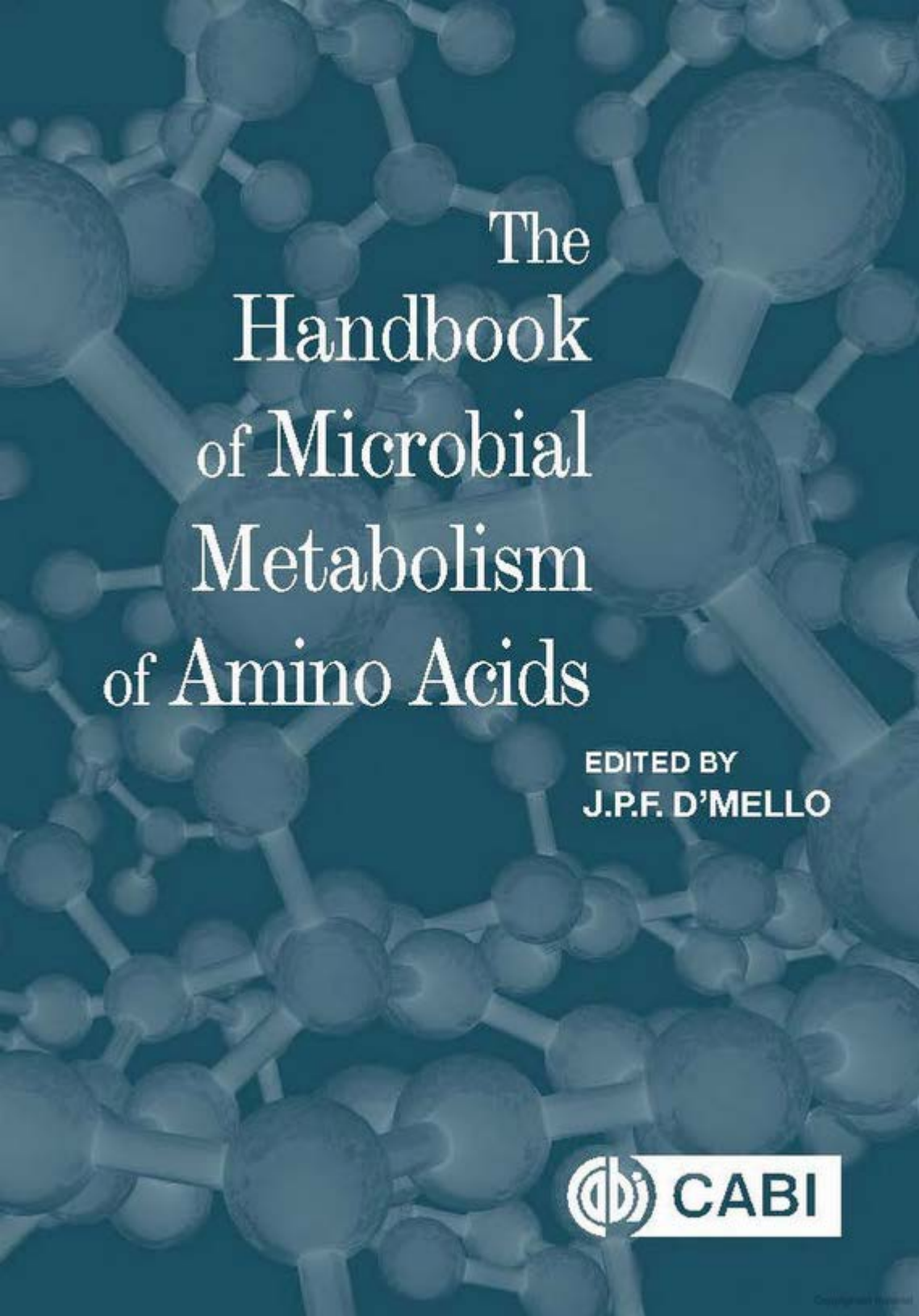
The
Handbook
of Microbial
Metabolism
of Amino Acids
EDITED BY
J.P.F. D'MELLO
CABI

1 Structural and Functional Properties of Glutamate Dehydrogenases

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1.1 Abstract

Glutamate dehydrogenases are homooligomeric enzymes that catalyse the reversible oxidative deamination of glutamate to 2-oxoglutarate, thereby linking nitrogen metabolism and the tricarboxylic acid cycle. Four different classes of the enzyme are known in microbes that differ in size and cofactor specificity. Despite some common features, the kinetic properties of the enzymes from different species vary considerably. We consider the relationship between the sequences, those structures that are available and the enzyme kinetics in the context of the metabolic role of the enzyme.

1.2 Introduction

Glutamate dehydrogenase (GDH) catalyses the reversible reductive amination of 2-oxoglutarate to glutamate:

$$\text{2-Oxoglutarate} + \text{NAD(P)H} + \text{NH}_4^+ \rightleftharpoons \text{Glutamate} + \text{NAD(P)}^+ + \text{H}_2\text{O} + \text{H}^+. \quad (1.1)$$

The enzyme may be NAD^+ specific (EC 1.4.1.2), $NADP^+$ specific (EC 1.4.1.4) or able to use either cofactor (EC 1.4.1.3). Irrespective of the cofactor, the standard transformed Gibbs free energy of reaction, $\Delta_r G'^0 \approx -40$ kJ mol^{-1} (Olson and Anfinsen, 1953; Strecker, 1953; Engel and Dalziel, 1967; Subramanian, 1978; Cook *et al.*, 1980) and the reaction operates in either direction depending on the relative magnitudes of

$$Q = [\text{Glutamate}][\text{NAD(P)}^+]/[\text{2-Oxoglutarate}][\text{NAD(P)H}][\text{NH}_4^+],$$

where Q is the reaction quotient and

$$K' = \exp\left(-\Delta G'^0/RT\right) \approx 10^{-7},$$

where K' is the apparent equilibrium constant, R is the gas constant and T is the absolute temperature. If $Q < K'$, the reaction will tend to form glutamate.

