Renaturation of Formiminotransferase–Cyclodeaminase from Guanidine Hydrochloride. Quaternary Structure Requirements for the Activities and Polyglutamate Specificity†

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ABSTRACT: Formiminotransferase–cyclodeaminase denatured in 6 M guanidine hydrochloride (Gdn·HCl) refolds and reassembles to the native octameric structure upon dilution into buffer. Both enzymic activities are recovered to greater than 90%, and the renatured enzyme "channels" the formiminotetrahydropteroylpentaglutamate intermediate. Under conditions where the two activities are recovered simultaneously, the rate-limiting step in reactivation is first order with respect to protein, with $k = 1.9 \times 10^{-5}$ s^{-1} at 22 °C and $\Delta E^{\ddagger} \simeq 15$ kcal mol^{-1}. In the presence of 1.5 M urea, renaturation is arrested at the level of dimers having only transferase activity. Subsequent dialysis to remove the urea leads to recovery of deaminase activity and formation of octamer. Kinetic studies with mono- and pentaglutamate derivatives of the folate substrates demonstrated that native and renatured enzyme as well as deaminase-active dimers [Findlay, W. A., & MacKenzie, R. E (1987) *Biochemistry 26*, 1948–1954] have much higher affinity for polyglutamate substrates, while the transferase-active dimers do not. These results indicate that the transferase activity is associated with one type of subunit–subunit interaction in the native tetramer of dimers and that the polyglutamate binding site and the deaminase activity are associated with the other interface. A dimeric transferase-active fragment generated by limited proteolysis of the native enzyme can also be renatured from 6 M Gdn·HCl, confirming that it is an independently folding domain capable of reforming one type of subunit interaction.

The bifunctional enzyme formiminotetrahydrofolate:glutamate formiminotransferase (EC 2.1.2.5)–formiminotetrahydrofolate cyclodeaminase (EC 4.3.1.4) isolated from porcine liver is composed of eight identical subunits (M_r 62 000) arranged in a ring (Beaudet & MacKenzie, 1976). This unusual octameric quaternary structure consists of two types of subunit–subunit interaction (MacKenzie et al., 1980) forming a tetramer of dimers arrangement. The enzyme catalyzes two sequential reactions involved in histidine degradation in mammalian liver and has much greater affinity for the naturally occurring polyglutamate derivatives of folates as substrates.

formiminoglutamate + $H_4PteGlu_n$ ⇌ formimino-$H_4PteGlu_n$ + glutamate

formimino-$H_4PteGlu_n$ ⇌ 5,10-methenyl-$H_4PteGlu_n$ + NH_4^+

With the longer polyglutamates ($n \geq 4$), direct transfer of the formimino-$H_4PteGlu_n$[1] from the transferase to the deaminase catalytic site is observed, with complete "channeling" of the pentaglutamate (Paquin et al., 1985). A model has been proposed, with the intermediate "anchored" to the enzyme via noncovalent attachment to a polyglutamate binding site while the pteroyl moiety is transferred between the catalytic sites (MacKenzie & Baugh, 1980).

Only four high-affinity sites for binding tetrahydropteroylpolyglutamates were observed per octamer (Paquin et al., 1985), and kinetic evidence suggested a single polyglutamate site per pair of transferase–deaminase catalytic sites. These results raised the possibility that the basic functional unit of the enzyme is a dimer, which would have both activities and the ability to channel polyglutamate substrate between the active sites. However, dissociation of formiminotransferase–cyclodeaminase by low concentrations of urea under different conditions generated monofunctional dimers with either transferase or deaminase activity and led to the suggestion that these dimers isolate different subunit–subunit interactions (Findlay & MacKenzie, 1987). This would mean that the dimer could not be the basic functional unit of this enzyme and that the activities require the integrity of alternate subunit interfaces in the native octamer.

In this paper we report conditions under which formiminotransferase–cyclodeaminase can be renatured from Gdn·HCl. Isolation and characterization of dimeric intermediates further defined the relationship between the enzymic activities and quaternary structure and established the location of the polyglutamate binding site.

