A Difference in the Sequence of Steps in the Reactions Catalyzed by Two Closely Homologous Forms of Glutamate Dehydrogenase†

Steven J. Maniscalco, Swapan K. Saha,* Patrick Vicedomine,‡ and Harvey F. Fisher*

Laboratory of Molecular Biochemistry, Department of Biochemistry, University of Kansas Medical Center, and Veterans Affairs Medical Center, Kansas City, Missouri 64128

Received April 24, 1995; Revised Manuscript Received September 8, 1995⊗

ABSTRACT: Glutamate dehydrogenase from beef liver (bl GDH) and the corresponding enzyme from *Clostridium symbiosum* (cs GDH) each catalyze the same sequence of chemical events in the oxidative deamination of L-glutamate. This catalysis involves interactions between at least six conserved functional groups, each of which appears to occupy the same geometric position with respect to the substrate molecule in both enzyme−coenzyme−L-glutamate reactive ternary complexes. In both cases steady-state *V*/*K* pH profiles indicate the requirement for the transfer to the solvent of a single proton from the same abnormal lysine for L-glutamate to bind and react; the p*K* of that lysine is the same for both enzymes. Here we report studies of the proton traffic between enzyme and solvent using direct pH-stat back-titration and indicator dye measurements on dead-end inhibitor ternary complexes, simultaneous transient-state time courses of proton and product, and transient-state kinetic isotope studies on both enzymes. We find that in the cs GDH catalyzed reaction the single proton is released only *after* the hydride transfer step whereas in the bl GDH reaction this proton release occurs *prior* to the hydride transfer step, despite the fact that the substrate molecule undergoes the same sequence of chemical events in both reactions. Interpreting these results in the context of the X-ray crystallographic structures of cs GDH and its NAD binary complex and of thermodynamic studies of bl GDH and its complexes, we conclude that the difference in the relative times of proton release in the two enzyme-catalyzed reactions must be ascribed to a difference in the sequence of active site cleft-opening and -closing events in the two otherwise identical reaction sequences. We suggest a possible biological significance to this unusual method of modulating a common reaction to suit differing metabolic roles.

The reactions catalyzed by all hexameric glutamate dehydrogenases (and probably all L-α-amino acid dehydrogenases) appear to involve a very specific mechanistic feature. This feature consists of the nucleophilic attack on the α-carbon atom of the α-iminoglutarate intermediate (which results from the preceding hydride transfer step) by a water molecule hydrogen bonded to the deprotonated γ-nitrogen atom of a highly conserved lysine residue whose p*K* is abnormally low (Brunhuber & Blanchard, 1994). We have previously shown (Singh et al., 1993) that in bovine liver glutamate dehydrogenase (bl GDH)[1] the release of a proton from the γ-amino group of lysine 126 to the solvent occurs as the earliest observable step in the reaction. Here we show that in the same reaction catalyzed by *Clostridium symbiosum* glutamate dehydrogenase (cs GDH) this event occurs at a later point in the reaction scheme and discuss the possible cause and mechanistic significance of such an unexpected circumstance.

† This work was supported in part by Grant MCB-9221119 from the National Science Foundation, Grant GM-47108 from the General Medicine Institute of the National Institutes of Health, and the Department of Veterans Affairs.

* Authors to whom correspondence may be addressed at the Research Service, VA Medical Center, 4801 Linwood Blvd., Kansas City, MO 64128. Telephone (816) 861-4700 X7156; FAX (816) 861-1110.

‡ Present address: Chemistry Department, Brandeis University, P.O. Box 9110, Waltham, MA 02254-9110.

⊗ Abstract published in *Advance ACS Abstracts,* December 1, 1995.

[1] Abbreviations: cs GDH, *Clostridium symbiosum* glutamate dehydrogenase; bl GDH, bovine liver glutamate dehydrogenase; E, enzyme; O, oxidized coenzyme [NAD(P)]; R, reduced coenzyme [NAD(P)H]; I, α-iminoglutarate; C, α-carbinolamine; K, α-ketoglutarate; N, ammonia; KIE, kinetic isotope effect.

MATERIALS AND METHODS

C. symbiosum glutamate dehydrogenase (cs GDH) was prepared and purified following the methods of Rice et al. (1985). The enzyme was dialyzed against three changes of 0.5 mM potassium phosphate and 0.1 M sodium sulfate buffer at the required pH, centrifuged at 4 °C for 20 min at 1500 rpm, treated with approximately 1 mg/mL Norit A, and finally filtered through 0.45 μm Millipore filters. The enzyme concentration was measured spectrophotometrically at 280 nm using $\epsilon = 51.8$ cm^{-1} mM^{-1}. Bovine liver glutamate dehydrogenase (bl GDH) was purchased from Sigma as an ammonium sulfate suspension. This enzyme was dialyzed and filtered as was done with the *Clostridium* enzyme. The enzyme concentration was measured spectrophotometrically at 280 nm using $\epsilon = 54.4$ cm^{-1} mM^{-1}. NADP and NAD were purchased from Sigma and used without any further purification. L-Glutamic acid was purchased from Calbiochem. Phenol red was purchased from Sigma, and the indicator solution was freshly prepared prior to each experiment. L-[α-^{2}H]Glutamic acid was prepared following the method of Rife and Cleland (1980a). NMR analysis of the deuterated glutamic acid showed 100% conversion at the α-position.

