Glycine metabolism in anaerobes

Jan R. Andreesen
Institute of Microbiology, University of Halle, Weinbergweg 16a, D-06099 Halle, Germany

Key words: Stickland reaction, Clostridium, Eubacterium, Peptostreptococcus, glycine, sarcosine, creatine, betaine, purines, threonine, proline, glycine cleavage system, glycine reductase, selenoprotein, thioredoxin system

Abstract

Some strict anaerobic bacteria catalyze with glycine as substrate an internal Stickland reaction by which glycine serves as electron donor being oxidized by glycine-cleavage system or as electron acceptor being reduced by glycine reductase. In both cases, energy is conserved by substrate level phosphorylation. Except for the different substrate-activating proteins $P_B$, reduction of sarcosine or betaine to acetyl phosphate involves in Eubacterium acidaminophilum the same set of proteins as observed for glycine, e.g. a unique thioredoxin system as electron donor and an acetyl phosphate-forming protein $P_C$ interacting with the intermediarily formed Se-carboxymethylselenoether bound to protein $P_A$.

Introduction

Interest in glycine metabolism by anaerobic bacteria originates from its capacity to act preferentially as an electron acceptor in a so-called Stickland reaction due to the relatively high redox potential of $E'_o = -10$ mV for the pair glycine + 2e + 2H$^+$ / acetate being close to that of fumarate + 2e + 2H$^+$ / succinate (+33 mV) (Thauer et al. 1977). In addition, an energy conservation in this reaction has been shown quite early using Clostridium sticklandii (Stadtman and Elliot 1956, Stadtman et al. 1958). However, clear-cut evidence and proposed mechanisms by which a substrate level phosphorylation might happen were only given quite recently when purified protein preparations became available (Arkowitz & Abeles 1989, 1991; Barnard & Akhtar 1979; Buckel 1990; Garcia & Stadtman 1992; Schräder & Andreesen 1992; Tanaka & Stadtman 1979). Besides glycine, its N-methylated derivatives sarcosine and betaine can also act as electron acceptor to form acetyl phosphate (Hormann & Andreesen 1989). Thus, glycine can act as oxidant and reductant (Fig. 1). This is also true for C. sporogenes when it grows in coculture with hydrogen-consuming anaerobes (Winter et al. 1987). Proline seems to be a better oxidant for it will only be reduced to 5-aminovalerate and, actually, it represses glycine reduction in case of C. sporogenes and C. sticklandii (Uhde 1990; Venugopalan 1980), although a contradictory report exists for the latter organism (Schwartz et al. 1979). Like the proline-derivative hydroxyproline (Stickland 1934), also glycine derivatives such as sarcosine (N-methylglycine) and betaine (N,N,N-trimethylglycine)
Fig. 1. Glycine metabolism by *Eubacterium acidaminophilum*. 1 mol of glycine will be oxidized to 2 CO$_2$ and 3 <2H> involving glycine-cleavage system (glycine decarboxylase) and, 1, methylene-THF dehydrogenase; 2, N-5, 10-methenyl-THF cyclohydrolase; 3, N-10-formyl-THF synthetase; 4, formate dehydrogenase. The six electrons generated in oxidation reactions will reduce 3 mol of glycine to acetyl phosphate and ammonia. Thus, the total fermentation balance is: 4 glycine + 2 H$_2$O $\rightarrow$ 3 acetate + 2 CO$_2$ + 4 NH$_3$. The energy yield conserved as ATP is derived from 3 acetyl phosphate and one formyl-THF, thus, one ATP is formed per mol of glycine.

Sources of glycine and its derivatives

Glycine is the only proteinogenic amino acid without stereoisomers. Its synthesis (as that of purines) can easily be achieved using the primordial conditions relevant at the beginning of metabolism (Ferris et al. 1978). Besides its presence in dipeptides or tripeptides such as glutathione, glycine is used to form conjugates with e.g. cholic acids and benzoate. In addition, some proteins besides collagen are rather rich in glycine containing it up to 60% (Keller et al. 1988; Fratini et al. 1993). High concentrations of glycine (0.05 to 1.3 M) are quite inhibitory for many bacteria for it interferes with the biosynthesis of the peptidoglycan by disturbing the natural balance in incorporating glycine instead of alanine, thus creating L-forms or enhance inhibitors of alanine racemase (Dienes & Zamank 1952; Hammes et al. 1973; Heaton et al. 1987). In addition, N-hydroxyglycine produced from glycine by some fungi can inhibit some enzymes involved in degradation (Murai et al. 1992). However, the concentration of glycine is rather low in natural systems, being only about 0.05 mM in rumen fluid (Wright & Hungate 1967a). In addition, most glycine is formed or consumed intracellularly by: i) L-serine hydroxymethyltransferase involved in C$_1$-metabolism (Schirch & Strong 1989), ii) purine metabolism (Dürre & Andreesen 1982b; Vogels & van der Drift 1976), iii) threonine metabolism via threonine aldolase (Dainty 1970) or threonine dehydrogenase plus 2-amino-3-oxobutyrate CoA ligase (Golovshenko et al. 1983; Wagner 1994).

Sarcosine (N-methylglycine) is formed primarily from creatine and creatinine. The concentration of the latter is an important indicator for the efficiency of the kidney to keep a balanced nitrogen status. Creatine kinase is an important enzyme to supply the muscle with energy. All pathways of creatine or creatinine degradation that are studied in detail form finally sarcosine (Hermann et al. 1992). The N-acylated salts of sarcosine are used as anticorrosives and dispersing aids.

N, N-Dimethylglycine is produced in liver mitochondria during degradation of choline via betaine (Finkelstein & Martin 1984). Its further oxidation to sarcosine and glycine plus methylene-THF/formaldehyde is catalyzed by FAD-containing dehydrogenases (Lang et al. 1991; Porter et al. 1985). Anaerobic species of the genus *Sporomusa* (Möller et al. 1984), *Eubacterium limosum* (Müller et al. 1981), and *Desulfobacterium autotrophicum* and related strains (Heithuisen & Hansen 1989a) form dimethylglycine. However, only *Sporomusa* species are capable to transform it further by disproportionation to sarcosine and betaine (Möller et al. 1984; Oren 1990).

Betaine (N, N, N-trimethylglycine) is an important compatible solute to avoid osmotic stress in bacteria and can be present within the cell at about 1 M (Csonka 1989). Betaine can be excreted via an efflux system different from the uptake system (Lamark et al. 1992). Proline can be exchanged for betaine (Molenaar et al. 1993). Plants also use quarternary ammonium and tertiary sulfonium compounds as osmoregulants (Rhodes & Hanson 1993). Therefore, betaine is readily available in ecosystems. Its presence (2–5 mM) in media...