Variants of the pyruvate dehydrogenase subunit (E1; EC 1.2.4.1) of the Escherichia coli pyruvate dehydrogenase multienzyme complex with Y177A and Y177F substitutions were created. Both variants displayed pyruvate dehydrogenase multienzyme complex activity at levels of 11% (Y177A E1) and 7% (Y177F E1) of the parental enzyme. The $K_m$ values for thiamin diphosphate (ThDP) were 1.58 $\mu$M (parental E1) and 6.65 $\mu$M (Y177A E1), whereas the Y177F E1 variant was not saturated at 200 $\mu$M. According to fluorescence studies, binding of ThDP was unaffected by the Tyr$^{177}$ substitutions. The ThDP analogs thiamin 2-thiazolone diphosphate (ThTDP) and thiamin 2-thiothiazolone diphosphate (ThTTDP) behaved as tight-binding inhibitors of parental E1 ($K_i = 0.003$ $\mu$M for ThTDP and $K_i = 0.064$ $\mu$M for ThTTDP) and the Y177A and Y177F variants. This analysis revealed that ThTDP and ThTTDP bound to parental E1 via a two-step mechanism, but that ThTDP bound to the Y177A variant via a one-step mechanism. Binding of ThTDP was affected and that of ThTTDP was unaffected by substitutions at Tyr$^{177}$. Addition of ThDP or ThTDP to parental E1 resulted in similar CD spectral changes in the near-UV region. In contrast, binding of ThTTDP to either parental E1 or the Y177A and Y177F variants was accompanied by the appearance of a positive band at 330 nm, indicating that ThTTDP was bound in a chiral environment. In combination with x-ray structural evidence on the location of Tyr$^{177}$, the kinetic and spectroscopic data suggest that Tyr$^{177}$ has a role in stabilization of some transition state(s) in the reaction pathway, starting with the free enzyme and culminating with the first irreversible step (decarboxylation), as well as in reductive acetylation of the dihydrolipoamide acetyltransferase component.

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The Escherichia coli pyruvate dehydrogenase multienzyme complex (PDHc) consists of three subunits, pyruvate dehydrogenase (E1; EC 1.2.4.1), dihydrolipoamide acetyltransferase (E2; EC 2.3.1.12), and dihydrolipoamide dehydrogenase (E3; EC 1.8.1.4), and converts the product of glycolysis (pyruvate) to acetyl-CoA. The E1 subunit catalyzes the thiamin diphosphate (ThDP)-dependent decarboxylation of pyruvate to 2-oxohydroxyethylidene-ThDP, which in turn undergoes reductive acetyl transfer to form S-acetyldihydrolipoamide-E2. ThDP and Mg$^{2+}$ are essential cofactors that appear also to be involved in the hysteretic regulation of E1 activity (1, 2). The E1 component of E. coli PDHc comprises two identical subunits with molecular masses of 99,474 Da each (3). Since the early publication by Gutowski and Lienhard (4), the thiamin 2-thiazolone diphosphate (ThTDP) and thiamin 2-thiothiazolone diphosphate (ThTTDP) analogs of ThDP have been considered to be “transition state” analog-type inhibitors of the ThDP-dependent decarboxylations. The structural similarity of these C-2=O and C-2=S bonds, with the expected bond polarizations, to the 2-oxohydroxyethylidene-ThDP (the enamine) intermediate and the transition state preceding it, i.e. the decarboxylation step, was noted. Although ThTDP and ThTTDP are indeed potent inhibitors of E. coli PDHc, these compounds do not appear to be particularly notable inhibitors of several other ThDP enzymes: yeast transketolase (5), wheat germ pyruvate decarboxylase (6), and E. coli pyruvate oxidase (7). A fluorescence titration of brewers’ yeast apopyruvate decarboxylase by ThDP and ThTTDP revealed that ThTTDP is not a transition state analog of this enzyme (8). ThTTDP is, however, a slow- and tight-binding inhibitor of the human E1 enzyme (9).

Equipped with an excellent overexpression system for the E1 subunit, we decided to reexamine this issue, in part, to determine whether any special structural features of E. coli PDHc E1 are responsible for the highly specific inhibition. We now present data indicating that the kinetics of inhibition of E. coli PDHc E1 by ThTDP and ThTTDP reflect properties of tight-binding reversible inhibitors.

The recently solved three-dimensional structure of E. coli PDHc E1 revealed the presence of a tyrosine (Tyr$^{177}$) near the ThDP site, in a highly conserved region of the bacterial E1 subunit, we decided to reexamine this issue, in part, to determine whether any special structural features of E. coli PDHc E1 are responsible for the highly specific inhibition. We now present data indicating that the kinetics of inhibition of E. coli PDHc E1 by ThTDP and ThTTDP reflect properties of tight-binding reversible inhibitors.