V/*K* measurements were carried out as described previously (Singh et al., 1993).

The pH titration experiments were carried out on a Radiometer PHM Research pH meter interfaced with a TTT 80 titrator and ABU 80 autoburet. The solutions were thoroughly degassed with argon prior to each experiment, and the experiments were carried out under an argon

0006-2960/96/0435-89$12.00/0

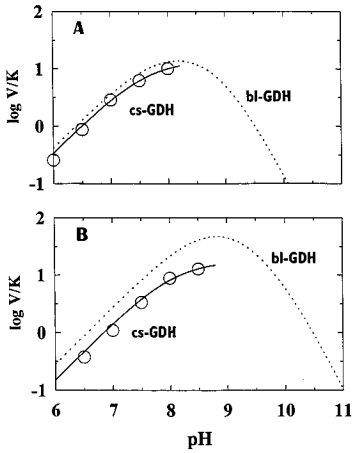


FIGURE 1: pH dependence of *V*/*K* values for bl and cs GDH at 10 °C (panel A) and at 25 °C (panel B). *V*/*K* measurements were done at [glutamate dehydrogenase] = 0.022−0.044 μM, [NAD] = 1 mM, and [L-glutamate] = 0.2−150 mM. The buffer contained 0.1 M potassium phosphate with either 10 mM MES for pH <7.6 or 10 mM CHES for pH >7.6. The open circles indicate the *V*/*K* results for cs GDH. The solid line represents the fit of the above data to a single pK_a equation with p*K* values of 7.6 at 25 °C and 8.1 at 10 °C. The dotted lines represent the *V*/*K* plot for bl GDH simulated with the data published by Rife and Cleland (1980b) (panel A) and Singh et al. (1993) (panel B), respectively. The *V*/*K* values for bl GDH in both panels have been divided by a factor of 1000 to permit easier visual comparison.

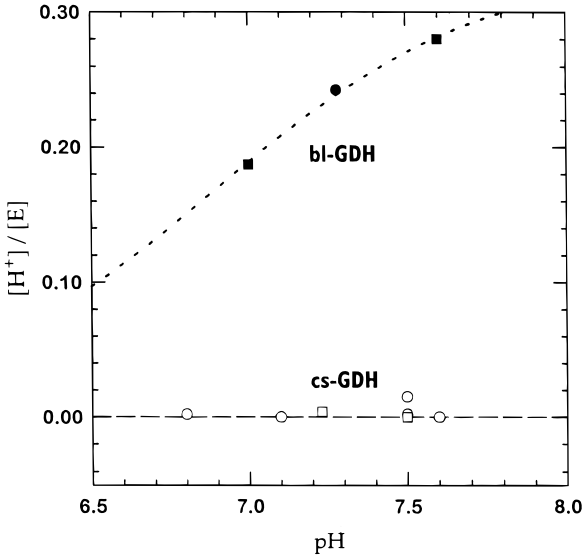


FIGURE 2: pH dependence of the number of protons released per mole of enzyme during the formation of the bl and cs GDH−NADPH−isophthalate complexes. The open and closed circles represent the data obtained by pH-stat back-titration method for cs GDH and bl GDH, respectively. The open and closed squares represent the data obtained from conventional spectrophotometric static experiments. The reaction conditions used for cs GDH and bl GDH were cs GDH = 80 mM, NAD = 380 μM, and isophthalic acid = 2 mM in 0.5 mM potassium phosphate and 0.1 M sodium sulfate buffer. For bl GDH, the conditions were bl GDH = 45 μM, $NADP^+$ = 380 μM, and isophthalic acid = 2 mM. The reactions were carried out at 25 °C for both cases.

atmosphere as described previously (Fisher et al., 1986). The static indicator dye experiments were carried out in a HP-8450A diode array spectrophotometer using phenol red as the indicator. The solutions containing the substrate, the coenzyme, and the dye on both sides were first balanced in the spectrophotometer, and then a small volume of the enzyme solution was added. To compensate for the enzyme, an equal volume of buffer was added to the reference cell. The difference in absorbance was converted into moles of H^+ produced per mole of enzyme using a standard curve obtained by titrating the indicator-containing solution with standardized HCl.