Materials and Methods

The formiminotransferase–cyclodeaminase enzyme was prepared and assayed as described previously (Drury et al., 1975) and stored at −20 °C as a stock solution of 2.0–2.5 mg/mL in 0.1 M potassium phosphate (pH 7.3), 40% glycerol, and 35 mM 2-mercaptoethanol. The purified enzyme had a transferase specific activity of 41 μmol min^{-1} mg^{-1} and yielded a single band on SDS–PAGE. The transferase-active fragment was generated by chymotryptic cleavage of the enzyme in the

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[1] Abbreviations: $H_4PteGlu_n$, tetrahydropteroylpolyglutamate with a total of n glutamates; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; Gdn·HCl, guanidine hydrochloride; NHCH-$H_4PteGlu_n$, formiminotetrahydropteroylpolyglutamate.

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presence of folate and purified by gel filtration (MacKenzie et al., 1980). It was then concentrated 6-fold by dialysis in Spectra/Por 3 membrane tubing (Fisher Scientific) against 40% poly(ethylene glycol) 8000 (J. T. Baker Chemical Co.), 0.1 M potassium phosphate (pH 7.3), and 35 mM 2-mercaptoethanol. After a second dialysis into 40% glycerol, 0.1 M potassium phosphate (pH 7.3), and 35 mM 2-mercaptoethanol, it was stored at −20 °C as a stock solution of 0.75 mg/mL, with a specific activity of 31 μmol min^{-1} mg^{-1}. It also gave a single band on SDS–PAGE.

Ultrapure guanidine hydrochloride and urea were purchased from Canadian Scientific Products, folic acid and formiminoglutamate (hemibarium salt) were from Sigma Chemical Co., and bis(sulfosuccinimidyl) suberate and high-purity Triton X-100 were from Pierce Chemical Co. Pteroylpentaglutamate was purchased from Dr. B. Schircks Laboratories (Jona, Switzerland). (6*RS*)-H_4PteGlu was prepared chemically (Drury et al., 1975), and (6*S*)-H_4PteGlu and (6*S*)-H_4PteGlu$_5$ were prepared enzymatically (MacKenzie & Baugh, 1980). Other chemicals were reagent grade: Tween 80 and EDTA from Fisher Scientific, sodium and potassium phosphates from J. T. Baker Chemical Co., 2-mercaptoethanol from Eastman Kodak Company, and DTT from Boehringer-Mannheim.

Renaturation. Assays for the two enzyme activities and chemical cross-linking by bis(sulfosuccinimidyl) suberate were performed as previously described (Findlay & MacKenzie, 1987). The enzyme was denatured by a 4-fold dilution from the stock solution of 2.0–2.5 mg/mL into 8 M Gdn·HCl (final concentration 6 M) in 0.1 M potassium phosphate (pH 7.3), 1 mM DTT, and 1 mM EDTA. After 30-min incubation at room temperature, renaturation was accomplished by dilution of at least 100-fold into renaturation buffer containing 0.1 M potassium phosphate (pH 7.3), 40% glycerol, 1 mM DTT, 1 mM EDTA, and 0.1% Triton X-100 or 0.05% Tween 80, and the recovery of the two activities was followed with time. All renaturations were carried out at a final concentration of 0.06 M Gdn·HCl. Controls showed that native enzyme under the same conditions retained almost 100% activity in the presence of Tween 80 and over 90% activity in the presence of Triton X-100 for several days at room temperature. For renaturation of the transferase-active fragment the same procedure was used, starting with a stock solution of 0.75 mg/mL. To reduce sample volumes in the cross-linking experiments, only a 10-fold dilution into buffer was used, followed by dialysis against the same buffer.

The program KINFIT (Knack & Röhm, 1981) was used to determine initial velocities of reactivation under various conditions by performing either an initial velocity or a first-order fit of the data for the activity versus time profiles.