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Inhibition of E. coli Pyruvate Dehydrogenase E1 Subunit

Tyrosine residues involved in ThDP binding were also identified in the crystal structures of human branched-chain α-ketoacid dehydrogenase (11), 2-oxoisovalerate dehydrogenase from Pseudomonas putida (12), and benzoylformate decarboxylase from P. putida (13). Therefore, the Y177A and Y177F variants of E. coli PDHc E1 were created, and the binding of ThTDP and ThTTDP to parental E1 and the Y177A and Y177F variants of E. coli decarboxylase from the University of Sheffield (14) was applied to a column of Amberlite CG-50 H+ cation-exchange resin, washed with 20 mM KH2PO4 (pH 7.0), and stored at 4 °C. The enzyme was rechromatographed on a Sephadex G-25 column equilibrated with 10 mM KH2PO4 (pH 7.0) and stored at 4 °C.

Construction of the Y177A and Y177F Variants of E1—Parental PDHc E1 and the Y177A and Y177F variants were studied using progress curve analysis, circular dichroism, and fluorescence spectroscopy. The results indicate that Tyr177 in E. coli PDHc E1 probably interacts with the covalent adduct(s) formed with ThDP during the reaction cycle, but not with ThDP itself. Finally, we report the observation and characterization of a new CD signature, never before reported, formed upon binding of ThTTDP to either the PDHc or the E1 subunit.

Experimental Procedures

Bacterial Strains, Plasmids, and Growth Conditions—E. coli strain JRG0456 (aceE mutant with a chloramphenicol resistance cassette in the aceE gene) was grown in LB broth (15); E2 and E3 genes are intact; provided by Dr. G. Furey (University of Sheffield) transformed with pGS878 was used for overexpression of the aceE gene encoding the E1 subunit of E. coli PDHc.

The presence of mutations was verified by sequencing the entire E1 gene with the following specific primers, using the ABI Prism Ready-Action Reaction dye terminator cycle sequencing kit (PerkinElmer Life Sciences): pdhe1, 5′-CCGCAACATCCATCCCATCC-3′; pdhe2, 5′-GAG-TATCCGGGTAATCTGGACGTAAGAC-3′; pdhe3, 5′-CAGTTCCGCGGCGTTATGGAATGAC-3′; pdhe4, 5′-CGTAAAGGATACACCCGGTAAACC-GTATGAC-3′; pdhe5, 5′-GAAATCCGACGGTTGCTGGTGTTCTATATC-3′; pdhe6, 5′-CCGCCCAGACCGCTACGATACGAC-3′; and pdhe8, 5′-GCAGAAGCTTTGACGCAGAGGCAC-3′ (for Y177F) and 5′-TGCCCTCTCCCTCCGTCCGACCGA-3′ (for Y177A).

Overexpression of Parental E and the Y177A and Y177F Variants—E. coli cells transformed with the corresponding plasmids were grown for 16 h at 37 °C in LB medium containing 50 μg/ml ampicillin and were used to inoculate 1000 ml of the same medium. The cells were grown to an A600 of 0.6–0.7 and then induced with isopropyl-β-D-thiogalactopyranoside (1 mM final concentration), harvested after 5–6 h, washed with 20 mM KH2PO4 (pH 7.0), and stored at −20 °C.

Purification of E1 and the Y177A and Y177F Variants—Purification of parental E1 and its tyrosine variants was carried out following the protocol described previously (14). E1 was also resolved from a single-lipid domain PDHc as reported previously (2). The synthesis of ThDP was similar to that of ThTTDP described above. Three spots were detected on TLC with Rf values of 0.89, 0.67, and 0.50 for thiamin-2-thiazolone, thiazin-2-thiazolone monophosphate, and ThDP, respectively. 1H NMR (D2O) δ 2.26 (3H, s), 2.50 (3H, s), 3.15 (2H, t), 4.20 (2H, t), 5.40 (2H, s), and 7.58 (1H, s); 31P NMR (D2O versus phosphoric acid) δ −3.20 (α) and −12.9 (β).

Inhibition of E. coli Pyruvate Dehydrogenase E1 Subunit

The synthesis of ThDP was similar to that of ThTTDP described above. Three spots were detected on TLC with Rf values of 0.89, 0.67, and 0.50 for thiamin-2-thiazolone, thiazin-2-thiazolone monophosphate, and ThDP, respectively. 1H NMR (D2O/DCI) δ 2.25 (3H, s), 2.70 (3H, s), 3.10 (2H, br), 4.20 (2H, br), and 7.85 (1H, s); 31P NMR (D2O versus phosphoric acid) δ −3.18 (α) and −13.93 (β).

Activity and Related Measurements—The E1 activity was measured by reconstituting holo-PDHc activity with added E2-E3 subcomplex (the ratio of E1 to E2-E3 subcomplex was 1.5) using a Varian DMS-300 spectrophotometer or a Cobas-Bio centrifugal analyzer (Roche Molecular Biochemicals), monitoring the pyruvate-dependent reduction of NAD+ at 340 nm. The reaction medium contained (in a 1-ml (DMS 300) or 0.25-ml (Cobas Bio) test volume) 0.1 mM Tris-HCl (pH 8.0), 1 mM MgCl2, 2 mM sodium pyruvate, 2.5 mM NAD+, 0.1–0.2 mM CoA, 0.2 mM ThDP, and 2.6 mM dithiothreitol at 30 °C. The reaction was initiated by adding CoA. Steady-state velocities were taken from the linear portion of the progress curve. One unit of activity is defined as the amount of NADH produced (μmol/min/mg of E1).