In bacteria, GDH can be involved in the assimilation of NH_4^+ (Kanamori *et al.*, 1987; Bruggeman *et al.*, 2005), in glutamate synthesis (Helling, 1994) and in controlling the intracellular concentration of 2-oxoglutarate to ensure the operation of 2-oxoglutarate dehydrogenase (ODH) and the whole tricarboxylic acid (TCA) cycle (Struhl and Maganasik, 1976; Janes *et al.*, 2001; Doucette *et al.*, 2011). We consider the structural and kinetic properties of the different forms of GDH, their taxonomic distribution and the roles of the enzyme in the metabolism of bacteria.

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 The Handbook of Microbial Metabolism of Amino Acids (ed. J.P.F. D'Mello)

1.3 Enzyme Structure

Four broad classes of GDH, designated GDH1–4 (Andersson and Roger, 2003), have been distinguished using structural and functional criteria. Both GDH1 and GDH2 have monomers of about 50 kDa and are distinguished by their cofactor specificity: GDH1 is NAD^+ specific, whereas GDH2 is $NADP^+$-specific. In contrast, the monomers of GDH3 and GDH4 are of about 115 and 180 kDa, respectively (Fig. 1.1), but are not as well characterized.

Both GDH1 and GDH2 are about 450 residues in length, are highly conserved and are much more similar to one another than they are to GDH3 and GDH4 (Fig. 1.2). In the alignment on which Fig. 1.2 is based, the identity among the 14 GDH1 and GDH2 sequences shown is 21–89% (40–96% similarity; average overall distance = 0.80 substitutions per site), whereas the seven GDH3 sequences have 17–65% identity (32–79% similarity; mean overall distance = 1.22 substitutions per site) and the seven GDH4 sequences are more highly conserved (31–65% identity; 47–77% similarity; overall mean distance = 0.88 substitutions per site).

1.3.1 Substrate and cofactor binding

The residues involved in binding the substrates or the cofactor are mostly completely conserved among GDH1 and GDH2 sequences (Fig. 1.3), and some of these are also conserved in GDH3 and GDH4 sequences. Of particular note among the latter are the lysine residue K126 and the aspartate residue D166, which, in concert with the α-amino group of the substrate glutamate and a water oxygen, form a tetrad capable of a wide range of hydrogen bonding patterns that may be catalytically significant (Fisher and Maniscalco, 2002).

The determinants of the cofactor specificity of GDH have not yet been identified unambiguously. It has been proposed that the Gly-X-Gly-X-X-Gly/Ala motif in the P-loop of the Rossmann fold is involved in determining the cofactor specificity of dehydrogenases (Bellamacina, 1996). An alanine in the final position (P6 or the alanine A244 in *Escherichia coli*) is supposed to favour $NADP^+$ binding, whereas a glycine favours NAD^+ binding, but the *Clostridium symbiosum* GDH1 has an alanine and the *Psychrobacter* sp. TAD1 GDH2 has a serine, so neither of these conform to this model. No consensus about P6 is apparent from the GDH3 and GDH4 sequences. Similarly, the presence of a negatively charged group near the 2′-OH and 3′-OH of adenosine (P7 or D263 in *E. coli*) has been suggested to favour NAD^+ rather than $NADP^+$ binding. However, as Sharkey *et al.* (2013) observed, there is an aspartate (or a glutamate in *Peptinophilus asaccharolyticus*) in this position in most GDH sequences, with the exceptions of the *C. symbiosum* GDH1 and *Pseudomonas aeruginosa* GDH4. Based on the modelling of $NADP^+$ binding to GDH2, it has been suggested that $NADP^+$

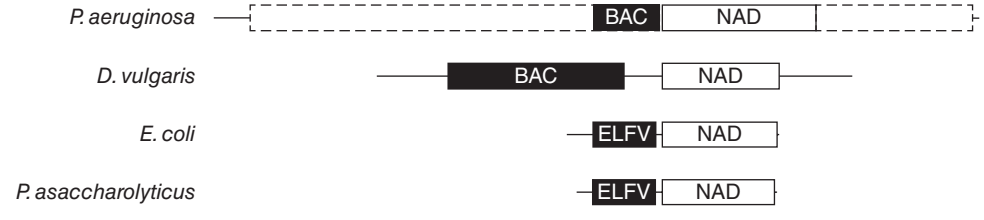

Fig. 1.1. The domains of representative bacterial glutamate dehydrogenases (GDHs). The *Peptoniphilus asaccharolyticus* (AAA25611.1), *Escherichia coli* (P00370.1), *Desulfovibrio vulgaris* (ABM29040.1) and *Pseudomonas aeruginosa* (WP_053816968.1) enzymes are examples of GDH1, GDH2, GDH3 and GDH4, respectively. The domains identified using Pfam (the protein families database; Finn *et al*., 2014) and CATH (class, architecture, topology, homology – a hierarchical protein domain classification system; Cuff *et al*., 2011) are an NAD-binding Rossmann-like domain (use of 'NAD' intends no implication on cofactor specificity), a bacterial GDH domain (BAC) and a domain common to glu/leu/phe/val dehydrogenases (the substrate-binding domain, ELFV). For the *P. aeruginosa* sequence, Pfam identified a more extensive BAC domain (the dashed rectangle) than did CATH (the black rectangle).

