Hypothesis

A unified scheme for carbon and electron flow coupled to ATP synthesis by substrate-level phosphorylation in the methanogenic bacteria

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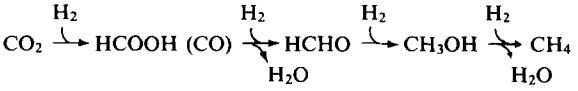
Due primarily to considerations of the nature of the electron transfer reactions which drive ATP synthesis in the methanogenic bacteria, substrate-level phosphorylation (SLP) has been generally ruled out. Recent studies, however, have cast doubt on an obligatory role for a transmembrane ion gradient in coupling. In this paper, a scheme is proposed for SLP coupled to methane formation. The essential features are phosphorylation of bound C_1 intermediate coupled to intermolecular electron transfer in a multifunctional protein complex containing a collection of bound C_1 and electron carriers. ATP synthesis is accomplished by subsequent phosphoryl transfer. The scheme is analogous to all known mechanisms of SLP and is compatible with most of the data published to date.

Energy metabolism *Oxidative phosphorylation* *Methanogen* *Electron transport* *ATP* *Anaerobiosis*

1. INTRODUCTION

1.1. *Methanogenic pathways*

Methanogens are capable of methane formation (and energy production) from a variety of simple substrates. Most species can carry out the energetically favorable 8-electron reduction of CO_2 by molecular hydrogen:



Although the scheme above represents the various free oxidation states during this 8-electron transfer, it is now accepted that, as originally suggested by Barker [1], this sequence involves only bound forms of the intermediates. This could allow for significant relative stabilization of these states and thus alter the effective free energy change involved for each of the individual electron transfer steps. Nevertheless, it is important to point out that the principal site of the negative free energy change of the overall process is the terminal, methane-producing step [2].

There are, in addition, a number of substrates which are both oxidized and reduced, accompanied by intermolecular electron transfer. One such pathway is methanol metabolism:

$$4CH_3OH \longrightarrow 3CH_4 + CO_2 + 2H_2O$$

Reducing equivalents produced from the 6-electron oxidation of one molecule of methanol to CO_2 result in the reduction of 3 additional molecules to methane. *Methanosarcina barkeri* is capable of methanogenesis from methylamines, which are metabolized analogously to methanol, after cleavage liberating ammonia. Similar pathways are functional for formate and carbon monoxide:

$$4HCOOH \longrightarrow CH_4 + 3CO_2 + 2H_2O$$

$$4CO + 2H_2O \longrightarrow 3CO_2 + CH_4$$

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The simplest methanogenic pathway is the reduction of methanol with hydrogen, which has recently been shown to be capable of providing all cellular energy requirements for at least one species [3]:

$$H_2 + CH_3OH \longrightarrow CH_4 + H_2O$$

All of the above pathways are energetically quite favorable overall, providing more than enough energy for stoichiometric ATP formation. This is not the case, however, for the cleavage of acetate, which is accomplished by several species:

$$CH_3COOH \longrightarrow CH_4 + CO_2$$

The low $\Delta G^{\circ\prime}$ for this reaction (−27.6 kcal/mol [4,5]) would apparently argue for a mechanism of energy conservation other than 1:1 ATP formation, and this realization has thus prompted the proposal of schemes for proton translocation coupled to intramolecular electron transfer [6,7].

1.2. *SLP or ETP in methanogens?*

Substrate-level phosphorylation (SLP) has been almost universally ruled out in the methanogenic bacteria because of the following considerations: (i) some species [5] are capable of autotrophic growth on gaseous H_2 and CO_2, with no additional carbon source; (ii) true electron transfer takes place, i.e. electrons are separated from protons in hydrogen [8]; (iii) as suggested originally by Lipmann [9] all known SLP reactions coupled to electron transfer involve the oxidation of an aldehyde to a carboxylate moiety, whereas methanogenesis from H_2/CO_2 involves reduction of carbon; (iv) as mentioned above, some species can derive energy from the cleavage of acetate, a reaction which yields barely enough energy for the synthesis of equimolar ATP.