The E1 activity was also measured in the model reaction with 2,6-dichlorophenolindophenol (2,6-DCPIP) using a Varian DMS 300 spectrophotometer by monitoring the reduction of 2,6-DCPIP at 600 nm. The reaction medium contained (in a 1-ml test volume) 50 mM KH2PO4 (pH 7.0), 1 mM MgCl2, 2.5 mM sodium pyruvate or 0.2 mM 2-hydroxyethyl- ThDP as substrate, 0.2 mM ThDP, and 0.1 mM 2,6-DCPIP at 30 °C. The reaction was initiated by adding the enzyme. One unit of activity is defined as the amount of 2,6-DCPIP reduced (μmol/min/mg of E1).

Inhibition of Parental E1 and the Y177A and Y177F Variants by Thiamin 2-Thiazolone and Thiamin 2-Thiazolone Diphosphates—ThTDP and ThTTDP were dissolved in 20 mM KH2PO4 (pH 7.0), and their concentrations were determined using ε235 = 10,600 M−1 cm−1 for ThTDP and ε519 = 10,900 M−1 cm−1 for ThTTDP (4). Parental E1 (0.035–0.10 μM) and the tyrosine variants (0.22–0.44 μM) were incubated in 50 mM KH2PO4 (pH 7.0) with 2 mM MgCl2 and 0.002–3.5 μM ThTDP or 0.005–3.5 μM ThTTDP in a total volume of 0.2 ml at 25 °C. At this concentration, the E2/E3 subcomplex and components required for assaying the PDHc activity were added to a volume of 1.0 ml, and the reaction was started by the addition of CoA.

Circular Dichroism—CD spectra were recorded on an Aviv Model 202 CD spectrometer at 25 °C. A 1-cm path length quartz cuvette was used for the near-UV region (230–350 nm). Data were collected at a wavelength step of 1.0 nm, an integration time of 1 s, and a bandwidth of 1 nm.

The concentration of the CD spectrum, parental E1 (1.5 mg/ml) and the tyrosine variants (0.22–0.44 μM) were incubated in 50 mM KH2PO4 (pH 7.0) containing 1 mM dithiothreitol, and variable concentrations of ThTDP or ThTTDP. The base line was normally recorded using 10 mM KH2PO4.

Data fitting and calculations utilized the KaleidaGraph, SigmaPlot, and DeltaGraph computer programs.

Fluorescence Spectroscopy—The fluorescence spectra of parental E1 and its tyrosine variants were recorded at 25 °C using an SLM8100 spectrophotofluorometer. The excitation wavelength was 290 nm, and the emission spectra were recorded in the 300–450-nm range in 3-m quartz cuvettes. The integration time was 0.5 s, and the scan rate was 1 nm/min. The concentration of parental E1 and its tyrosine variants was 0.05 mg/ml in 20 mM KH2PO4 (pH 7.0). The excitation and emission monochromator slit widths were 4 nm. The inner filter effect was corrected with the absorbance spectrum of ThDP, ThTTDP, or ThTTDP with Equation 1 (16, 17),

$$F_{\text{corr}} = F_{\text{lab}} (V/\alpha) \times \text{antilog}(0.45A + A_{\text{em}})$$

where $F_{\text{corr}}$ is the corrected value of the fluorescence intensity at a given point in the titration, $F_{\text{lab}}$ is the experimentally measured fluorescence intensity, $V$ is the initial volume of sample, $\alpha$ is the volume added at the excitation wavelength, and $A_{\text{em}}$ is the absorbance at the excitation wavelength.

The $K_v$ value for ThDP was calculated using Equation 2 (17),

$$\frac{\Delta F/\Delta [E]}{F_0} \times 100 = \left(\frac{\Delta F_{\text{corr}}/F_0}{\Delta [E]/K_v + \Delta [E]} \right)$$

where $\Delta F/\Delta [E]$ is the percent fluorescence quenching following the addition of E1.

2 P. Arjuman, N. Nemerin, A. Brunskill, K. Chandrasekhar, M. Sax, Y. Yan, F. Jordan, J. R. Guest, and W. Furey, submitted for publication.
addition of ThDP. The concentrations of ThTDP and ThTTDP used for the titration were comparable to the concentration of enzyme. Quadratic Equation 3 (18) was used to calculate $K_a$ values,

$$\frac{\Delta F/M}{F_{max}^{/F_0}} = \left(\frac{E_L + K_d}{2E_L} - \sqrt{\left(\frac{E_L + K_d}{2E_L}\right)^2 - 4E_LK_d}\right)$$

(Eq. 3)

where $(\Delta F/M)/F_{max}$ is the relative fluorescence; $E_L$ and $L$ are the total concentrations of E1 and ThTDP (or ThTTDP) used for the titration, respectively; $K_d$ is the dissociation constant; and $(\Delta F/M)/F_{max}$ × 100 is the percent of fluorescence quenched.