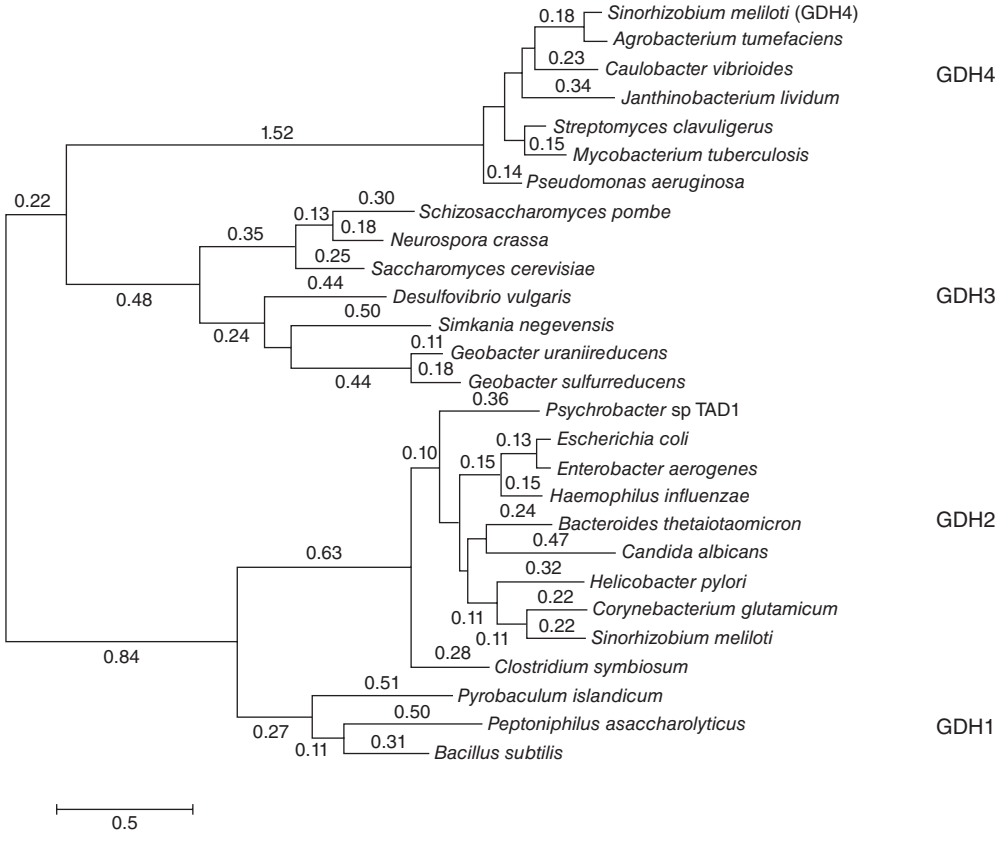

Fig. 1.2. The bootstrap consensus phylogram derived by maximum likelihood from an alignment of 27 glutamate dehydrogenase (GDH) amino acid sequences (375 gap-free positions) using molecular evolutionary genetics analysis (MEGA5; Tamura *et al*., 2011). The maximum likelihood was based on the JTT (Jones, Taylor, Thornton) matrix-based model (Jones *et al*., 1992) using 500 bootstrap replicates (Felsenstein, 1985). The branch lengths (number of substitutions per site) are indicated below the branches unless the value was less than 0.1. The shape parameter of the discrete gamma distribution was 1.637.

specificity may be conferred by: (i) three positively charged residues (lysine K286, and arginines R289, R292 in the *E. coli* enzyme); and (ii) a serine (S264 in *E. coli*). In this model, these four residues are adjacent to the 2′-phosphate of $NADP^+$ and stabilize the negative charge (Sharkey *et al*., 2013). While K286 is almost completely conserved among GDH1 and GDH2, and S264 is also highly conserved (although neither is present in the *Burkholderia thailandensis* sequence), R289 and R292 are both absent from the $NADP^+$-specific *Psychrobacter* enzyme (Di Fraia *et al*., 2000). Overall, as Sharkey *et al*. (2013) suggest, cofactor specificity cannot be reliably inferred from primary sequence.

1.3.2 Tertiary and quaternary structure

As with other dehydrogenases, GDH has a substrate-binding domain (ELFV), which is similar to those of other amino acid dehydrogenases, and an $NAD(P)^+$-binding domain (NAD), both of which can be identified from sequence homology alone (Fig. 1.2). Crystal structures are available for only GDH1 and GDH2, but these are similar to those of the human and bovine enzymes, except for the absence of the antenna domain. In each case, the ELFV domain consists of a series of β-strands sandwiched between α-helices and the NAD domain is a modified Rossmann fold. The nucleotide binds