By analogy with other known systems for electron transport phosphorylation (ETP), therefore, a chemiosmotic mechanism has been generally assumed to be responsible for coupling methane formation to ATP synthesis [5]. However, although it is clear that methanogens maintain a transmembrane electrical potential and possess an ion-translocating ATPase [5], there appears to be little evidence of an unequivocal nature to prove this assumption (see section 3). Indeed, very recently, Schönheit and Beimborn [10], with *Methanobacterium thermoautotrophicum* (Marburg), and our laboratory, with *Methanococcus voltae* [11,12] and *M. thermoautotrophicum* (delta-H) [12], have provided evidence for methanogenesis-driven ATP synthesis without the obligatory intermediacy of a transmembrane ion gradient.

In light of this evidence against ETP, this paper describes a possible mechanism for SLP consistent with virtually all data published to date and strictly analogous to other SLP reactions, and examines the resultant prediction of a unified scheme for carbon and electron flow for all methanogenic pathways.

2. POSTULATES OF THE MECHANISM

The mechanism proposed here is based on three postulates, described below.

2.1. *ATP synthesis occurs at the terminal two-electron transfer step*

The methyl reductase (MR) system is responsible for the terminal step in methanogenesis, involving the reduction of methylated coenzyme M to methane and reduced coenzyme [5]. There are several reasons to suggest energy coupling by this system, including the following: (i) the MR system is present in all methanogens, and is responsible for the methane-forming reaction from all substrates [13]; (ii) at least for CO_2 reduction by H_2 there is only enough free energy under physiological conditions for the synthesis of one ATP [4]; (iii) as mentioned above, most of the free energy change in the overall process occurs at this last step; (iv) controlled growth yield studies indicate that the cell yield is proportional to the moles of methane formed for a particular mode of metabolism, rather than the other electron transfer steps involved [14]; (v) in *M. thermoautotrophicum* the MR is the only step which is not common to dissimilatory and assimilatory pathways [15]; and (vi) the MR is the only electron transfer step coupled to ATP synthesis for H_2/methanol metabolism [3].

2.2. *Phosphoanhydride bond formation occurs by SLP coupled to intermolecular electron transfer between two C_1 moieties*

If strictly analogous to known SLP systems, phosphoanhydride bond formation in C_1

metabolism would occur coupled to the oxidation of the formaldehyde to the formate state, with the production of formyl phosphate (or its energetic equivalent):

$$\text{HCHO} + \text{HO-P}_i \longrightarrow \text{HCO-P}_i + 2\text{H}$$

$$\text{HCO-P}_i + \text{ADP-OH} \longrightarrow \text{HCOH} + \text{P}_i\text{-O-ADP}$$

$$\text{HCHO} + \text{HO-P}_i + \text{ADP-OH} \longrightarrow 2\text{H} + \text{HCOH} + \text{P}_i\text{-O-ADP}$$

Formyl phosphate has been implicated as a bound intermediate during ATP synthesis from purine degradation by certain clostridia via the reversal of formyltetrahydrofolate synthetase [16].

Assuming the formation of such a bound formyl phosphate species from the oxidation of bound formaldehyde and that this oxidation is coupled to the reduction of methyl coenzyme M via the MR system, fig.1 presents a possible minimal scheme for SLP coupled to methanogenesis. According to this scheme, formation of the high-energy phosphoryl bond occurs as a result of electron transfer between these two C_1 moieties. After transfer of the phosphate group to ADP, the formyl group is reduced to regenerate the formaldehyde moiety. Although the chemical mechanism is formally identical in principle to all SLP reactions coupled to electron transfer, electrons from hydrogen would enter the pathway via true electron transport, i.e. by initial oxidation by hydrogenase. As described in more detail below, there is substantial experimental evidence for intermolecular electron transfer between C_1 units.

2.3. *Electron and carbon flow occur via common protein-bound 'pools', not involving freely diffusable reducing equivalents or C_1 intermediates*

According to this postulate, electrons from all methanogenic substrates flow into and out of a collection of protein-bound oxidation-reduction centers, which functions for all methanogenic pathways. Each individual oxidation-reduction step for C_1 metabolism is also identical for different substrates. In addition, transfer of bound C_1 units via freely diffusable intermediates does not occur.