**RESULTS**

**Characterization of the Y177A and Y177F Variants of PDHc E1**—Site-directed substitutions were introduced into the *E. coli* E1 subunit at Tyr177 in the highly conserved region of bacterial E1 (see BLAST alignment in Fig. 1), and the Y177A and Y177F variants were created (Table I). Furthermore, a BLAST search for nearly exact matches did not reveal any related sequences in the heterotetrameric α2β2 E1 subunits, so the highly conserved sequence in Fig. 1 is present only in the α2 homodimers. The activities of parental E1 and the Y177A and Y177F variants measured with different assays are presented in Table I. After reconstitution with the E2-E3 subcomplex, compared with parental E1, the Y177A variant retained 11% and the Y177F variant retained 7% activity according to the overall PDHc reaction assay in the presence of 0.2 mM ThDP. The catalytic constant ($k_{cat}$) was ~10-fold lower for the Y177A variant compared with the value for parental E1 (Table I). Similar relative activity (7%) was obtained for the Y177A variant using 4,4′-dithiopyridine as a substitute for the E2 in PDHc, an E1-specific assay developed in this laboratory (data not shown) (19). According to the 2,6-DCPIP assay (a different E1-specific assay that measures E1 activity in the absence of the E2-E3 subcomplex as a second substrate and that is used extensively in the study of ThDP-dependent enzymes (9, 20, 21)), compared with parental E1, the activities were 20% (Y177A E1) and 49% (Y177F E1) with pyruvate as substrate and 49% (Y177A E1) and 68% (Y177F E1) with 2α-hydroxethyl-ThDP as substrate. These results suggest that both the interaction of E1 with the E2-E3 subcomplex and, to a lesser extent, the decarboxylation of pyruvate (the latter concluded on the basis of the 2,6-DCPIP experiments) are affected by the substitutions. The steady-state kinetic parameters for ThDP and pyruvate with parental E1 and the Y177A and Y177F variants are presented in Table II. The apparent $K_m$ for ThDP measured in the overall PDHc reaction in the presence of 0.2–200 μM ThDP was 6.65 μM for the Y177A variant compared with 1.58 μM for parental E1. The Y177F variant was not saturated by ThDP even at a concentration >200 μM, indicating that its activity could be increased with elevated ThDP concentrations (data not shown). A weak positive cooperativity ($n_H = 1.38$) was observed for ThDP binding to parental E1, which, however, changed to a negative cooperativity ($n_H < 1.0$) for the Y177F variant. We previously reported that for single-lipoyl domain PDHc (a single lipoyl domain rather than the three lipoyl domains found in wild-type *E. coli* PDHc), variants with the G231A, N258Q, and C259S substitutions in the ThDP-binding fold of the E1 subunit were not saturated by concentrations of ThDP as high as 5–10 mM and exhibited negative cooperativity at all concentrations of ThDP studied (2). Similar $K_m$ values for pyruvate were obtained for parental E1 (0.515 mM) and the Y177A (0.280 mM) and Y177F (0.531 mM) variants.

**TABLE I**

Activities of parental E1 and Y177A and Y177F variants of E1

| Enzyme | Pyruvate-NAD$^+$ oxidoreductase assay$^a$ | $k_{cat}$ | 2,6-DCPIP assay$^b$ | 2,6-DCPIP assay$^c$ |
|--------|-----------------------------------------|----------|-------------------|-------------------|
|        | units/mg E1 | s$^{-1}$ | units/mg E1 | milliunits/mg E1 |
| Parental E1 | 8.71 ± 0.39 | 28.89 ± 1.31 | 0.445 ± 0.028 | 9.86 ± 0.65 |
| (100%) | | | (100%) | (100%) |
| Y177A E1 | 0.989 ± 0.073 | 3.31 ± 0.25 | 0.087 ± 0.009 | 4.83 ± 1.44 |
| (11%) | | | (20%) | (49%) |
| Y177F E1 | 0.614 ± 0.063 | 2.16 ± 0.030 | 0.216 ± 0.030 | 6.71 ± 1.39 |
| (7%) | | | (49%) | (68%) |

$^a$ The activity of parental E1 and its variants was determined using the NADH assay in the presence of 0.2 mM ThDP.

$^b$ This assay was carried out with pyruvate as substrate.

$^c$ This assay was carried out with 2α-hydroxethyl-ThDP as substrate.