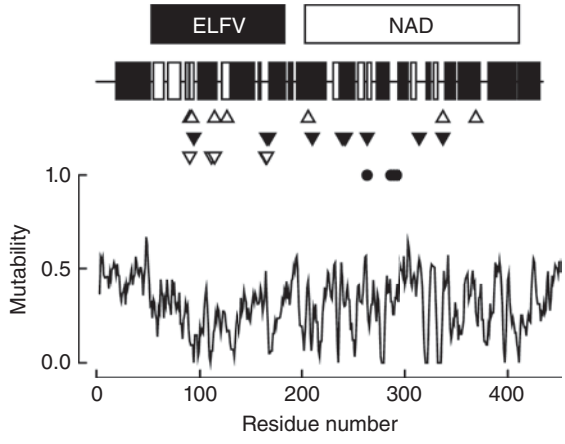

Fig. 1.3. Summary of the structural properties of the NAD⁺-specific glutamate dehydrogenase (GDH) from *Burkholderia thailandensis* and the mutability of GDH1 and GDH2 sequences. The triangles (▲, ▼, △) indicate residues that are hydrogen bonded to glutamate, 2-oxoglutarate or NAD⁺ based on the crystal structures of the *B. thailandensis* (4XGI) and *Clostridium symbiosum* (1BGV) enzymes. The circles (●) indicate the approximate position of the residues involved in determining the NADP⁺-specificity of the *Escherichia coli* enzyme (Sharkey *et al*., 2013). The secondary structure (4XGI) indicates only the positions of α-helices and β-strands (black and white rectangles, respectively). The mutability was calculated as described previously (Brown *et al*., 2014) from the sequence alignment of the GDH1 and GDH2 sequences identified in Fig. 1.2. Abbreviations: ELFV, a domain common to glu/leu/phe/val dehydrogenases (the substrate-binding domain); NAD, a NAD-binding Rossmann-like domain (use of 'NAD' intends no implication on cofactor specificity).

in a cleft between the domains and is ligated by residues from each (Fig. 1.3).

The substrate and cofactor are necessarily close together. In both the bovine enzyme and in *B. thailandensis* GDH1, the substrate binds towards the narrow end of the cleft and NAD⁺ binds further towards the wider end of the cleft with the nicotinamide moiety oriented towards the substrate. In each case, they are so close that the 3′-OH of NAD⁺ and the carboxyl group of the substrate are both H bonded to a single asparagine residue.

Most microbial GDHs are homohexamers, although the GDH2 of *Streptomyces fradiae* (Vančurová *et al*., 1989), the GDH3s of *Agaricus bisphorus* and *Neurospora crassa* (Smith *et al*., 1975) and the GDH4 of *P. aeruginosa* (Lu and Abdelal, 2001) are reportedly tetramers. Unusually, the GDH4 of *Psychrobacter* is dimeric and while some kinetic data have been reported (Camardella *et al*., 2002), the primary sequence is not yet available. The crystal structures that are available exhibit D_3 symmetry in which monomers are located at the vertices of triangles and these are stacked in pairs with the monomers approximately aligned. In each monomer the cleft between the domains opens away from, and the nicotinamide moiety of NAD⁺ is oriented towards, the threefold axis. It is inevitable that the tetrameric GDHs will have very different quaternary structures.

1.4 Enzyme Mechanism and Kinetics

The kinetics of GDH are complex, both because of the number of reactants and products (Eqn 1.1) and because of the variety of behaviours observed (Barton and Fisher, 1971; Bailey *et al*., 1982; Syed and Engel, 1987). For some forms of the enzyme the usual rectangular hyperbolic relationship between initial rate (v_0) and substrate concentration (s) is observed, but cooperative kinetics have also been reported (Table 1.1). Reports of non-linear double reciprocal, Eadie–Hofstee or other transformed plots are common for both the mammalian (Barton and Fisher, 1971; Bailey *et al*., 1982) and microbial enzymes (Table 1.1).

Table 1.1. Kinetic characteristics of some microbial glutamate dehydrogenases (GDHs). Where cooperativity is reported, the Hill coefficient (n_H) is given below the K_m or $s_{0.5}$ in parentheses.

		K_m or $s_{0.5}$ (n_H)				
Species	Cofactor	2-OG[a] (mM)	Glu[b] (mM)	NH_4^+ (mM)	NAD(P)$^+$ (μM)	NAD(P)H (μM)
GDH1						
Bacillus cereus DSM 31 (Jahns and Kaltwasser, 1993)	NAD$^+$	[c]	7.4	96	560	33
Bacillus subtilis ISW1214 (Khan *et al*., 2005)	NAD$^+$	0.65	0.34	55.6	80	70
Bacteroides thetaiotaomicron (Glass and Hylemon, 1980)	NAD$^+$		12.3		610	
	NADP$^+$	0.24	3.7	5.0	40	17
Peptostrepococcus asaccharolyticus (Hornby and Engel, 1984)	NAD$^+$	0.82	6.6	18.4	31	66
Psychrobacter sp. TAD1 (Camardella *et al*., 2002)	NAD$^+$	2.36	28.6	24.6	500	40 (1.8)
Pyrobaculum islandicum (Kujo and Ohshima, 1998)	NAD$^+$	0.066	0.17	9.7	25	5.0
	NADP$^+$				240	270
GDH2						
Escherichia coli B/r (Sakamoto *et al*., 1975)	NADP$^+$	0.64	1.3	1.1	42	40
Psychrobacter sp. TAD1 (Di Fraia *et al*., 2000)	NADP$^+$	6.8 (3.6)	67.9 (1.2)	4.5 (1.1)	52 (0.8)	23 (2.3)
Ruminococcus flavefaciens (Duncan *et al*., 1992)	NADP$^+$	0.41	62.19	19.22	–	34
Saccharomyces cerevisiae (DeLuna *et al*., 2001)	NADP$^+$	1.27 (1.5)	6.36	5.00	10.5	331
Saccharomyces cerevisiae (DeLuna *et al*., 2001)	NADP$^+$	0.29 (1.3)	9.79	5.96	14.1	113
GDH3						
Benjaminiella poitrassi (Joshi *et al*., 2013)	NAD$^+$	3.12	33.3	27.7	170	60
Agaricus bisporus (Kersten *et al*., 1999)	NAD$^+$	3.5	37.1	6.5	46	60
Neurospora crassa (Smith *et al*., 1975)	NAD$^+$	4.6	5.5	17	330	550
GDH4						
Janthinobacterium lividum (Kawakami *et al*., 2007, 2010)	NAD$^+$	9.1 (1.7)	7.1	20.9	2100	68 (1.8)
Pseudomonas aeruginosa (Lu and Abdelal, 2001)	NAD$^+$	–	4.4 (1.7)	–	130	–
Streptomyces clavuligerus (Miñambres *et al*., 2000)	NAD$^+$	1.33 (1.5)	2.27 (1.39)	33.9	51.16 (1.48)	19.87 (1.52)

[a]2-OG, 2-oxoglutarate.
[b]Glu, glutamate.
[c]Response is sigmoidal, but no data are reported.