The stopped-flow experiments were carried out in either a Hi-Tech SF-51 or an Applied Photophysics SX-17MV stopped-flow instrument equipped with dual photomultipliers and interfaced to an IBM compatible PC. After subtraction of baselines and averaging of 20−30 individual scans, the absorbance signals at 340 and 560 nm were converted to concentrations of product−complex produced and protons released, respectively, as described earlier (Fisher et al., 1988).

RESULTS

The pH dependence of *V*/*K* for the cs GDH catalyzed reaction is shown in Figure 1 along with its previously determined equivalent in the bl GDH catalyzed reaction. It can be seen that the same p*K* value occurs in both enzyme reactions. For bl GDH it has been firmly established that this p*K* is in fact that of lysine 126 and is characterized by its ability to form a stable complex with pyridoxal phosphate at low pH (Smith et al., 1970; Brown et al., 1973; Rife & Cleland, 1980b). Lilley and Engel (1988, 1992) showed that, in a highly analogous sequence in cs GDH, lysine 125 also forms an intermediate with pyridoxal phosphate at low pH, and the crystal structure of cs GDH described by the Sheffield group (Baker et al., 1992; Stillman et al., 1992) clearly shows that the γ-amino group of lysine 125 is in close proximity to the α-carbon atom of the bound substrate. Thus, the finding shown in Figure 1 of the occurrence of the same p*K* in the *V*/*K* plots of the two enzymes was to be expected. It should be noted, however, that a p*K* obtained from a *V*/*K* plot indicates only that such a protonic event must occur *somewhere* in the sequence that begins with the binding of L-glutamate and terminates with the release of the first product (NH_3, in this case).

A plot of the number of protons released per mole of enzyme vs pH involved in the formation of the enzyme−NAD(P)−isophthalate dead-end inhibitor complex as determined by two different physical measurements is shown in Figure 2. It can be seen that bl GDH shows the same behavior in this case as previously reported for the formation of the enzyme−NADPH (ER) binary complex (Fisher et al., 1986)—the destabilization of a proton from a group now well established as lysine 126 from its abnormally large $\Delta H°$ and interpreted as reflecting the closing of the active site cleft (evident from the X-ray crystal structural work). Figure 2, however, shows that no protonic traffic between the cs enzyme and the solvent occurs on formation of its corresponding ternary complex, even though we have found isophthalate to be an effective and fully competitive inhibitor for that enzyme (data not shown). Furthermore, preliminary isothermal titration studies of the formation of cs GDH stable complexes show thermodynamic properties which are similar to those which accompany the active-cleft-closing event ascribed to the $\Delta C_p°$ effects observed in the bl GDH case.

To explore the nature of the paradox presented by the results described above, we turn to transient-state kinetic approaches. The rection time courses of proton release and of reduced-coenzyme product formation (measured simul-

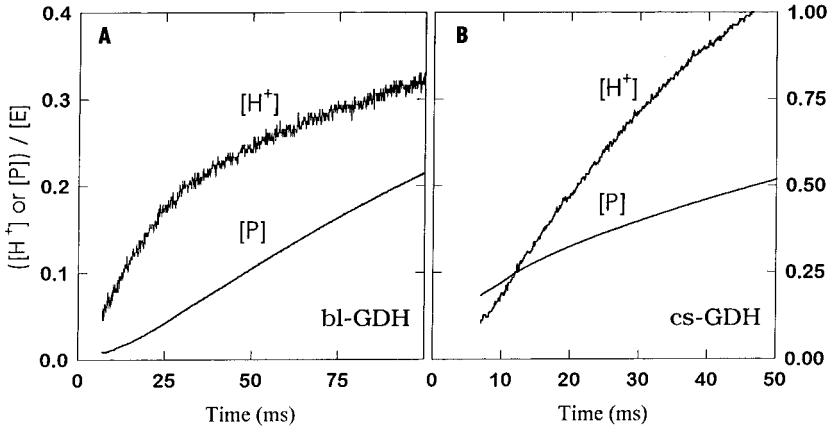


FIGURE 3: Reaction time courses of proton release and of reduced coenzyme product formation catalyzed by bl and cs GDH at pH 7.6, 10 °C. (A) bl GDH = 45 μM, $NADP^+$ = 380 μM, and L-glutamate = 45 mM. The dashed curve corresponds to the proton release whereas the solid curve corresponds to the product formation. (B) cs GDH = 20 μM, NAD = 380 μM, and L-glutamate = 45 mM. The dashed and solid curves correspond to the proton and product formulation, respectively.