**TABLE II**

Kinetic parameters for parental E1 and TY177A and TY177F variants of E1 determined using NADH assay

| Enzyme | $K_m$/ThDP$^a$ | $n_H$ | $k_{cat}/K_m$/ThDP$^b$ | $K_m$/pyruvate | $k_{cat}/K_m$/pyruvate$^b$ |
|--------|----------------|------|----------------------|----------------|----------------------------|
|        | μM            |      | μM$^{-1}$s$^{-1}$    | mM            | μM$^{-1}$s$^{-1}$          |
| Parental E1 | 1.58 ± 0.20 | 1.38 ± 0.19 | 18 | 0.515 ± 0.014 | 0.056 |
| Y177A E1 | 6.65 ± 0.47 | 1.0 | 0.498 | 0.290 ± 0.027 | 0.012 |
| Y177F E1 | NS$^c$ | <1.0 | | 0.531 ± 0.079 | |

$^a$ Apparent $K_m$.

$^b$ Values of $k_{cat}$ are presented in Table I.

$^c$ Not saturated at 200 μM ThDP.
Fig. 2. Stoichiometry of ThTDP (A) and ThTTDP (B) binding to parental E1. E1 (0.072 and 0.076 μM) was incubated in 50 mM KH₂PO₄ (pH 7.0) with 0.002–0.08 μM ThTDP or 0.04–0.7 μM ThTTDP and 2 mM MgCl₂ in a total volume 0.2 ml at 25 °C. After a 30-min incubation, a reaction mixture containing the E2-E3 subcomplex and all components required for assay of PDHc activity was added to a total volume of 1 ml. The reaction was initiated by the addition of 0.13 mM CoA.

(Tables II) at 0.2 mM ThDP, indicating that substitution at Tyr₁⁷⁷ in E1 does not affect pyruvate binding.

Inhibition of Parental E1 and Its Tyrosine 177 Variants by ThTDP and ThTTDP—The following evidence was obtained, suggesting that ThTDP and ThTTDP behave as tight-binding reversible inhibitors of E1. 1) It was shown that 0.8–0.94 mol of ThTDP/mol of E1 monomer and ∼2 mol of ThTTDP/mol of E1 monomer (Fig. 2, A and B) were required to inactivate parental E1. With the Y₁₇₇A variant, 1.61–1.75 mol of ThTDP/mol of Y₁₇₇A E1 monomer and 1.34–1.55 mol of ThTTDP/mol of Y₁₇₇A E1 monomer were required for inactivation. The Y₁₇₇F variant gave a molar ratio of inactivation by ThTDP similar to that observed with the Y₁₇₇A variant. 2) According to the Ackermann-Potter plot, using E1 resolved from single-lipoyl domain PDHc, ThTDP was found to behave as a tight-binding inhibitor (Fig. 3). A plot of velocity versus concentration of E1 for different concentrations of ThTDP was curved with an asymptote (Fig. 3). According to the Ackermann-Potter plot (22), the asymptote intersects the x axis at Eᵢ = 1. As shown in Fig. 3 (inset), for a ThTDP concentration of 0.03 μM, the total concentration of E1 titrated was 0.035 μM, indicating that the concentration of ThTDP required for inhibition is comparable to the concentration of enzyme, a diagnostic for tight-binding inhibition. When ThTTDP was tested at a concentration of 0.10 μM, the total amount of E1 titrated was ∼0.021 μM, indicating that at least a 5-fold molar excess of ThTTDP is required to inhibit E1 (data not shown). 3) Inhibition of parental E1 by ThTDP and ThTTDP is reversible. About 10% (ThTDP inactivation, followed by 16 h of dialysis) and 34% (ThTTDP inactivation, followed by 48 h of dialysis) of the activity of parental E1 was recovered upon dialysis against 50 mM potassium phosphate buffer (pH 7.5).

Determination of Kᵢ Values for ThTDP and ThTTDP Using Reaction Progress Curve Analysis—The progress of the overall PDHc reaction was monitored by recording the formation of NADH at 340 nm as a function of time (23, 24). A family of progress curves for the reaction, started by the addition of parental E1 and the E2-E3 subcomplex to assay medium containing 30 μM ThDP and different concentrations of ThTDP, is shown in Fig. 4A. In the absence of ThTDP, a linear rate of NADH release was observed during the first 10 min of the reaction (Fig. 4A). In the presence of ThTDP (Fig. 4A) and ThTTDP (data not shown), a time-dependent decrease in NADH production was observed, indicating a slow attainment of equilibrium between parental E1, ThDP, and ThTTDP or parental E1, ThDP, and ThTTDP. Since the concentrations of ThTDP and ThTTDP were at least 10-fold higher than the concentration of parental E1, resulting in the conversion of <5% of pyruvate to product (P) during the reaction, the progress curves were treated according to Equation 4,

\[ P = v₀ \times t + \frac{vₛ - v₀}{k} \times (1 - e^{-kt}) \]  (Eq. 4)

where \( v₀ \) is the initial velocity, \( vₛ \) is the steady-state velocity, and \( k \) is the pseudo first-order rate constant for the approach to the steady-state phase. The progress curve analysis revealed that the initial velocity of NADH production (\( v₀ \)) decreased with increasing concentration of ThTDP or ThTTDP (data not shown), indicating that the equilibria drawn below are reached rapidly: E1 + ThDP ↔ E1·ThDP and E1 + ThTTDP ↔ E1·ThTTDP or E1 + ThTDP ↔ E1·ThTDP. The dependence of the pseudo first-order rate constant (\( k_{app} \)) on the concentration of ThTDP or ThTTDP was hyperbolic, indicating saturation (Fig. 4, B and C). The data suggest that the inhibition of parental E1 by ThTDP and ThTTDP follows mechanism A (Scheme 1) (24, 25) for competitive tight-binding inhibitors.