Rife and Cleland (1980b) proposed a model of the catalytic mechanism of GDH which is largely consistent with the crystal structures that have been found (Stillman *et al*., 1993) and subsequent analyses of the kinetics (Fisher, 2005). The model shown in Fig. 1.4 is essentially that of Rife and Cleland (1980b) for bovine liver GDH. The enzyme-bound 2-oxoglutarate is aminated, transiently forming a quaternary complex (ERNK in Fig. 1.4), to a 2-carbinolamine (2-hydroxy-2-iminoglutarate). This is dehydrated to 2-iminoglutarate which is then reduced to glutamate. At this point, the complex is protonated and then water is released

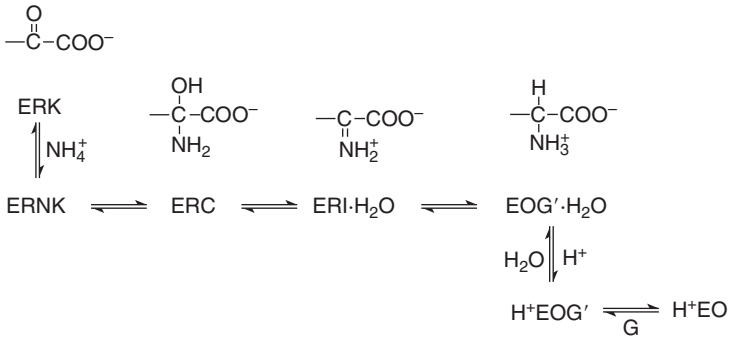

Fig. 1.4. Catalytic mechanism of bovine glutamate dehydrogenase (GDH) adapted from Rife and Cleland (1980b), Maniscalco *et al*. (1996) and Fisher (2005). The sequence of binding of NADH and 2-oxoglutarate is not shown. The structural fragments above each intermediate represent C1 and C2 of the ligand starting with 2-oxoglutarate (left) and ending with glutamate (right). The standard abbreviations used below the fragments are: C, 2-carbinolamine; E, enzyme; G, glutamate; I, 2-iminoglutarate; K, 2-oxoglutarate; N, NH_4^+; O, NAD^+; R, NADH.

from bovine liver GDH, although these steps are reversed in the *C. symbiosum* enzyme (Maniscalco *et al*., 1996), before glutamate is released (Fig. 1.4).

The most commonly used initial rate (v_0) equation for GDH is the empirical expression

$$\frac{e}{v_0}=\phi_0+\frac{\phi_1}{s_1}+\frac{\phi_2}{s_2}+\frac{\phi_3}{s_3}+\frac{\phi_{12}}{s_1s_2}+\frac{\phi_{13}}{s_1s_3}+\frac{\phi_{23}}{s_2s_3}+\frac{\phi_{123}}{s_1s_2s_3} \quad (1.2)$$

or (see Eqn 1.3 at bottom of the page):

where e is the concentration of GDH, the s_i (s_1, s_2, s_3) are the concentrations of NAD(P)H, NH_4^+ and 2-oxoglutarate, and different combinations of rate and dissociation constants are distinguished by particular subscripts to ϕ. In Eqns 1.2 and 1.3, e/ϕ_0 is the maximum velocity (V_{max}), the K_m for s_i is ϕ_i/ϕ_0 and all substrates are independent and have rectangular hyperbolic kinetics. This expression is based on a general random binding order model involving the formation of a quaternary complex, but it includes, as special cases, other models, including those with specific binding sequences (Dalziel, 1969). For example, some authors have concluded that there was, in at least one direction, an ordered sequence of binding to the bovine enzyme (Frieden, 1959; Rife and Cleland, 1980a), whereas others have argued that the data are consistent with the random binding order and have obtained estimates of all eight parameters (Engel and Dalziel, 1970). The implication of the mechanism shown in Fig. 1.4 is that binding is at least partially ordered: NH_4^+ binds after NAD(P)H and 2-oxoglutarate and, in the opposite direction, glutamate binds after $NAD(P)^+$.

The parameters of Eqn 1.2 are often obtained sequentially from linearized plots (Dalziel, 1969) which necessarily introduces bias into the estimates and may explain, in part, why it is sometimes difficult to obtain values for some parameters (Engel and Dalziel, 1970). Unsurprisingly, it is also clear that Eqn 1.2 cannot account for the reported cooperative kinetics (Di Fraia *et al*., 2000; Miñambres *et al*., 2000) of GDH and does not account for substrate inhibition (Sharkey and Engel, 2008) or the activation by high NAD^+ concentrations (Olson and Anfinsen, 1953; Engel and Dalziel, 1970). Other empirical expressions have been devised to account for some of these phenomena, and of these three

$$v_0=\frac{s_1s_2s_3e}{\phi_0s_1s_2s_3+\phi_1s_2s_3+\phi_2s_1s_3+\phi_3s_1s_2+\phi_{12}s_3+\phi_{13}s_2+\phi_{23}s_1+\phi_{123}}, \quad (1.3)$$

are of particular interest. First, Engel and Ferdinand (1973) proposed a rational rate equation:

$$v_0 = \frac{\sum_{j=1}^{q} D_j s^j e}{N_0 + \sum_{i=1}^{q} N_i s^i}, \qquad (1.4)$$