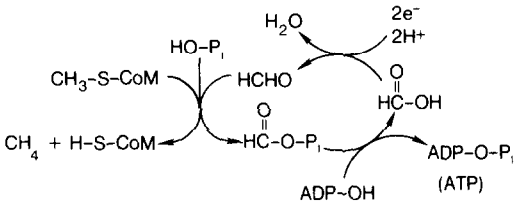

Fig.1. A minimal scheme for SLP coupled to the methyl reductase. For simplicity, intermediates are designated as the free intermediates.

Probably the strongest evidence for such a common pool of reducing equivalents comes from studies of methanogenesis during mixotrophic metabolism, i.e. the simultaneous utilization of different substrates. Earlier studies showed that, although the normal route for acetate catabolism involves the simultaneous reduction of the C-2 methyl group to methane and the oxidation of the carboxyl to carbon dioxide, the addition of methanol results in the oxidation of the C-2 which provides reducing equivalents for the reduction of the methanol to methane [17]. This result has been verified with several species of methanogen [18–20].

Recent studies have suggested that all methanogens utilize the same three carriers at the various oxidation levels between CO_2 and CH_4, methanofuran (formyl), tetrahydromethanopterin (formyl, hydroxymethyl, and methyl), and coenzyme M (methyl) [13]. Escalante-Semerena and Wolfe [21] have shown that formaldehyde (via methylene tetrahydromethanopterin) undergoes a disproportionation reaction in crude extracts of formate-grown cells involving the oxidation of one molecule to CO_2 with the resulting 4 electrons reducing a second molecule to methane. This intermolecular electron transfer between two C_1 units thus involves all 4 of the oxidation-reduction steps in the reduction of CO_2 to CH_4, and supports the conclusions above. In addition, preparations from H_2/CO_2-grown cells passed through Sephadex G-25 (thus presumably devoid of soluble electron transfer components) carry out the oxidation of two formaldehyde moieties and the concomitant reduction of a third to methane at rates comparable to those of crude extract, with the addition of only those small molecular mass components required for the MR. This shows that at least 3 of the 4 oxidation-reduction steps involved in methano-

genesis from CO_2 (including the terminal MR step) do not occur via the intermediacy of a soluble electron transfer carrier which might serve as an electron 'shuttle', and thus supports the existence of a common protein-bound pool of reducing equivalents and of C_1 intermediates. Aggregation of proteins catalyzing electron transfer in several methanogens has been reported [22–26]. In addition, a closely analogous system could be C_1-tetrahydrofolate synthase from *Saccharomyces cerevisiae*, where evidence has recently appeared for overlapping active sites in this multifunctional enzyme complex [27] (for a discussion of the possibility that electron transfer occurs via H^+ reduction and subsequent oxidation ('hydrogen cycling' [28]), see section 3).

2.4. *Integration of methanogenic pathways*

The previous considerations would predict that (with the possible exception of acetate) all methanogenic pathways would share 3 common components: the MR system, a site for the interconversion of various C_1 derivatives of tetrahydromethanopterin (THMPT), and a common pool of reducing equivalents. Fig.2 presents schemes for carbon and electron flow for methane formation from the 4 most commonly studied substrates (H_2/CO_2, methanol, formate, and acetate), based on these common features and the mechanism of SLP described above. The species variation in the production of methane from certain substrates is proposed to be due to the lack of the enzymatic machinery necessary for electron or C_1 delivery to the central pools.