\[ E₁ + \text{ThDP} \rightarrow E₁·\text{ThDP} + \text{pyruvate} \rightarrow E + P \]

\[ k₁ \downarrow k \]

\[ E₁·\text{ThTDP} \rightarrow k₃ \downarrow k₂ \]

\[ E₁·\text{ThTTDP} \]

\[ k₄ \]

\[ E₁·\text{ThTTDP} \]

\[ \text{Scheme 1} \]
According to mechanism A, the rate of combination between parental E1 and ThDP or ThTDP (or parental E1 and ThDP or ThTTDP) may be similar, but the binding of ThTDP or ThTTDP to parental E1 is accompanied by a slower rate compared with the E1/ThDP complexes. The rate constants $k_2$ and $k_{-2}$ and $K_d$ values ($k_{2}/k_{-2}$) that characterize the equilibrium between parental E1 and the E1/ThDP and E1/ThTTDP complexes were calculated using Equation 5 and are presented in Table III.

$$k_{app} = \frac{k_2 \times L}{L + K_d \left(1 + \frac{S}{K_m}\right)} + k_{-2} \quad \text{(Eq. 5)}$$

The progress curves were also recorded for the Y177A variant in the presence of ThTDP. A plot of $k_{app}$ against ThTDP concentration is linear, indicating a simpler mechanism of inhibition than that outlined for parental E1 (Fig. 5). Mechanism B describes the inhibition of the Y177A variant by ThTDP (Scheme 2) (24, 25).

Scheme 2

According to mechanism B, the Y177A E1 + ThTDP $\leftrightarrow$ Y177A E1-ThTDP $+ \text{pyruvate} \rightarrow$ Y177A E1 + P

$$k_1 \not= k_{-1} \quad \text{Y177AE1-ThTDP}$$

The CD spectra of the Y177A and Y177F variants (maximum observed at 325 nm) titrated with ThTTDP (data not shown). The CD spectra displayed positive CD bands in the 259 and 265 nm regions, which increased in magnitude upon addition of ThTDP or ThTTDP, as well as an extensive negative band in the 268–285 nm region (Fig. 6), similar to that reported for E1 isolated from pigeon breast muscle (26) and human recombinant E1 (27). The positive CD bands in the 287 and 293 nm regions are likely due to tyrosine and/or tryptophan and were also observed with parental E1 (Fig. 6). No CD bands were in evidence in the 300–350 nm region of the spectra. In contrast, the addition of ThTTDP to parental E1 (Fig. 7) produced significant changes in the CD spectrum of E1, which were different from those produced by the addition of ThDP and ThTDP. The addition of ThTTDP decreased the positive CD bands in the 259 and 265 nm regions while generating a broad positive CD band with a maximum near 330 nm (Fig. 7). The amplitude of the positive band at 330 nm increased with increasing ThTTDP concentration, exhibiting saturation with inhibitor (Fig. 7, inset). Fitting the data to Equation 7 provides an estimate for $K_d$ of $0.450 \pm 0.258 \mu M$ for the binding of ThTTDP to parental E1,

$$\text{CD} = \frac{(E_0 + I_0 + K_d) - \sqrt{(E_0 + I_0 + K_d)^2 - 4E_0 \cdot K_d}}{2E_0/\text{CDmax}} \quad \text{(Eq. 7)}$$

where $E_0$ and $I_0$ are the total concentrations of parental E1 and ThTDP and $K_d$ is the dissociation constant for the parental E1-ThTTDP complex. The appearance of a CD band at 330 nm was specific for E1. It was not detected in the near-UV CD spectrum of the E91D variant of yeast pyruvate decarboxylase (this E91D variant can be purified as an apo-yeast pyruvate decarboxylase, enabling us to replace ThDP with other ThDP analogs (28)) titrated with ThTTDP, indicating that ThTTDP probably does not form a tight complex with this enzyme (data not shown). The CD maximum at 330 nm was also observed with E. coli single-lipoyl domain PDHC and with E1 resolved from E. coli PDHC as well as with PDHC from porcine heart (maximum observed at 325 nm) titrated with ThTTDP (data not shown). The CD spectra of the Y177A and Y177F variants were similar to that of parental E1, and the CD maximum at 330 nm was observed for both variants upon addition of ThTDP. The $K_d$ values for ThTTDP binding were $0.644 \pm 0.237 \mu M$ (Y177A) and $0.365 \pm 0.103 \mu M$ (Y177F), compared...
with $K_d = 0.450 \pm 0.258 \, \mu M$ for parental E1, indicating that the substitution of Tyr$^{177}$ in the E1 subunit did not significantly affect ThTTDP binding.