which can describe an almost unlimited range of behaviour, as has been considered in some detail (Bardsley and Childs, 1975; Bardsley *et al.*, 1980; Brown and Simcock, 2013). Second, Kurganov (2000) proposed that the K_m varies continuously with v_0/V_{max} and modified the standard Michaelis–Menten expression accordingly:

$$v_0 = \frac{V_{max} s}{\left(K_0 + \left(K_{lim} - K_0\right)\frac{v_0}{V_{max}}\right) + s}, \qquad (1.5)$$

where K_0 and K_{lim} are the effective values of K_m as v_0/V_{max} approaches 0 and 1, respectively. This can be written as

$$s = \left(K_0 - K_{lim}\right)\frac{v_0}{V_{max}} + K_{lim}\frac{v_0}{V_{max} - v_0}, \qquad (1.6)$$

so if $K_{lim} < K_0$, the linear v_0–s regime dominates and the transition to the saturated regime occurs over a small range of s. If $K_{lim} > K_0$, a biphasic double reciprocal plot is generated which is reminiscent of those often reported for GDH (Barton and Fisher, 1971; Bailey *et al.*, 1982; Syed and Engel, 1987). Both Eqns 1.4 and 1.5 account for only one of the substrates and, like Eqn 2, their main purpose is to provide a means of summarizing particular aspects of the kinetics of GDH. This weakness is overcome, to some extent, by the third example, Eqn 1.6 – the expression devised by Barton and Fisher (1971) to account for the kinetics of the oxidative deamination reaction. Then again, this involves 13 parameters, few of which have any obvious mechanistic significance. A complete kinetic model is yet to be devised for the enzyme.

Despite rate equations such as shown in Eqns 1.2–1.5, the usual practice has been to estimate the K_m (or $s_{0.5}$ where there is cooperativity) and V_{max} by independently varying the concentration of each reactant. The small selection of data in Table 1.1 prompts hypothesis that there might be some differences between GDHs. For example, it appears that the K_m values for both NH_4^+ and $NAD(P)^+$ may tend to be larger in GDH1 than in GDH2 (Table 1.1). While this is merely speculation, careful analysis of the available data is warranted.

1.5 Genomics

The taxonomic distribution of the forms of GDH is uneven. Both GDH1 and GDH2 are widespread among bacteria and fungi, but GDH3 and GDH4 have more restricted distributions (Andersson and Roger, 2003). Based on the data available in Pfam (the protein families database; Finn *et al.*, 2014), homologues of GDH4 (based on the presence of the BAC domain shown in Fig. 1.1, Pfam accession code PF05088) can be found among the α-, β- and γ-proteobacteria (149, 43 and 146 species, respectively), and in the actinobacteria (84 species), as well as in a small number of fungi (11 species) and metazoa (five species). Among the bacteria that have GDH4 homologues are a number of *Mesorhizobium* spp., *Sinorhizobium* spp. and *Bradyrhizobium* spp., and some, such as *Sinorhizobium meliloti* (Fig. 1.2), also have other forms of GDH. However, rhizobia generally lack significant GDH activity (Ali *et al.*, 1981; Bravo and Mora, 1988; Rossi *et al.*, 1989), and several species of bacteria lack any GDH sequence (Andersson and Roger, 2003).

One of the many challenges of genomic analysis is the determination of the presence or absence of a gene (Cordwell, 1999; Osterman and Overbeek, 2003), but it is just one of many. Even if it can be confirmed that a gene occurs in a genome, that need not mean that it is expressed or, if it is, that the product has only the anticipated consequence. The identification of any homologue of a gene can be an even greater challenge if only because of insufficient data. For example, GDH4 is quite different from GDH1 (see Fig. 1.1) and so a database search may not identify all of the four forms of the enzyme. Depending on the circumstances, even the detection of a particular enzyme activity need not be unambiguous. For example, ODH was reported to be present in *Mycobacterium tuberculosis* (Cordwell, 1999) based on the presence of the genes and the oxidation of the substrate, but even if the genes are expressed they need not be incorporated into ODH (Tian *et al.*, 2005b) and the apparent ODH activity can be due to other enzymes entirely

(Tian *et al.*, 2005a). Further, there is the growing realization that an enzyme might have more than one function. For example, mammalian GDH may link lysosomes to microtubules (Rajas *et al.*, 1996) and bind mRNA (Preiss *et al.*, 1995), perhaps regulating the translation of proteins targeted to the mitochondria, while the aconitase apoprotein is involved in the regulation of iron metabolism (Beinert *et al.*, 1997; Brzóska *et al.*, 2006).

Nevertheless, the presence or absence of an enzyme such as GDH is biochemically and physiologically significant because it links carbon and nitrogen metabolism (Fig. 1.5). Notwithstanding, the TCA cycle has many variants (Weitzman, 1981; Huynen *et al.*, 1999) and so this metabolic intersection can be especially complex. Bearing in mind the intrinsic difficulties already described, several species do not have genes encoding any of the GDHs (Andersson and Roger, 2003), including *Aquifex aeolicus*, *Archaeoglobus fulgidus*, *Borrelia burgdorferi*, *Methanobacterium thermoautotrophicum* and *Mycoplasma pneumoniae* (Cordwell, 1999). All five of the named species just listed also lack ODH activity, and two of them, *B. burgdorferi* and *M. pneumoniae*, do not have isocitrate dehydrogenase (IDH) activity either (Cordwell, 1999). This type of listing can be extended indefinitely (see Table 1.2). While such a list does not show the extent to which alternative reactions can substitute for any of these enzymes, it does prompt consideration of the possible metabolic variants.