All modes of methanogenesis can be divided into two half-cell reactions, an oxidation of either H_2 or a C_1 intermediate to yield reducing equivalents and a transfer of these electrons to another C_1 intermediate to yield methane. Table 1 lists the appropriate two-electron half-cell reactions for all known methanogenic pathways in terms of the free intermediates, along with the corresponding standard reduction potentials. Of significance energetically is that all generated reducing equivalents are quite negative in potential, with the only positive step being the terminal reaction resulting in methane formation, the MR. This is, of course, simply an electrochemical expression of the energetic favorability of this last step; the mechanistic implication of this is that all

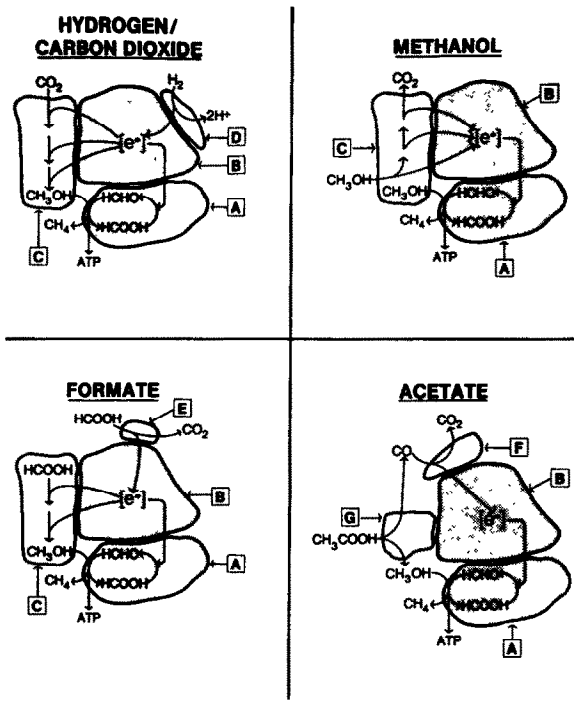

Fig.2. Minimal schemes for carbon and electron flow for 4 methanogenic modes. The shaded components are proposed to be common to all pathways. Designated components: (A) methyl reductase; (B) electron-transferring component, representing a collection of relatively low-potential electron transfer centers; (C) methanofuran- and tetrahydromethanopterin-converting enzyme activities; (D) hydrogenase; (E) formate dehydrogenase; (F) carbon monoxide dehydrogenase; (G) aceticlastic reaction center.

methanogenic pathways could deliver electrons to a common collection of electron transfer centers of relatively negative $E^{\circ\prime}$, with ATP synthesis occurring coupled to the subsequent 'funneling' of the flow of these low-potential electrons through the final, most positive half-cell reaction.

3. DISCUSSION

There are several reports of the existence of a transmembrane electrical potential in methanogens, and of ion-translocating ATPase activity [5]. However, since similar systems exist for organisms known to derive energy solely by SLP and function

Table 1

Two-electron half-cell reactions of methanogenesis (A) and roles for methanogenic metabolism (B)

(A)

Half-cell reaction	$E^{\circ\prime}$ (V)
(1) $2H^+ + 2e^- \longrightarrow H_2$	−0.421
(2) $CO_2 + 2H^+ + 2e^- \longrightarrow CO + H_2O$	−0.317
(3) $CO + 2H^+ + 2e^- \longrightarrow HCHO$	−0.455
(4) $CO_2 + 2H^+ + 2e^- \longrightarrow HCOOH$	−0.414
(5) $HCOOH + 2H^+ + 2e^- \longrightarrow HCHO + H_2O$	−0.542
(6) $HCHO + 2H^+ + 2e^- \longrightarrow CH_3OH$	−0.189
(7) $CH_3OH + 2H^+ + 2e^- \longrightarrow CH_4 + H_2O$	0.162

(B)

Substrate(s)	Reducing half-cell(s)	Oxidizing half-cell(s)
H_2/CO_2	(1)	(4)–(7)
$CH_3OH(CH_3NH_2)$	(6)–(4)	(7)
H_2/CH_3OH	(1)	(7)
HCOOH	(4)	(5)–(7)
CO	(2)	(3),(6),(7)
CH_3COOH[a]	(2)	(7)

[a] This is treated as for the free C_1 intermediates subsequent to hydrolysis; the overall energetics thus are not accurately reflected in the calculated value for $\Delta E^{\circ\prime}$

for the maintenance of intracellular ion homeostasis and for membrane transport [29], these data are not a priori evidence for a chemiosmotic mechanism of ATP synthesis coupled to electron transport.