In additional experiments, the following results were obtained. 1) Parental E1 saturated by ThTTDP and dialyzed overnight against 50 mM KH$_2$PO$_4$ (pH 7.5) still retained the CD band with a maximum at 330 nm. The CD intensity was 85% compared with non-dialyzed E1, indicating that this band originates from tightly bound ThTTDP. 2) ThTDP replaced ThTTDP in its complex with E1. The intensity of the CD band at 330 nm for the E1-ThTTDP complex was reduced by 30–39% upon addition of ThTDP followed by a 24-h incubation, indicating that ThTDP and ThTTDP are bound at the same site of E1. 3) ThDP was easily replaced by ThTTDP in its complex with E1. The CD band at 330 nm appeared immediately upon addition of ThTTDP to the E1-ThDP complex and retained the same intensity after 15 h of incubation with ThTTDP, indicating that ThDP and ThTDP are also bound at the same site of E1.

**Table III**

$K_i$ and rate constants for ThTDP and ThTTDP binding to parental E1

| Compound | $K_i$ | $k_2$ | $k_{-2}$ |
|----------|-------|-------|---------|
| ThTDP    | 0.003 ± 0.001 | 0.053 ± 0.005 | 0.031 ± 0.006 |
| ThTTDP   | 0.064 ± 0.022 | 0.035 ± 0.003 | 0.041 ± 0.002 |

**Fig. 5.** A, progress curve analysis for the inhibition of the Y177A variant of E1 by ThTDP. The Y177A variant (0.154 \, \mu M) was preincubated with the E2-E3 subcomplex (E1/E2-E3 subcomplex molar ratio = 3.8:1.0) and was added to 1 ml of assay medium containing all components required for assay of PDHc activity and 200 \, \mu M ThDP in the absence (line 1) or presence of 15 (line 2), 20 (line 3), 30 (line 4), 40 (line 5), 50 (line 6), 70 (line 7), 80 (line 8), and 90 (line 9) \, \mu M ThTDP. The reaction was started by the addition of CoA, and the formation of NADH at 340 nm was recorded for 18 min at 30 °C. B, dependence of $k_{app}$ on the concentration of ThTDP. The Data from A were fitted to Equation 6 (see “Results”).
Inhibition of E. coli Pyruvate Dehydrogenase E1 Subunit

Fluorescence Studies of the Binding of ThDP, ThTDP, and ThTTDP to Parental E1 and Its Tyrosine 177 Variants—It was reported previously that E. coli PDHc and E1 resolved from PDHc have an intrinsic fluorescence, probably due to tryptophan, that diminishes upon ThDP binding in the presence or absence of pyruvate (29, 30). We have now shown that, upon addition of ThDP, ThTDP, or ThTTDP (Fig. 8), the intrinsic fluorescence of parental E1 was diminished. An emission maximum at 334 nm resulted from excitation at 290 nm, which very likely corresponds to tryptophan residues. The fluorescence quenching resulting from addition of ThDP, ThTDP, or ThTTDP (Fig. 8) exhibited saturation behavior, which was fitted to Equations 1–3 (see “Experimental Procedures”) to estimate the $K_d$ values. The $K_d$ obtained for the binding of ThDP to parental E1 (Table IV) is in good agreement with the data presented previously for E. coli PDHc ($K_d = 1.49$ μM) (29) and with our value of 3.73 μM reported for single-lipoyl domain PDHc (2). The $K_d$ values for ThTDP and ThTTDP for parental E1 were at least 10 times lower than the $K_d$ for ThDP itself (Table IV). According to the data in Table IV, the Y177A and Y177F substitutions did not significantly affect the binding of ThDP. The $K_d$ values for ThDP binding were 2.01 μM (Y177A) and 3.87 μM (Y177F), nearly the same as the $K_d$ value of 1.84 μM obtained for parental E1.

Titration of the Y177A and Y177F variants with ThDP quenched 8 and 7% of the fluorescence, respectively, compared with 16% for parental E1, indicating that Tyr 177 substitution in the E1 subunit affects ThDP binding. The binding of ThTTDP to the Y177A and Y177F variants of E1 was not affected and exhibited 45 and 40% fluorescence quenching, respectively, similar to that observed with parental E1 (57%) (Table IV). The $K_d$ values were 0.113 ± 0.035 μM (parental E1), 0.052 ± 0.007 μM (Y177A E1), and 0.029 ± 0.040 μM (Y177F E1), lower than the corresponding values obtained from the CD titrations. The pronounced quenching of the fluorescence of parental E1 and the tyrosine 177 variants by ThTTDP could be explained by the different substituents at the C-2 atom in the thiazolium ring of ThDP, ThTDP, and ThTTDP, specifically the C=S bond in the last one. It was reported that the thiol of N-acetylcysteine makes it a stronger quencher of the fluorescence of 3-methylindole compared with the quenching produced by other amino acids tested (31). We ran a model study in which we monitored changes in fluorescence of tryptophan (2 μM) upon addition of ThTDP and ThTTDP. No significant changes were observed with concentrations as high as 100 μM, indicating that specific binding of ThTDP and ThTTDP to parental E1 and the tyrosine 177 variants contributed to the observed quenching of protein fluorescence.