Fluorescence. A Perkin-Elmer LS-5 fluorescence spectrophotometer was used with excitation at 290 nm and emission scanned from 310 to 400 nm. Slit widths were 3 mm for excitation and 5 mm for emission, and samples were prepared exactly as in the renaturation experiments. Duplicate scans were averaged, and the background was subtracted.

Gel Filtration. A 48-mL column (1.6 × 24 cm) of AcA-34 (Pharmacia) was equilibrated with 3–4 column volumes of 0.1 M potassium phosphate (pH 7.3), 40% glycerol, 1 mM EDTA, 5 mM DTT, and 0.1% Triton X-100, containing 1.5 M urea when required. Samples of 0.5 mL of native enzyme in renaturation buffer containing 1.5 M urea, enzyme renatured in the presence of 1.5 M urea, and enzyme renatured in the absence of urea were applied separately and eluted at room temperature with the same buffer as used for equilibration, and 30-drop (0.47-mL) fractions were collected and assayed for transferase activity. Native enzyme and transferase fragment diluted in renaturation buffer were used as markers.

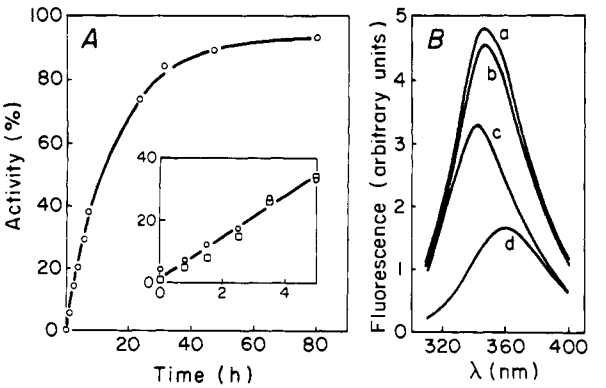


FIGURE 1: Renaturation of formiminotransferase–cyclodeaminase after dilution from 6 M Gdn·HCl. Final protein concentration was 76 nM subunit (4.7 μg/mL) in renaturation buffer (0.1 M potassium phosphate, 40% glycerol, 1 mM DTT, and 1 mM EDTA) containing 0.05% Tween 80 and 0.06 M Gdn·HCl. (A) Recovery of activity with time after dilution; expanded scale for the first 5 h is shown in the inset. Symbols: (○) percent of original transferase activity; (□) percent of original deaminase activity. (B) Spectrum of intrinsic tryptophan fluorescence of (a) native enzyme, (b) enzyme renatured for 48 h, (c) enzyme renatured for 5 min, and (d) enzyme in 6 M Gdn·HCl.

Kinetic Experiments. Transferase activity was assayed in 0.5 mL of 0.1 M potassium phosphate (pH 7.3), 5 mM formiminoglutamate, 35 mM 2-mercaptoethanol, and various concentrations of (6*S*)-H_4PteGlu$_n$ (n = 1 or 5), at 30 °C. The reaction was initiated by addition of enzyme, stopped after 3 min with 25 μL of 5 N HCl, and then heated in boiling water for 1 min and cooled on ice. For blanks the acid was added with the enzyme. A Beckman DU-7 spectrophotometer was used to measure the absorbance at 350 nm due to (6*R*)-5,10-methenyl-H_4PteGlu$_n$.

Deaminase activity was assayed in 0.5 mL of 0.1 M potassium phosphate (pH 7.3), 35 mM 2-mercaptoethanol, and various concentrations of (6*S*)-5-formimino-H_4PteGlu$_n$ (n = 1 or 5), prepared as reported previously (Paquin et al., 1985). After addition of enzyme, the absorbance at 355 nm was monitored by using the Beckman DU-7 spectrophotometer to observe the production of methenyl-H_4PteGlu$_n$ at 23 °C. For blanks, the rate was measured in the absence of enzyme.

After subtraction of the blanks, the data for both activities were fit to the Michaelis–Menten equation to determine values of K_m by use of the computer program KINFIT (Knack & Röhm, 1981) with an unweighted fit.