As described above, the value for $\Delta G^{\circ\prime}$ for the cleavage of acetate (−27.6 kJ/mol [5]) compared to that for the synthesis of ATP (31.5 kJ/mol [30]) has been used to rule out SLP [6]. There are, however, numerous caveats in the application of this argument. The physical chemical interpretation of the proximity of these two values is that one molecule of ATP can theoretically be synthesized to maintain an approx. 1:5 ratio of ATP and ADP, in contrast to the ratio of 1:340000 with no energy input. Although this assumes 100% efficiency of coupling, SLP might in general be expected to be more efficient than ETP due to the presence of ion 'leaks' in the latter mechanism (e.g. the efficiency of coupling ATP synthesis to electron transfer for glyceraldehyde-3-phosphate dehydrogenase is quite close to 100% [31]). In this regard, it is important to point out that growth on acetate is extremely slow, with doubling times of the order of days, and there is evidence that methane production from acetate is at least somewhat reversible [32]. This could mean that, in contrast to most other biological energy-producing reactions, the process could occur relatively reversibly and thus with close to the theoretically maximum efficiency. Indeed, on irreversible thermodynamic grounds it would be expected that in general 100% efficiency can be achieved only at zero growth rate [33]. A long doubling time would also lead to a decrease in the magnitude of growth rate-dependent maintenance and thus to an increase in cellular energy conservation [34] (this may actually be the phenomenological manifestation of the irreversible thermodynamic considerations referred to above). Additionally, the appropriate thermodynamic parameter is $\Delta G'$ (instead of $\Delta G^{\circ\prime}$), the free energy available under physiological conditions. Although it is impossible in most instances to estimate this value from the literature (due to variations in pH and gas composition, among other parameters), most growth studies are conducted under a low partial pressure of methane (e.g. with constant sparging or periodic exchange) which thus greatly increases the overall energetic favorability of this reaction. A definitive answer to this question will come only from a study of ATP production and growth under carefully controlled conditions. Finally, even the parameter ΔG is not completely appropriately applied to biochemical systems [35], especially in the case where cellular reactions occur in microenvironments which differ from dilute solutions, which (as described above) might occur in methanogens.

It has been proposed [5] that proton translocation coupled to methanogenesis occurs by transmembrane electron flow, from hydrogen oxidation on one side of an ion-impermeable membrane and reduction of C_1 unit(s) to methane on the other ('hydrogen cycling'). For methanogenic pathways utilizing C_1 units as electron donor, protons are proposed to be reduced to H_2 which then diffuses through the membrane to the same site of oxidation (a membrane-associated hydrogenase) as for metabolic modes utilizing H_2 as donor.

Although suggested initially, the possibility of an intracellular location for membrane structures involved in chemiosmotic coupling to methanogenesis has apparently been eliminated [36]. This means that (assuming an orientation for ion movement analogous to all other known systems) the direction of electron transfer from hydrogen would be from the extracellular to the intracellular compartment, presumably involving a periplasmic location for hydrogenase. Contrary to this expectation is the demonstration that in whole cells protons are separated from electrons upon hydrogen oxidation (i.e. hydrogenase activity) in the internal cell compartment [8]. There is also no evidence from assays of hydrogen-dependent dye reduction for an extracellular location, i.e. all activity requires cell disruption. In addition, although there have been reports of sedimentation of certain electron transfer activities upon ultracentrifugation [37] and of the tendency of proteins involved in methanogenesis to aggregate (as mentioned above), there is no strong evidence for an integral membrane location for any electron transfer segment. Finally, hydrogen cycling in acetate metabolism is strongly argued against by the recent finding that in *M. acetivorans* (which lacks appreciable hydrogenase activity) electrons for the MR can be provided by CO but not by H_2 ([38]; see fig.2).