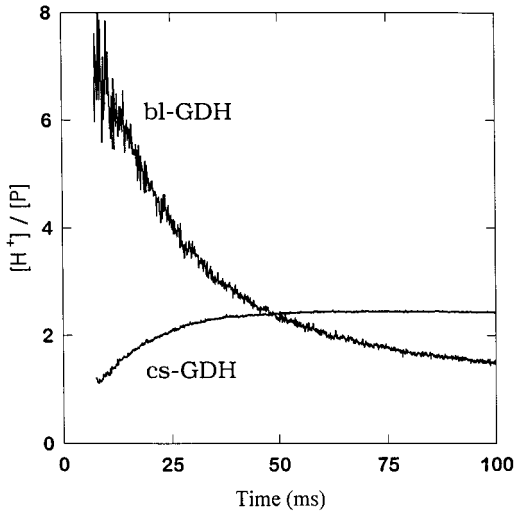


FIGURE 4: Proton/product ratio time courses calculated from the data in Figure 3. The dashed line corresponds to the $[H^+]/[P]$ (mol/mol) ratio for cs GDH whereas the solid curve represents the ratio for the bl GDH reaction.

taneously in indicator−dye stopped-flow experiments) are shown in panels A and B of Figure 3 for bl and cs GDH, respectively.[2] While the bl enzyme shows an immediate proton burst accompanying a pronounced lag in reduced coenzyme−product formation, the cs enzyme appears to show quite the opposite behavior; product formation is rapid and immediate (much of the burst occurring in the experimental dead time), while proton release seems to lag behind. These effects are shown more clearly in Figure 4, in which the molar ratio of protons released to product formed shows a rapidly *falling* value for bl GDH in contrast to the slowly *rising* value for cs GDH.

To confirm the inference from the spectroscopic kinetic data that in the cs GDH reaction the proton release step *follows* the hydride transfer step (the opposite order of events to those which occur in the bl GDH reaction), we have carried out transient-state kinetic isotope effect (KIE) studies of the proton release time courses (again using the indicator−dye stopped-flow technique). The proton release time courses of the reaction using L-[α-^{1}H]- and L-[α-^{2}H]glutamate for each enzyme are shown in panels A and B of Figure 5. In both panels A and B the experimental data for the unlabeled (H) and the α-deuterio-L-glutamate reactions are shown in thick lines in the upper panel of the figure. The best single-term or two-term exponential fits to the data are shown by the corresponding thin lines. The time courses of the deviations of the data from the thin smoothing curves are shown in the lower panel in each case.[3] The corresponding KIE's for both enzymes are shown in Figure 6. We have shown that, in a sequential reaction, a KIE significantly greater than 1 that decreases with time indicates that the signal being measured arises from an event which follows the isotope-sensitive step, while a rising KIE indicates that the signal being measured reflects an event which precedes the isotope-sensitive step.[3] The opposite time dependences of the proton KIE's shown in Figure 6 reinforce the conclusion reached from the other studies described above: the specific proton release step and the hydride transfer step do indeed occur in a different order in these two enzyme-catalyzed reactions, despite the fact that in all other respects they give every indication that their chemical mechanisms are identical.

DISCUSSION

To understand the basis of the "step-reversal" phenomenon demonstrated by the experimental results described thus far, it is necessary to consider the currently known sequence of chemical events that occurs in the reaction, the series of individual enzyme complexes which accomplish those chemical changes, and the clues from X-ray crystal structure studies which reveal at least some of the specific functional group interactions between the enzyme and the substrate which define the nature of the various complexes.

[2] The determination of the precise zero A_{560} value presents some difficulty due to the extreme sensitivity of the signal in such weakly buffered solutions. We have found that the average of the unmixed E−NAD and the E−L-glutamate baselines using the identical solutions mixed in the experimental shots provides the most valid zero-time value for the proton signal. The curves shown in Figure 3 have been corrected in this way.

[3] We have demonstrated and discussed this phenomenon in our previous paper (Saha et al., 1994). A full theoretical treatment of such transient-state kinetic isotope effects, and an experimental demonstration of their existence along with the process of obtaining $KIE_{obs}(t)$ from experimental data, is presented in the preceding paper (Fisher & Saha, 1996).