Channeling was monitored by following the time course of appearance of the products of the two activities at 30 °C for up to 2 min as reported previously (Paquin et al., 1985), with 0.5 mL of assay mix containing 0.1 M potassium phosphate (pH 7.3), 35 mM 2-mercaptoethanol, 5 mM formiminoglutamate, and 25 μM (6*S*)-H_4PteGlu$_5$.

RESULTS

Preliminary experiments to establish optimal conditions for renaturation of formiminotransferase–cyclodeaminase indicated that both 40% glycerol and a low concentration (≤0.1% w/v) of nonionic detergent (Tween 80 or Triton X-100) are required for complete renaturation of this enzyme. In the absence of detergent, less than 40% of the activities could be recovered.

Figure 1A shows the time course of recovery of the transferase activity after a 100-fold dilution into renaturation buffer [0.1 M potassium phosphate (pH 7.3), 40% glycerol, 1 mM DTT, and 1 mM EDTA] containing 0.05% Tween 80. The

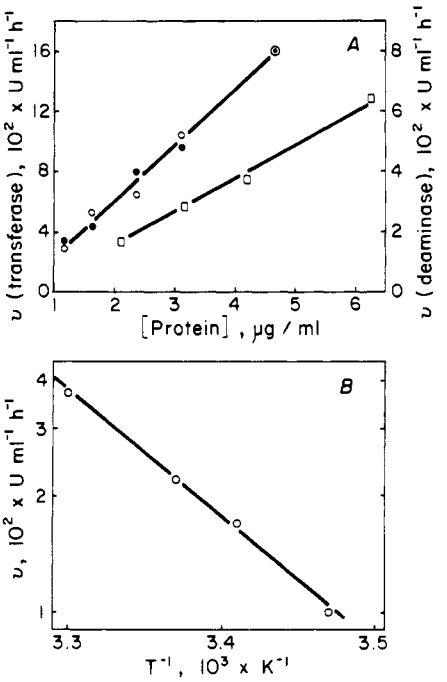


FIGURE 2: Effects of enzyme concentration and temperature on initial rates of reactivation (v) after dilution from 6 M Gdn·HCl into renaturation buffer containing 0.05% Tween 80. (A) Initial velocity values at different protein concentrations obtained by initial velocity fit of the transferase (○) and deaminase (□) reactivation profiles and by first-order fits of the transferase (●) profiles using the program KINFIT (Knack & Röhm, 1981). (B) Arrhenius plot of transferase reactivation at a protein concentration of 4.7 μg/mL. Initial velocity values were obtained as in (A).

renatured enzyme has over 90% of both activities after 48 h, and under these conditions the two activities are recovered simultaneously as shown in the inset in Figure 1A. Figure 1B shows that renatured enzyme has ~95% of the intrinsic tryptophan fluorescence of native enzyme and that denatured enzyme recovers ~70% of the native fluorescence in the first 5 min after dilution. The fluorescence intensity of the enzyme in 6 M Gdn·HCl is much lower, and the wavelength of maximum emission is shifted from 345 to 360 nm, consistent with unfolding of the polypeptide chain.

The rate-limiting step for reactivation under these conditions is a first-order process. The initial rate of reactivation of both the transferase and deaminase activities varies linearly with enzyme concentration as shown in Figure 2A, and the rate constant for reactivation is $(1.9 \pm 0.2) \times 10^{-5}$ s^{-1} at 22 °C. Figure 2B is an Arrhenius plot of the initial rate of transferase reactivation at temperatures from 15 to 30 °C. Since it is linear, a single step appears to be rate-limiting over this range of temperatures and the energy of activation is ~15 kcal mol^{-1}.

When Tween 80 is replaced by Triton X-100 in the renaturation buffer, the transferase and deaminase activities are recovered at different rates, as shown in Figure 3A. The transferase is reactivated somewhat more quickly than it is in the presence of Tween 80, but the deaminase is reactivated significantly more slowly. After 48 h, over 80% of both activities has been recovered (90% of control). Since we observe two different initial rates, there appear to be two rate-limiting steps during renaturation under these conditions, and reactivation of each activity is apparently first order (not shown).