1.6 Metabolic Context

GDH is an important link between amino acid and carbon metabolism through the TCA cycle (Fig. 1.5). Specifically, the 2-oxoglutarate produced by IDH (EC 1.1.1.42), is a substrate for

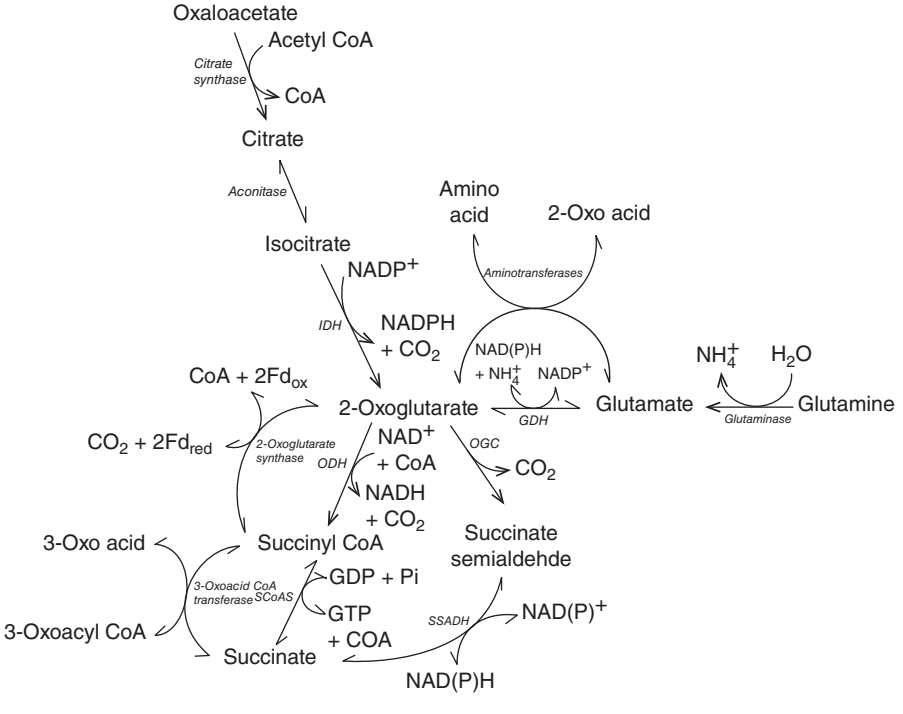

Fig. 1.5. Glutamate dehydrogenase (GDH) is the link between the tricarboxylic acid (TCA) cycle and nitrogen metabolism. The oxidative branch of the TCA cycle and several reactions involving glutamate and 2-oxoglutarate are shown, but the reactions that actually operate vary with the conditions and between species, as discussed in the text. Abbreviations: IDH, isocitrate dehydrogenase; ODH, 2-oxoglutarate dehydrogenase; OGC, 2-oxoglutarate decarboxylase; SCoAS, succinyl-CoA synthetase; SSADH, succinate semialdehyde dehydrogenase.

Table 1.2. The presence (+) or absence (–) of glutamate dehydrogenase (GDH), isocitrate dehydrogenase (IDH), 2-oxoglutarate dehydrogenase (ODH) and/or succinyl-CoA synthetase (SCoAS) in several species for which a complete genome sequence is available. The data for IDH, OGDH and SCoAS were adapted from Cordwell (1999).

Species	GDH	IDH	ODH	SCoAS
Aquifex aeolicus	–	+	–	+
Archaeoglobus fulgidus	–	+	–	+
Bacillus subtilis	+	+	+	+
Borrelia burgdorferi	–	–	–	–
Chlamydia trachomatis	–	–	+	+
Corynebacterium glutamicum	+	+	–	+
Escherichia coli	+	+	+	+
Haemophilus influenzae	+	–	+	+
Helicobacter pylori	+	+	–	–
Methanobacterium thermoautotrophicum	–	+	–	+
Methanococcus jannaschii	?	+	–	+
Mycobacterium tuberculosis	+	+	–	?
Mycoplasma genitalium	?	–	–	–
Mycoplasma pneumoniae	–	–	–	–
Pyrococcus horikoshii	+	–	–	–
Synechocystis sp. PCC6803	+	+	–	+
Treponema pallidum	?	–	–	–

both GDH and the ODH complex, which consists of multiple copies of a thiamine pyrophosphate-dependent dehydrogenase (E1; EC 1.2.4.2), dihydrolipoamide succinyltransferase (E2; EC 2.3.1.61) and dihydrolipoamide dehydrogenase (E3; EC 1.8.1.4). While GDH catalyses a reversible reaction, the citrate synthase, IDH and ODH reactions are irreversible. In fact, 2-oxoglutarate is among the key metabolites in the metabolic network of *E. coli* and glutamate is the most highly connected metabolite in this network (Wagner and Fell, 2001).

The interaction of the great variety of enzymes and reactions of the TCA cycle observed among microbes (Weitzman, 1981; Huynen *et al.*, 1999) with GDH has profound metabolic implications. Considered simply, the three enzymes directly involved in 2-oxoglutarate metabolism are IDH, ODH and GDH. In *E. coli*, IDH and GDH2 are $NADP^+$ dependent with K_m = 17 μM $NADP^+$ (Hurley *et al.*, 1996) and 42 μM NADPH (Table 1.1), respectively, while ODH is NAD^+ dependent with K_m = 400 μM NAD^+, and is also subject to product inhibition (K_i = 1 mM) (Schmincke-Ott and Bisswanger, 1981). However, this difference in cofactor specificity is less significant than it might seem because *E. coli* has two transhydrogenases, one soluble (Cao *et al.*, 2011), and the other in the plasma membrane (Bizouarn *et al.*, 2000), that keep $[NAD^+]/[NADH] \approx 19$ and $[NADP^+]/[NADPH] \approx 1.2$ (Andersen and von Meyenburg, 1977). The K_m for 2-oxoglutarate is similar for GDH2 (0.64 mM; Table 1.1) and ODH (0.1 mM; Gupta and Dekker, 1980), but GDH2 also requires NH_4^+ (K_m = 1.1 mM; Table 1.1), so the flux through ODH would tend to be greater than that through GDH2.