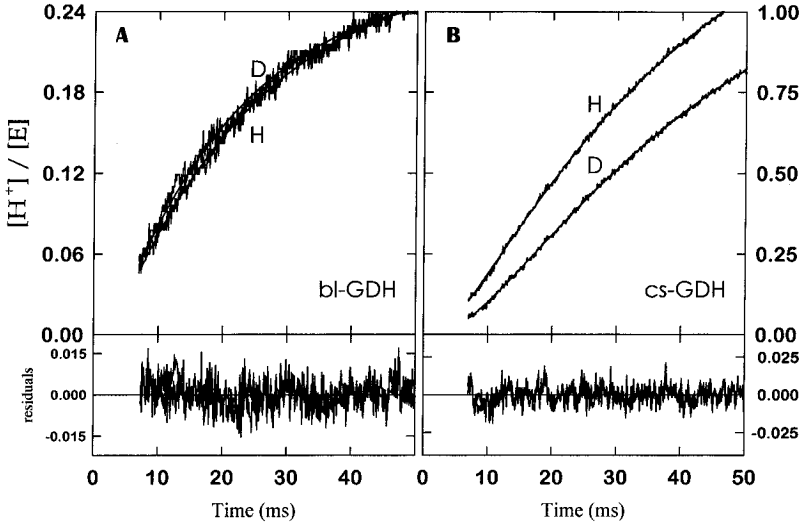


FIGURE 5: Proton release time courses for the bl and cs enzyme catalyzed reactions of α-protio- and α-deuterio-L-glutamate. The thick lines in all four panels are the experimental data for the unsubstituted (H) and the isotopically substituted (D) reactions for each enzyme. Zero-time intercepts have been subtracted from each curve for reasons discussed in footnote 1 of the accompanying paper. The thin lines are smoothing functions. The lower panels show the deviations on an expanded scale, as described in the text.

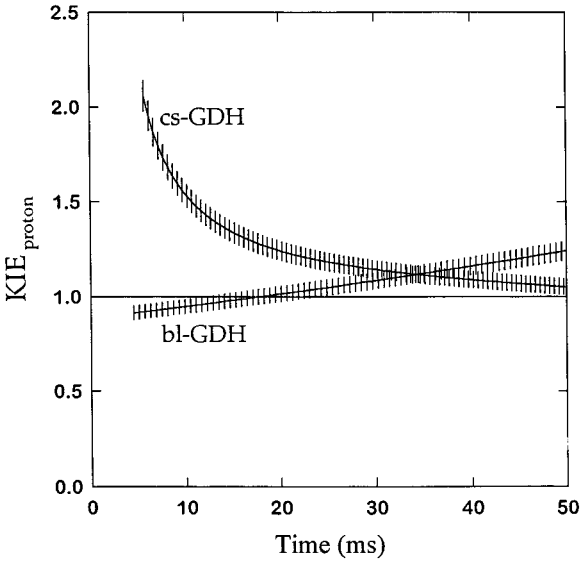


FIGURE 6: Time courses of KIE_{obs} for the reactions catalyzed by cs GDH and by bl GDH. The vertical marks on each curve represent the estimated error calculated as described in the preceding paper (Fisher & Saha, 1996).

The sequence of complexes shown in Scheme 1 have been shown to occur in the bl GDH catalyzed reaction (Saha et al., 1994).

The early events in the forward reaction can be described in terms of the sequence of chemical bond changes that occur in the α-carbon atom of the substrate as shown in Scheme 2 (Singh et al., 1993). The assignment of intermediate substrate structures to specific enzyme complexes for the bl GDH catalyzed reaction is shown below the scheme.

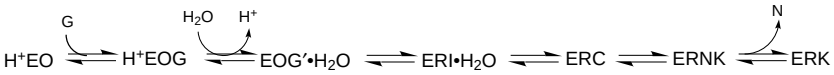
The functional group interactions inferred from the X-ray crystal structure studies of the cs GDH E−O and E−G complexes are shown schematically in Figure 7. The five protein functional groups of cs GDH whose spatial locations precisely fit the chemical catalysis requirements are all conserved in bl GDH as are eight other groups listed in the legend to Figure 6, whose function is to define the substrate binding pocket.

Some years ago it was shown that only compounds that had the equivalent of two carboxylate groups separated by the same negative charge distance of L-glutamate itself (7.45 Å) were strict competitive inhibitors of the bl GDH catalyzed forward reaction (Caughey et al., 1957). Isophthalate, a planar molecule whose interproton charge distance can be accurately measured, was found to be one of the tightest binders of this group. The finding reported here, that isophthalate is also a tight competitive inhibitor of the corresponding cs GDH reaction, establishes the fact that the distance between Lys 90 and Lys 114 in bl GDH must be identical to that between Lys 89 and Lys 113 in cs GDH. We have also reported here that the only *V*/*K* p*K* observed for cs GDH is the same as that which appears in the equivalent *V*/*K* profile of bl GDH. We have previously assigned that p*K* to the abnormal Lys 126 of bl GDH and [following the suggestion of Rife and Cleland (1980b)] have ascribed its function as one of positioning a bound H_2O molecule for the chemically required nucleophilic attack on the α-carbon atom of the bound substrate (Singh et al., 1993). The cs GDH structure shows that the corresponding abnormal Lys 125 is in fact ideally located for just such a function in that enzyme also.[4]