The addition of 1.5 M urea to the renaturation buffer completely prevents recovery of the deaminase activity, while further increasing the initial rate of reactivation of the transferase (Figure 3B). However, the final extent of reactivation is less than 50% of the initial transferase activity, even

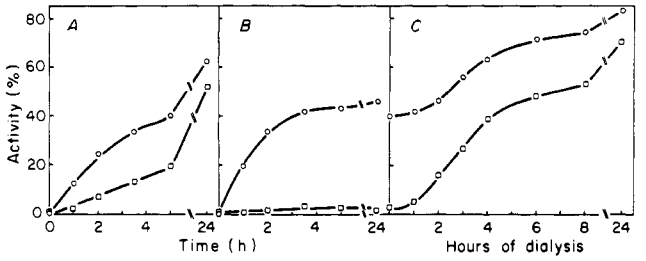


FIGURE 3: Effect of urea on reactivation of formiminotransferase–cyclodeaminase after dilution from 6 M Gdn·HCl into buffer containing Triton X-100. Final enzyme concentration was 100 nM subunit (6.25 μg/mL) in renaturation buffer containing 0.1% Triton X-100 and (A) no urea or (B) 1.5 M urea, or (C) during dialysis after 20-h renaturation in 1.5 M urea. Symbols: (○) percent of original transferase activity; (□) percent of original deaminase activity.

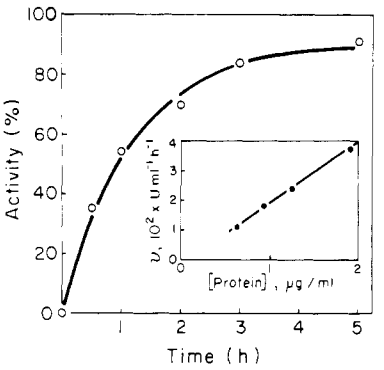


FIGURE 4: Reactivation of the proteolytically derived transferase fragment after dilution from 6 M Gdn·HCl. Final protein concentration was 1.88 μg/mL fragment (48 nM monomer) in renaturation buffer containing 0.1% Triton X-100. Inset shows initial velocity values obtained by computer fit of reactivation profiles at different protein concentrations using the program KINFIT (Knack & Röhm, 1981).

after 24 h. Dialysis against renaturation buffer to remove the urea leads to reactivation of the deaminase as well as an increase in transferase activity, as shown in Figure 3C. After 48 h of dialysis, over 85% of both activities is recovered (not shown).

As reported previously (MacKenzie et al., 1980), limited proteolysis of the enzyme by chymotrypsin in the presence of folic acid generates a dimeric transferase-active fragment (2 × 39 kDa). This fragment can also be completely renatured after denaturation in 6 M Gdn·HCl, under the same conditions as the native enzyme. Figure 4 shows recovery of the transferase activity with time for the dimeric fragment, and the inset illustrates the initial rate of reactivation at different protein concentrations. The rate-limiting step in renaturation is apparently first order for the fragment also and has a rate constant $k = (2.4 \pm 0.3) \times 10^{-4}$ s^{-1}, which is about 5-fold faster than the value obtained for the native enzyme (Figure 3A). It is also slightly faster (≤2-fold) than that for reactivation of the transferase alone in 1.5 M urea (Figure 3B).

Cross-linking of the enzyme with the bifunctional reagent bis(sulfosuccinimidyl) suberate under the various conditions shows that octameric structure is regained after 48 h of renaturation in the presence of either Tween 80 or Triton X-100, and after 24 h of renaturation in the presence of 1.5 M urea, followed by removal of the urea by dialysis for 48 h (Figure 5A). Cross-linking of the renatured proteolytic fragment (Figure 5B) shows that it recovers its dimeric structure as it is reactivated.