Under aerobic conditions, *E. coli* operates a complete TCA cycle, but in anaerobic conditions it lacks ODH activity and the pathway operates as two branches: an oxidative branch leading to the production of 2-oxoglutarate and a reductive branch yielding succinate (Hager and Kornberg, 1961; Amarasingham and Davis, 1965). *E. coli* only assimilates NH_4^+ under aerobic conditions and GDH is involved in this process in energy- or glucose-limited conditions, when the concentration of NH_4^+ is sufficiently high, whereas glutamine synthetase (GS; EC 6.3.1.2) and glutamate synthase (GOGAT, E. C. 1.4.1.13) are responsible in energy-rich conditions (Helling, 1994, 1998). In glucose-limited conditions, GDH and ODH share the available 2-oxoglutarate.

However, when ODH activity is low and 2-oxoglutarate accumulates, GDH2 can utilize the available NH_4^+ and the NADPH generated by

IDH to produce glutamate (if $Q < K'$). For example, the *E. coli* mutant 309-1, which constitutively lacks the capacity to oxidize 2-oxoglutarate, excretes glutamate when grown aerobically on glucose in the presence of succinate (Davis *et al.*, 1959; Shiio *et al.*, 1961). A similar *Enterobacter aerogenes* mutant produces 2-oxoglutarate from glucose in anaerobic conditions (Stouthamer, 1966). Conversely, if the 2-oxoglutarate supply is low, then GDH2 can oxidatively deaminate glutamate (using $NADP^+$; $K_m = 40\ \mu M$) to produce more, providing that $Q > K'$. It should be noted though that while glutamate is an effective inducer of ODH activity (Amarasingham and Davis, 1965) and *E. coli* can use glutamate as a sole nitrogen source, the maximum growth rate is low whether or not glucose is limiting (Halpern and Umbarger, 1961; Senior, 1975).

Corynebacterium glutamicum (formerly *Brevibacterium flavum* and *B. lactofermentum*) has significant $NADP^+$-dependent GDH activity (Shiio *et al.*, 1959), which is presumably GDH2 (Shiio and Ozaki, 1970), along with appreciable IDH activity (Shiio *et al.*, 1961), but its ODH activity declines during growth (Kawahara *et al.*, 1997) and, in at least some circumstances, it can be undetectable (Shiio *et al.*, 1961). During aerobic growth on glucose, 2-oxoglutarate, or in the presence of NH_4^+, glutamate accumulates (Shiio *et al.*, 1959), and this can be substantially reduced by biotin supplementation (Shiio *et al.*, 1962). Deleting the gene for the E1 enzyme of ODH completely inactivates ODH and has little effect on the accumulation of glutamate (Asakura *et al.*, 2007), whereas deletion of the GDH gene reduces the intracellular concentration of glutamate and increases those of NH_4^+ and 2-oxoglutarate (Müller *et al.*, 2006).

The oxidation of citrate and isocitrate by *M. tuberculosis* can be estimated from the rate of O_2 uptake, but this requires $NADP^+$ and is independent of CoA (Murthy *et al.*, 1962). This is inconsistent with the operation of ODH, which requires NAD^+ and CoA, and arises from the replacement of ODH and succinyl CoA synthetase (SCoAS; EC 6.2.1.4) by a combination of reactions catalysed by 2-oxoglutarate decarboxylase (OGC; EC 4.1.1.71)

$$\text{2-Oxoglutarate} \rightleftharpoons \text{Succinate semialdehyde} + CO_2$$

and succinate semialdehyde dehydrogenase (SSADH; EC 1.2.1.16)

$$\text{Succinate semialdehyde} + \text{NAD(P)}^+ + H_2O \rightleftharpoons \text{Succinate} + \text{NAD(P)H} + 2H^+$$

(Tian *et al.*, 2005a). The K_m for 2-oxoglutarate of OGC is 0.48 mM (Tian *et al.*, 2005a) is much lower than that of *M. smegmatis* (5 mM; Sarada *et al.*, 1980), the only GDH2 that has been characterized from *Mycobacterium* spp., which, if a similar situation pertains in *M. tuberculosis*, would tend to favour the operation of OGC/SSADH rather than GDH. While these, and other (Piston *et al.*, 1999), alternative reactions are interesting, what is remarkable is that *M. tuberculosis* has genes encoding the enzymes of ODH, but that they are used for other purposes (Maksymiuk *et al.*, 2015). This reinforces a point made previously: it is unwise to infer the presence of a gene product or an expected biological function from the presence of a gene.

1.7 Conclusions

Of the four forms of GDH in microbes, GDH1 and GDH2 have been well characterized, but GDH3 and GDH4 are less well understood. Crystal structures are available for GDH1 and GDH2 and much work has been done to analyse the kinetics of these enzymes, but there is little information on GDH3 or GDH4, and much remains to be done to generate a coherent whole from the rapidly growing volume of data. Some of the difficulty arises from the extraordinary metabolic and physiological flexibility of bacteria and fungi, the consequence of which is that genera (and sometimes species) have less in common than might be anticipated. This leads to the conclusion that more data are only part of the answer, and that it is also necessary to develop the means to integrate the data in meaningful ways.

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