We have previously shown that a bound α-iminoglutarate intermediate must occur in the bl GDH reaction (Fisher et al., 1984), and since it is unlikely that such a hydride transfer could occur starting from the protonated amino form of L-glutamate, that amino proton must be lost prior to hydride transfer. On that basis, Rife and Cleland (1980b) postulated that an enzyme carboxylate group must abstract that proton in the initial step after binding. Our proton release studies (Singh et al., 1993) showed the absence of the release of such a proton to the solvent. The location of Asp 165 in the cs GDH structure is ideal for such a proton abstraction, agrees with our negative proton release-to-solvent experi-

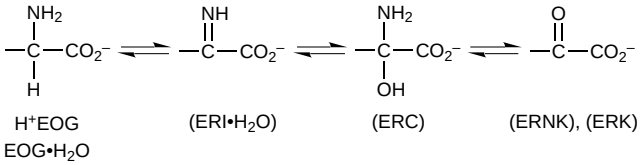
[4] Brunhuber et al. (1994) have noted that a "GGGK" motif is widely conserved among the α-amino acid dehydrogenases as a group. The "GGAK" sequence (which includes Lys 126) is itself highly conserved among the mammalian GDH's (Smith et al., 1975) and represents a very close analogy to this motif.

Scheme 1[a]



[a] E is bl GDH, O is NADP (oxidized coenzyme), R is NADPH (reduced coenzyme), G is L-glutamate, I is α-iminoglutarate, C is α-carbinolamine (α-hydroxy-α-iminoglutarate), K is α-ketoglutarate, and N is ammonia.

Scheme 2



mental findings on cs GDH, and, as Stillman et al. (1992, 1993) have noted, agrees with the step postulated by Rife and Cleland (1980b) for the bl GDH reaction.[5]

These structure−function correlations make it clear that the same set of principal functional groups is involved in both the bl and cs GDH reactions, that these functional groups have the same relative locations on the peptide chains in each species, and that, *at the time of specific chemical bond changes*, they occupy closely corresponding geometrical positions.

Given this near identity of both the chemical reaction sequences and active site functional group geometry, how then can we account for the reverse order of the hydride transfer and the proton release steps of the bl and cs GDH reactions?

In a previous paper we have postulated a mechanism for the bl GDH reaction that involves the closing of the active site cleft, an event which provides a hydrophobic environment suitable for the subsequent hydride transfer step (Singh et al., 1993). This closure, in the bl GDH case, appears to require the removal or neutralization of the four positive charges carried on the three conserved enzyme lysine ϵ-amino groups and the substrate α-amino group. The latter charge is removed by transfer to Asp 165 (as we have already noted) while the substrate carboxylate groups form salt bridges, neutralizing the charge on two of the three lysine groups. The spontaneous loss of the remaining proton as an H^+ transferred to the solvent from the abnormal Lys 126 then triggers three rapidly ensuing events: the cleft closes, a single water molecule becomes hydrogen bonded to the $-NH_2$ group of Lys 126, the bulk solvent is expelled, and the α-hydrogen atom of the substrate is brought close enough to the C-4 atom of the bound nicotinamide ring to permit hydride transfer. We have recently shown that the attainment of this necessary closure in distance is signaled by the appearance of a pre-hydride weakly absorbing but highly fluorescent charge-transfer complex (Saha et al., 1994). The phenomenon is due to the apposition of the unshared electron pair of the unprotonated substrate α-amino nitrogen atom above and quite close to the NADP nicotinamide ring. In the bl GDH reaction both the proton release step and the appearance of the charge-transfer signal appear very rapidly

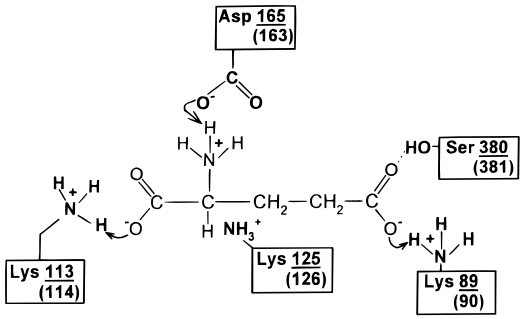


FIGURE 7: Conservation of enzyme−substrate functional group interactions between cs GDH and bl GDH. For each enzyme functional group the underlined number in the box refers to the position of that residue in the cs GDH sequence (Baker et al., 1992). The number enclosed in parentheses refers to the position of that residue in the bl GDH sequence (Smith et al., 1995). The functional group interactions indicated are those inferred from the cs GDH−L-glutamate complex crystal structure (Stillman et al., 1993). Not shown are five specific Gly residues, a Val, and an asparagine residue which line the active site pocket and define the substrate binding site in the cs GDH structure. All eight of these residues are conserved, occupying closely corresponding positions in the bl GDH sequence.