Cross-linking of the enzyme renatured in 1.5 M urea showed some dimer and no higher forms (not shown), and gel filtration on an AcA-34 column was used to confirm the size of the active species. As shown in Figure 6, completely renatured

Table I: Values of K_m for the Folate Substrates[a] of Native and Renatured Enzyme and of Monofunctional Dimers

substrate	previous value[b] (μM)	control enzyme (μM)	renatured enzyme (μM)	dimers	
				transferase (μM)	deaminase (μM)
(6*S*)-H_4PteGlu	48 ± 14	43 ± 21	21 ± 5	27 ± 1	
(6*S*)-H_4PteGlu$_5$	0.7 ± 0.3	2.1 ± 1.7	0.9 ± 0.2	70 ± 30	
(6*S*)-5-NHCH-H_4PteGlu	149 ± 14	114 ± 26	52 ± 12		79 ± 14
(6*S*)-5-NHCH-H_4PteGlu$_5$	2.0 ± 0.7	3.4 ± 0.5	2.0 ± 0.6		17 ± 7

[a] Values are expressed as averages ± standard deviations for three separate determinations. [b] From Paquin et al. (1985); determined for native enzyme.

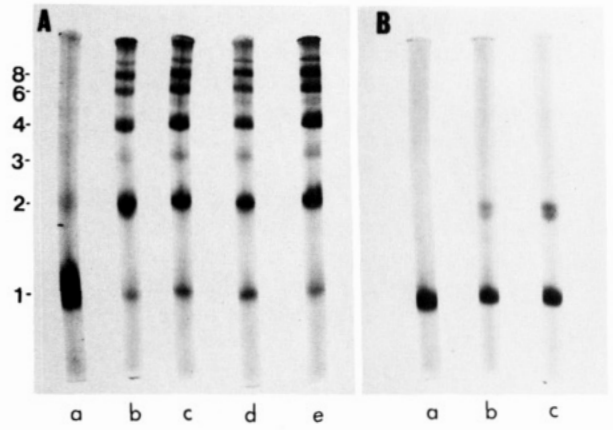
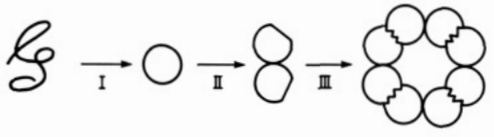


FIGURE 5: SDS–PAGE analysis (Weber & Osborn, 1969) of formiminotransferase–cyclodeaminase and the proteolytically derived fragment cross-linked after renaturation under different conditions (as described under Materials and Methods) in the presence of 0.1% Triton X-100 unless otherwise indicated. (A) Each 4% polyacrylamide tube gel contains 20 μg of formiminotransferase–cyclodeaminase cross-linked with 0.55 mg/mL bis(sulfosuccinimidyl) suberate (a) at zero time, (b) after 48 h in the presence of 0.05% of Tween 80 (no Triton X-100), (c) after 48 h, or (d) after dialysis for 48 h following 20-h renaturation in 1.5 M urea or (e) native enzyme diluted in renaturation buffer. (B) Each 5% polyacrylamide tube gel contains 10 μg of fragment cross-linked with 1.5 mg/mL bis(sulfosuccinimidyl) suberate (a) at zero time or (b) after 6 h or (c) fragment diluted in renaturation buffer (not denatured). The number of subunits in each cross-linked species is indicated.

Scheme I



enzyme and native enzyme in the presence and absence of 1.5 M urea elute at the same position, confirming that they are all octameric. Enzyme renatured in the presence of 1.5 M urea appears to be dimeric since it elutes much later than the octamer but earlier than the transferase fragment (M_r ~ 80 000). It appears that the presence of 1.5 M urea during renaturation prevents formation of the second subunit–subunit association present in native enzyme, thereby generating a dimeric transferase-active species. This may be an active intermediate in the complete renaturation process, as shown in Scheme I. Refolding of the denatured polypeptides to form structured monomers is indicated by step I, followed by reassociation of the monomers to form transferase-active dimers (step II) which undergo further association (step III) to give the native octamer.