Evidence for a chemiosmotic scheme for ETP in *Ma. barkeri* has recently been presented [39]. The addition of ionophores (tetrachlorosalicylanilide (TCS), gramicidin, valinomycin) to cells results in a simultaneous decline in membrane potential and cell ATP level, a result which (considered alone) is compatible with either ETP or SLP [40,41]. However, it was shown that addition of TCS inhibits the oxidation of methanol, a result which was cited as evidence for a direct link between electron transfer and ion movement *without* the intermediacy of ATP. An alternative interpretation is that methanol oxidation is very sensitive to alterations in intracellular ion composition, normally controlled by an ion-translocating ATPase; evidence for this possibility includes the finding that sodium depletion from the medium results in the same effect on this electron transfer reaction as TCS addition [42]. A role has been proposed for sodium movement in internal ion homeostasis in methanogens [11].

Probably the strongest experimental evidence against SLP is the lack of a demonstration of strict coupling between methane formation and ATP synthesis in cell-free systems where, unlike in whole cells, catabolic mechanisms of rapid ATP hydrolysis would be minimal (as well as the formation of storage compounds other than ATP such as 2,3-cyclic diphosphoglycerate [43] or glycogen [44]). Indeed, ATP addition is actually required to activate the MR system [45], making the experiment technically difficult. Although several studies have reported an apparent lack of such coupling in whole cells, this phenomenon was observed under relatively nonphysiological conditions (e.g. during recovery from oxygen exposure [46], under high gassing rates [47], or under nitrogen or sulfur starvation [48]), where maintenance energy could provide a substantial drain on the ATP pool. In any event, it may well be that significant progress in the molecular mechanisms of energy transduction in the methanogens will be accomplished only after a cell-free system capable of electron transfer-driven ATP synthesis is developed.

In conclusion, in this paper I have attempted to present a plausible scheme for SLP in the methanogenic bacteria. The main purpose is not so much to advocate this particular mechanism as to show that reasonable possibilities can be envisaged, and thus to suggest that the elimination of SLP on theoretical grounds alone may be inappropriate.

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REFERENCES

[1] Barker, H.A. (1956) in: Bacterial Fermentations, pp.1–27, John Wiley, London.
[2] Stadtman, T.C. (1967) Annu. Rev. Microbiol. 21, 121–142.
[3] Miller, T.L. and Wolin, M.J. (1983) J. Bacteriol. 153, 1051–1055.