[5] While there is no question that the substrate molecule of L-glutamate must lose a proton from its α-amino group prior to the hydride transfer step, some confusion has existed as to precisely where in the reaction course this event actually occurs. Rife and Cleland (1980b) originally suggested that the substrate α-amino hydrogen is transferred to an enzyme carboxyl group, pK = 5.2, as the first step after the binding of the amino protonated form of L-glutamate in the forward reaction. On the basis of a study of the pH dependence of the oxidation of L-proline by bl GDH, Srinivasan and Fisher (1985) concluded that the protonic assignments of Rife and Cleland could be interpreted in an opposite fashion (with the unprotonated form of L-glutamate binding to the E−O complex). Singh et al. (1993), observing that the formation of the E−O−isophthalate complex produced a proton release equivalent to that of the L-glutamate reaction, concluded (correctly) that the ascending pK observed in both steady- and transient-state studies could not arise from the L-glutamate amino group. They also maintained (incorrectly) that glutamate binds with its amino group unprotonated. If this were so, however, V/K and transient-state studies would both show the release of *two* protons rather than the single one observed. The X-ray crystal structure of the cs GDH−G complex clearly shows a conserved aspartate residue in the (Baker et al., 1992) precise position required to abstract a proton from the substrate α-amino group. Such an internal proton transfer need not necessarily release a proton to the solvent at this point in the reaction sequence. We now conclude that the substrate binding event does indeed proceed precisely as Rife and Cleland originally predicted.

[6] There is a possible alternative explanation for the delayed release of the proton in the cs GDH catalyzed reaction which we have explained here in terms of changes in the cleft-opening sequence. It is at least conceivable that the critical proton ionization of the conserved abnormal lysine residue (125) actually does occur before the hydride transfer in both enzyme reactions but that in the cs GDH case that proton is transferred internally to another enzyme residue rather than directly to the solvent as in bl GDH. This proton would then be released to the solvent well after completion of the hydride transfer step, again by a late cleft-opening event. While we cannot formally exclude this possibility, there are several facts which are difficult to explain on this basis. First of all, an examination of the crystal structure of the cleft surfaces has failed to reveal any residue which has both the chemical structure and geometric position to serve such a function. A second fact that is difficult to explain by the internal transfer hypothesis is the close agreement of the V/K pK values and ΔH_{ion}'s of bl and cs GDH. Finally (in work to be described elsewhere) we have found that the late transient proton release demonstrated here has a pK and ΔH_{ion} that agree very well with the corresponding V/K function of the cs enzyme. We believe, therefore, that the cleft-opening phase-shift mechanism suggested in the text is better supported by the facts than the alternative internal transfer hypothesis.

and at similar rates ($t \cong 5-10$ ms). In the much more rapid cs GDH reaction the charge-transfer phenomenon appears to be completely formed in the instrumental dead time, while the proton release step is delayed until after the hydride transfer has occurred. It can be seen, however, from Scheme 2 that no necessary violation of the sequence of strictly chemical events is caused by this discrepancy. The presence of the lysine-bound water molecule is not actually required until after hydride transfer and the consequent formation of the substrate imine. In the bl GDH mechanism we have ascribed the early requirement for the presence of the enzyme-bound water molecule to the lack of access to solvent of the closed active site in later steps. Recognizing these facts, we now must ascribe the difference in the mechanistic time courses of the two enzyme forms to differences in the sequence of cleft-opening and -closing events in the two enzymes rather than to any fundamental changes in the sequence of chemical events they catalyze.[6]

While we cannot at this time completely describe the cleft-opening and -closure sequence of the cs GDH reaction, there are a few solidly established facts which appear to support our conclusion. The crystal structure results of Baker et al. (1992) show that, in the free cs GDH molecule, the cleft is open. Formation of the E−NAD complex causes no alteration of conformation. Later crystal structure work from that same laboratory (Stillman et al., 1993), however, showed that the E−G complex is closed at least to a degree so that the bulk solvent is now expelled. In the case of bl GDH, thermodynamic measurements of the formation of the E−NADPH binary complex indicate that the free enzyme is closed to the extent of 90% at pH 7.6, 25 °C (Fisher et al., 1980; Subramanian et al., 1978). These findings, then, do support the notion of some discordance in the number and/or timing of conformational changes between the two GDH-catalyzed reactions.

Finally, we may note that we have shown some thermodynamic evidence that these energetics of the conformational changes observed between the various complexes of bl GDH are complementary to those of the free energy profile of the chemical component, in very good agreement with the Lumry compensation theory of enzyme catalysis (Lumry, 1986).