Table I shows K_m values determined with mono- and pentaglutamate substrates for native and renatured enzyme, and for the two monofunctional dimers. For the transferase activity, the K_m values for (6*S*)-H_4PteGlu are similar in the three cases, but the transferase dimers generated by renaturation

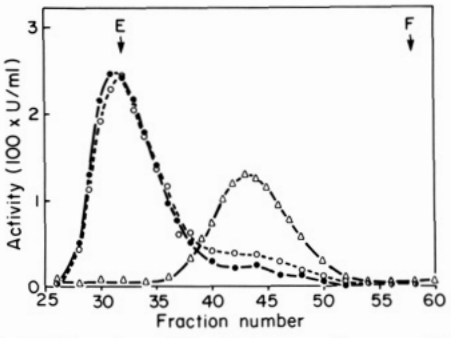


FIGURE 6: Gel filtration of formiminotransferase–cyclodeaminase renatured in the presence and absence of 1.5 M urea. After dilution from 6 M Gdn·HCl into renaturation buffer containing 0.1% Triton X-100, 0.5 mL (~3 μg of protein) was applied to a 48-mL column of AcA-34 as described under Materials and Methods. Fractions of 0.47 mL were collected and assayed for transferase activity. Symbols: (○) native enzyme diluted in renaturation buffer containing 1.5 M urea; (●) enzyme renatured for 50 h in the absence of urea; (△) enzyme renatured for 20 h in the presence of 1.5 M urea. E indicates the elution position of native enzyme (M_r ~500 000) in the absence of urea, and F indicates that of the proteolytically derived transferase-active fragment (M_r ~80 000).

in 1.5 M urea have >30-fold higher K_m values for the pentaglutamate derivative than either native or renatured enzyme. Addition of 1.5 M urea to native enzyme had no effect on the K_m values (not shown). The K_m values for (6*S*)-5-formimino-H_4PteGlu determined for native and renatured enzyme, and for deaminase dimers formed by dissociation of the enzyme in 3 M urea in the presence of folic acid (Findlay & MacKenzie, 1987), are similar for the three cases, and the K_m values for the pentaglutamate derivative are ~25-fold lower for both the native and renatured enzyme and ~5-fold lower for the deaminase dimers. It appears that the deaminase dimers have polyglutamate specificity while the transferase dimers do not, suggesting that the polyglutamate binding site requires the same subunit–subunit interface as the deaminase activity.

Formiminotransferase–cyclodeaminase renatured for ≥48 h in renaturation buffer containing 0.1% Triton X-100 channeled 85% of the (6*S*)-5-formimino-H_4PteGlu$_5$ produced by the first reaction. The same extent of channeling was observed with native enzyme incubated under the same conditions, but enzyme diluted into cold renaturation buffer and assayed immediately exhibited 100% channeling (data not shown).

Discussion

Formiminotransferase–cyclodeaminase is an excellent system for studying both site–site interactions involved in the channeling of a noncovalently bound intermediate and the relationship between structure and function of an oligomeric enzyme with two types of subunit–subunit interaction. In this paper we have used renaturation of formiminotransferase–cyclodeaminase from 6 M Gdn·HCl to further probe the relationship of the two activities to quaternary structure and to

help localize the two catalytic sites and the polyglutamate binding site.

Jaenicke (1982) has reviewed work on denaturation and renaturation of many oligomeric enzymes and has proposed the following general scheme for renaturation of an octameric enzyme:

$$n\mathrm{U} \xrightarrow{\text{uni}} n\mathrm{M}' \xrightarrow{\text{uni}} n\mathrm{M} \xrightarrow{\text{bi}} (n/2)\mathrm{D}' \xrightarrow{\text{uni}} (n/2)\mathrm{D} \xrightarrow{\text{bi}} (n/4)\mathrm{T}' \xrightarrow{\text{uni}} (n/4)\mathrm{T} \xrightarrow{\text{bi}} (n/8)\mathrm{O}' \xrightarrow{\text{uni}} (n/8)\mathrm{O}$$

where U is unfolded polypeptide and M, D, T, and O are monomer, dimer, tetramer, and octamer, respectively, with the primed forms representing different conformational states. This scheme involves a series of alternating first- and second-order reactions, but the final symmetry of formiminotransferase–cyclodeaminase raises the possibility that four dimers may associate directly to form the circular octamer, resulting in a fourth-order step.

