad (2)$$

Note that in this stoichiometry the product side shows a slight hydrogen deficiency (~1%), which can be explained by the necessity of some reducing equivalents for biomass synthesis. The amount of methyl group of acetate oxidized is just enough to supply the electrons necessary to reduce the assimilated carbon dioxide to biomass.

We hypothesize that ATP formation in these organisms occurs as a result of the formation of a proton gradient that is produced without the activity of intermolecular electron transport. The energy released by acetate cleavage could, at 100% efficiency, lead to a Δp of −290 mV, the potential energy for one proton to complete the circuit. The measured Δp for *Escherichia coli* is −237 mV and similar values have been found for other organisms [9].

In the following, we propose two hypothetical mechanisms by which a protonmotive force could be established in the absence of a redox reaction, using the energy released from the decarboxylation of acetate.

2. Schemes

Figure 1A shows a hypothetical flip–flop scheme. A protein molecule lying across the membrane binds an acetate molecule from the outside. To re-establish the protein's electrical neutrality, a proton from the cytoplasm is bound at the opposite end. Acetate is subsequently cleaved into methane and bicarbonate, and the energy released by this process causes a conformational change in the protein leading to the translocation of the proton to the outside. After the products of acetate decarboxylation have left the binding site, the protein returns to its initial configuration, and ATP is synthesized by a proton-translocating ATPase. This flip–flop mechanism has not perforce to lead to a major rearrangement of large parts of the protein in space. Even a simple intramolecular electron translocation with a synchronous proton transport would suffice, a process similar to the bacteriorhodopsin proton pump in *Halobacterium halobium* [10].

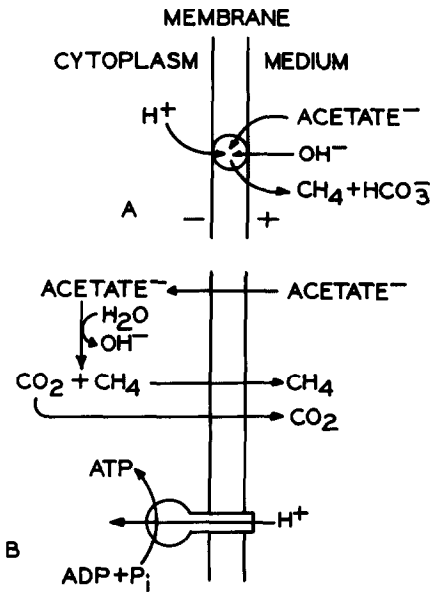

Fig.1A. Formation of a pH gradient by a flip–flop mechanism. The energy liberated by the anaerobic decarboxylation of acetate drives the protons across the membrane.

Fig.1B. The difference in acidity between substrate and product establishes a pH gradient.

Figure 1B presents a different mechanism for establishing a pH gradient. This second mechanism is based on the difference in acidity constants (pK values) of the substrate (acetate) and the non-neutral product (bicarbonate). In this scheme, the ionized acetate is transported into the cell and is split to methane and bicarbonate. (If acetate travels in the non-ionized form across the membrane, the necessary proton can be picked up from the membrane upon entering, and then left behind for re-use after the acetate molecule reaches the cytoplasm.) The pH in the cell rises due to the lower acidity of bicarbonate compared to acetate, a fact which results in the creation of a pH gradient (inside alkaline). The resulting protonmotive force can then be used to drive ATP synthesis. If acetic acid rather than acetate is taken up by the cells, the above situation is just reversed, but still leads to the formation of a proton gradient. Since at pH 7 the ratio of acetate/acetic acid is ~200, the exclusive use of acetic acid will, according to eq. (2), leave hydroxyl ions on the outside. This results

in a situation where the Δp vector is directed from the cytoplasm to the medium (outside alkaline).

3. Discussion

Recently Wolfe [4] has speculated that the carbon of the methyl group of acetate is first oxidized to the –II redox state and subsequently re-reduced to the –IV state in methane and that the resulting electron flow would generate a Δp for ATP synthesis. Our flip–flop mechanism is conceptually similar to Wolfe's scheme, but does not need a redox system. Although the 'acetate organism' does have a redox system and coenzyme M, it is not clear that these components are involved in energy generation, since some redox processes are needed for biomass formation (eq. (2)).

In our second proposed mechanism, the key point is that the formation of methane from acetate requires a proton from water, and since the neutral methane molecule escapes from the system carrying this proton with it, the direction of the Δp vector depends solely on whether the needed proton is picked up inside the cell (if acetate is the active species) or outside the cell (if acetic acid is the active species).

Under either condition, neither electron transport nor substrate-level phosphorylation are necessary to synthesize ATP. The proposed mechanisms may represent the most primitive type of energy generating system.

Acknowledgements

This work was supported by a research contract with the US Department of Energy. We thank the following for reading and commenting on this paper: Franklin Harold, A. L. Lehninger, J. Lanyi, T. Langworthy. The development of these hypotheses was aided by numerous conversations with J. G. Zeikus.

References

[1] Mitchell, P. (1966) Biol. Rev. Cambr. Phil. Soc. 41, 445–502.
[2] Thauer, R. K., Jungermann, K. and Decker, K. (1977) Bacteriol. Rev. 41, 100–180.
[3] Woese, C. R., Magrum, L. J. and Fox, G. E. (1978) J. Mol. Evol. 11, 245–252.
[4] Wolfe, R. S. (1979) in: Microbial Biochemistry (Quayle, J. R. ed) vol. 21, pp. 270–300.
[5] Zehnder, A. J. B. (1978) in: Water Pollution Microbiology (Mitchell, R. ed) vol. 2, pp. 349–376, Wiley, New York.
[6] Pine, M. J. and Barker, H. A. (1956) J. Bacteriol. 71, 6744–6748.
[7] Smith, M. R. and Mah, R. A. (1978) Appl. Environ. Microbiol. 36, 870–879.
[8] Zehnder, A. J. B., Huser, B., Brock, T. D. and Wuhrmann, K. (1979) in preparation.
[9] Harold, F. M. (1977) Ann. Rev. Microbiol. 31, 181–203.
[10] Wagner, G., Hartmann, R. and Oesterhelt, D. (1978) Eur. J. Biochem. 89, 169–179.