It is reasonable to assume that this modulation of a fixed detailed chemical sequence for two closely homologous enzymes is needed to meet the differing metabolic roles played by the same enzyme in different organisms (and conceivably between isoenzymes in different tissues of the same organism). *C. symbiosum* is an organism which uses L-glutamate as its sole carbon source. The 20-fold faster transient burst rate and the much faster enzyme−product complex release rate of its form of GDH are thus suited to such a role. On the other hand, while the precise metabolic role of bl GDH is still a matter of dispute, this enzyme is known to provide the only *de novo* source of α-amino acids in mammals, requiring its operation in the reverse mode. The observations reported here may possibly be expected to reflect such circumstances. If so, it would be quite surprising if such behavior patterns were limited to this single enzyme.

ACKNOWLEDGMENT

We thank David W. Rice of the University of Sheffield for sharing with us the unpublished X-ray crystal structure coordinates of cs GDH.

REFERENCES

Baker, P. J., Britton, K. L., Engel, P. C., Farrants, G. W., Lilley, K. S., Rice, D. W., & Stillman, T. J. (1992) *Proteins: Struct., Funct., Genet. 12*, 75−86.

Brown, A., Culver, J. M., & Fisher, H. F. (1973) *Biochemistry 12*, 4367−4373.

Brunhuber, N. M. W., & Blanchard, J. S. (1994) *Crit. Rev. Biochem. Mol. Biol. 29*, 415−467.

Brunhuber, N. M. W., Banergee, A., Jacobs, W. R., Jr., & Blanchard, J. S. (1994) *J. Biol. Chem. 269*, 16203−16211.

Caughey, W. S., Similey, J. D., & Hellerman, L., (1957) *J. Biol. Chem. 224*, 591.

Fisher, H. F. (1988) *Adv. Enzymol. 61*, 1−46.

Fisher, H. F., & Viswanathan, T. S. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 2747−2751.

Fisher, H. F., & Saha, S. K. (1996) *Biochemistry 35*, 83−88.

Fisher, H. F., Medary, R. T., Wykes, E. J., & Wolf, C. S. (1984) *J. Biol. Chem. 259*, 4105−4110.

Fisher, H. F., Maniscalco, S., Wolfe, C., & Srinivasan, R. (1986) *Biochemistry 25*, 2910−2915.

Fisher, H. F., Maniscalco, S., Singh, N., & Adediran, S. A. (1988) *J. Biol. Chem. 263*, 11704−11710.

Fisher, H. F., Maniscalco, S., Singh, N., Mehrotra, R. N., & Srinivasan, R. (1992) *Biochim. Biophys. Acta 1119*, 52−56.

Lilley, K. S., & Engel, P. C. (1988) *Biochem. Soc. Trans. 16*, 875−876.

Lilley, K. S., & Engel, P. C. (1992) *Eur. J. Biochem. 207*, 533−540.

Lumry, R. (1986) in *The Fluctuating Enzyme* (Welch, G., Ed.) p 4, Wiley-Interscience, New York.

Rice, D. W., Hornby, D. P., & Engel, P. C. (1985) *J. Mol. Biol. 181*, 147−149.

Rife, J. E., & Cleland, W. W. (1980a) *Biochemistry 19*, 2321−2327.

Rife, J. E., & Cleland, W. W. (1980b) *Biochemistry 19*, 2328−2333.

Saha, S. K., Maniscalco, S. J., Singh, N., & Fisher, H. F. (1994) *J. Biol. Chem. 269*, 29592−29597.

Singh, N., Maniscalco, S. J., & Fisher, H. F. (1993) *J. Biol. Chem. 268*, 21−28.

Smith, E. L., Landon, M., Piszkiewicz, D., Brattin, W. J., Langley, T. J., & Melamed, M. D. (1970) *Proc. Natl. Acad. Sci. U.S.A. 67*, 724−730.

Smith, E. L., Austen, B. M., Blumenthal, K. M., & Nyc, J. F. (1975) in *The Enzymes* (Boyer, P. D., Ed.) Vol. 11, pp 293−367, Academic Press, New York.

Srinivasan, R., & Fisher, H. F. (1985) *Biochemistry 24*, 618−622.

Srinivasan, R., Viswanathan, T. S., & Fisher, H. F. (1988) *J. Biol. Chem. 263*, 2304−2308.

Stillman, T. J., Baker, P. J., Britton, K. L., Rice, D. W., & Rodgers, H. F. (1992) *J. Mol. Biol. 224*, 1181−1184.

Stillman, T. J., Baker, P. J., Britton, K. L., & Rice, D. W. (1993) *J. Mol. Biol. 234*, 1131−1139.

Subramanian, S., Stickel, D. C., Colen, A. H., & Fisher, H. F. (1978) *J. Biol. Chem. 253*, 9370−8374.

BI9509098