Because reassembly requires several second (or higher) order steps, it was rather unexpected that even at very low protein concentrations ($\sim$1 μg/mL) all reactivation steps observed were apparently first order. In the presence of Tween 80, both activities are recovered simultaneously and to $\geq$90% in 48 h. The rate-limiting step has a rate constant $k = (1.9 \pm 0.2) \times 10^{-5}$ s^{-1} at 22 °C and an activation energy of $\sim$15 kcal mol^{-1}. It must correspond to either a step in the initial refolding of monomers or a conformational change after one or more association steps. The intrinsic tryptophan fluorescence spectra confirm that the enzyme is unfolded in 6 M Gdn·HCl but suggest that major refolding occurs within the first 5 min after dilution. Chemical cross-linking indicates that fully reactivated enzyme has recovered its native octameric structure.

In the presence of Triton X-100, the initial rate of transferase reactivation is more than twice that of the deaminase, indicating that there are two rate-limiting steps under these conditions, both of which appear to be first order. In the presence of a low concentration of urea, renaturation is arrested at the level of a transferase-active dimer which we propose is also an intermediate in the complete renaturation to octamer in the presence of Triton X-100. One rate-limiting step would therefore be either refolding of monomer or a conformational change forming active dimer. The second rate-limiting step would be somewhere on the dimer to octamer pathway, since it appears that formation of octamer is required for expression of both activities. The reactivated enzyme has also recovered the ability to channel the formimino-H_4PteGlu$_5$ intermediate between the catalytic sites.

The proteolytically derived transferase fragment comprises 63% of the polypeptide chain and contains one subunit interface of the native octamer. It can be completely renatured under the same conditions as the enzyme, confirming that it is an independently folding domain. The rate-limiting step here is also first order, and the initial rate is slightly ($\leq$2-fold) faster than that of the transferase dimer.

The results of these renaturation experiments provide independent confirmation of the proposal that the two activities require alternate subunit interfaces in the native octamer, which was based on the isolation of two monofunctional dimers by dissociation of formiminotransferase–cyclodeaminase in urea (Findlay & MacKenzie, 1987).

Both activities of native formiminotransferase–cyclodeaminase have been shown to exhibit much lower K_m values ($\geq$50-fold) for polyglutamate forms of their folate substrates (Paquin et al., 1985). We have shown here that the deaminase dimers formed by dissociation in urea in the presence of folate (Findlay & MacKenzie, 1987) show much higher affinity for pentaglutamate than monoglutamate substrate. However, the transferase dimer has lower affinity for the pentaglutamate substrate, similar to results obtained previously with the proteolytically derived transferase fragment, where a 4-fold increase in the K_m for H_4PteGlu$_5$ versus H_4PteGlu was reported (MacKenzie et al., 1980). Since both the transferase dimer and fragment have no polyglutamate specificity, while the deaminase dimer has $\sim$5-fold higher affinity for the pentaglutamate substrate, it appears that the four polyglutamate sites observed by binding studies (Paquin et al., 1985) are formed by the interfaces required for deaminase activity.

There have been a number of recent reports of enzyme active site formation at interfaces between subunits, mainly based on X-ray crystallographic studies. In dimers with a single isologous interaction such as aspartate aminotransferase and glutathione reductase, two active sites are formed at the interface (Ford et al., 1980; Thieme et al., 1981). For enzymes with heterologous interactions between subunits, such as the trimeric aspartate carbamoyltransferase (Krause et al., 1985) and glutamine synthetase with twelve identical subunits (Almassy et al., 1986), a single active site is formed at each interface.

Formiminotransferase–cyclodeaminase appears to be unusual in that catalytic sites for two different reactions require two kinds of subunit–subunit interfaces but that there appears to be a single site associated with each isologous interaction. X-ray crystallography is now required to determine if the active sites of this enzyme are formed by the interfaces between subunits, which would explain why the native octameric structure is required for expression of the two activities and for channeling of polyglutamate intermediates.

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