[4] Thauer, R.K., Jungermann, K. and Decker, K. (1977) Bacteriol. Rev. 41, 100–180.
[5] Daniels, L., Sparling, R. and Sprott, G.D. (1984) Biochim. Biophys. Acta 768, 113–163.
[6] Wolfe, R.S. (1979) Int. Rev. Biochem. 21, 270–300.
[7] Zehnder, A.J.B. and Brock, T.D. (1979) FEBS Lett. 107, 1–3.
[8] Spencer, R.W., Daniels, L., Fulton, G. and Orme-Johnson, W.H. (1980) Biochemistry 19, 3678–3683.
[9] Lipmann, F. (1941) Adv. Enzymol. 1, 99–162.
[10] Schönheit, P. and Beimborn, D.B. (1985) Eur. J. Biochem. 148, 545–550.
[11] Crider, B.P., Carper, S.W. and Lancaster, J.R. jr (1985) Proc. Natl. Acad. Sci. USA 82, 6793–6796.
[12] Lancaster, J.R. jr, Carper, S.W., Crider, B.P., Gillies, K. and Rogers, K.R. (1985) in: Bioinorganic Chemistry '85 (Xavier, A.V. ed.) VCH Publishers, in press.
[13] Jones, W.J., Donnelly, M.I. and Wolfe, R.S. (1985) J. Bacteriol. 163, 126–131.
[14] Hippe, H., Caspari, D., Fiebig, K. and Gottschalk, G. (1979) Proc. Natl. Acad. Sci. USA 76, 494–498.
[15] Stupperich, E. and Fuchs, G. (1984) Arch. Microbiol. 139, 14–20.
[16] Buttlaire, D.H., Balfe, C.A., Wendland, M.F. and Himes, R.H. (1979) Biochim. Biophys. Acta 567, 453–463.
[17] Pine, M.J. and Vishniac, W. (1957) J. Bacteriol. 73, 736–742.
[18] Smith, M.R. and Mah, R.A. (1978) Appl. Environ. Microbiol. 36, 870–879.
[19] Zinder, S.H. and Mah, R.A. (1979) Appl. Environ. Microbiol. 38, 996–1008.
[20] Krzycki, J.A., Wolkin, R.H. and Zeikus, J.G. (1982) J. Bacteriol. 149, 247–254.
[21] Escalante-Semerena, J.C. and Wolfe, R.S. (1984) J. Bacteriol. 158, 721–726.
[22] McKellar, R.C. and Sprott, G.D. (1979) J. Bacteriol. 139, 231–238.
[23] Jacobson, F.S., Daniels, L., Fox, J.A., Walsh, C.T. and Orme-Johnson, W.H. (1982) J. Biol. Chem. 257, 3385–3388.
[24] Jin, S.-L.C., Blanchard, D.K. and Chen, J.-S. (1983) Biochim. Biophys. Acta 748, 8–20.
[25] Fauque, G., Teixeira, M., Moura, I., Lespinat, P.A., Xavier, A.V., Der Vartanian, D.V., Peck, H.D. jr, LeGall, J. and Moura, J.G. (1984) Eur. J. Biochem. 142, 21–28.
[26] Rouviere, P.E., Escalante-Semerena, J.C. and Wolfe, R.S. (1985) J. Bacteriol. 162, 61–66.
[27] Appling, D.R. and Rabinowitz, J.C. (1985) Biochemistry 24, 3540–3547.
[28] Odom, J.M. and Peck, H.D. jr (1981) FEMS Microbiol. Lett. 12, 47–50.
[29] Kobayashi, H. (1985) J. Biol. Chem. 260, 72–76.
[30] Rosing, J. and Slater, E.C. (1972) Biochim. Biophys. Acta 267, 275–290.
[31] Meyerhof, O. and Oesper, P. (1947) J. Biol. Chem. 170, 1–22.
[32] Zehnder, A.J.B. and Brock, T.D. (1979) J. Bacteriol. 137, 420–432.
[33] Kedem, O. and Caplan, S.R. (1965) Trans. Faraday Soc. 61, 1897–1911.
[34] Hellingwerf, K.J., Lolkema, J.S., Otto, R., Neijssel, O.M., Stouthamer, A.H., Harder, W., Van Dam, K. and Westerhoff, H.V. (1982) FEMS Microbiol. Lett. 15, 7–17.
[35] Welch, G.R. (1985) J. Theor. Biol. 114, 433–446.
[36] Sprott, G.D., Sowden, L.C., Colvin, J.R., Jarrell, K.F. and Beveridge, T.J. (1984) Can. J. Microbiol. 30, 594–604.
[37] Sauer, F.D., Mahadevan, S. and Erfle, J.D. (1984) Biochem. J. 221, 61–69.
[38] Nelson, M.J.K. and Ferry, J.G. (1984) J. Bacteriol. 160, 526–532.
[39] Blaut, M. and Gottschalk, G. (1984) Eur. J. Biochem. 141, 217–222.
[40] Thauer, R.K. and Fuchs, G. (1979) Naturwissenschaften 66, 89–94.
[41] Maloney, P.C. (1977) J. Bacteriol. 132, 564–575.
[42] Blaut, M., Müller, V., Fiebig, K. and Gottschalk, G. (1985) J. Bacteriol. 164, 95–101.
[43] Seely, R.J. and Fahrney, D.E. (1984) J. Bacteriol. 160, 50–54.
[44] König, H., Nusser, E. and Stetter, K.O. (1985) FEMS Microbiol. Lett. 28, 265–269.
[45] Roberton, A.M. and Wolfe, R.S. (1969) Biochim. Biophys. Acta 192, 420–429.
[46] Roberton, A.M. and Wolfe, R.S. (1970) J. Bacteriol. 102, 43–51.
[47] Schönheit, P., Moll, J. and Thauer, R.K. (1980) Arch. Microbiol. 127, 59–65.
[48] Gunnarsson, L.A.H. and Ronnow, P.H. (1982) FEMS Microbiol. Lett. 14, 317–320.