**DISCUSSION**

The results of this study suggest that ThTDP and ThTTDP exhibit kinetic properties characteristic of tight-binding reversible inhibitors according to the following criteria. 1) Titration of parental E1 by ThTDP and ThTTDP revealed that 0.8–0.94 mol of ThTDP/mol of E1 monomer and −2 mol of ThTTDP/mol of E1 monomer are required to inactivate E1. 2) The Ackermann-Potter plot is consistent with ThTDP behaving as a tight-binding inhibitor. 3) Inhibition of parental E1 by ThTDP and ThTTDP is partially reversible in dialysis experiments. Additional evidence of reversibility of ThTTDP binding to E1 was obtained in experiments in which the CD band at 330 nm induced by ThTTDP was reduced in magnitude upon addition of ThTDP. 4) According to reaction progress curve analysis, ThTDP and ThTTDP behave as competitive tight-binding inhibitors with a two-step mechanism of inhibition shown in Scheme 1. It is noteworthy that ThTTDP was also reported to be a tight-binding inhibitor of human E1 (9).

Several methods were used to determine the strength of binding of ThDP and ThTTDP to E. coli E1, including reaction progress curve analysis, CD, and fluorescence spectroscopy. Importantly, progress curve analysis could yield $K_i$ values (nanomolar range) inaccessible by other methods. The lower limit of $K_i$ for ThTDP was 0.003 μM. The graphical procedure of Henderson (36) led to a $K_i$ of 0.013 μM for parental E1 and E1 resolved from single-lipoyl domain PDHc (data not shown). Gutowski and Lienhard (4) reported a $K_i$ of 0.5 nM for inhibition of E. coli PDHc by ThTDP. According to these results, ThTDP binds more tightly to E. coli E1 than to several other ThDP-dependent enzymes such as yeast transketolase ($K_i = 28$ nM) (5), wheat germ pyruvate decarboxylase ($K_i = 2$ μM) (6), and E. coli pyruvate oxidase ($K_i = 0.2$ μM) (7). A $K_i$ of 0.064 μM was determined for the binding of ThTTDP to E. coli E1 using progress curve analysis, a value significantly higher than that for ThDP, but very similar to the $K_i$ of 0.0757 ± 0.001 μM reported for the binding of ThTTDP to human E1 (9). For the binding of ThTTDP to parental E1, CD provided a $K_i$ of 0.450 μM, and fluorescence spectroscopy provided a $K_i$ of 0.113 μM. For comparison, a $K_i$ of 7 μM was estimated for the binding of ThTTDP to brewers’ yeast pyruvate decarboxylase (8).

An important new finding from our studies is the observation and characterization of a positive CD band in the 330 nm region upon addition of ThTTDP to parental E1. The same band was not observed upon addition of either ThDP and ThTDP to E1; but we showed (by competition experiments) that all three ThDP analogs occupy the same locus. The addition of ThTDP to a sample in which the 330 nm CD band was evident from mixing ThTTDP and E1 diminished the amplitude of the band. In addition, the 330 nm CD band was also observed when ThTTDP was added to 1) E1 resolved from PDHc, 2) E. coli single-lipoyl domain PDHc, and 3) PDHc from mammalian sources. We wish to emphasize that this positive band is quite distinct from the broad negative band at 300–350 nm with a maximum at 320 nm, which was assigned to the formation of a charge transfer complex between ThDP and transketolase (32, 33) and also reported for pigeon breast muscle E1 (26) and mammalian E1 (27), but only as a very weak band at best for E. coli E1 (2). The appearance of a broad positive CD band at 330 nm was observed upon addition of ThTTDP only; and more...
likely, it is related to the formation of chiral ThTTDP at the active center by virtue of the V conformation enforced on all ThDP enzymes studied to date (34). We also note that the absorption spectrum of ThTTDP has a maximum at 319 nm, and this electronic transition could be the source of the observation. Finally, we also note that, so far, we have not observed the same 330 nm CD band on the related yeast pyruvate decarboxylase when ThTTDP was added to its apo-E91D variant at the highest concentrations. Therefore, to date, we have observed the new band only for solutions of ThTTDP and PDHc or the E1 component.

The results on the Tyr 177 variants suggest that this residue does affect the potent binding of ThTDP. 1) The molar ratio of ThTDP or ThTTDP inhibitor to Y177A variant monomer is the same, in contrast to that observed with parental E1, which is inactivated with a 1:1 stoichiometry. 2) According to reaction progress curve analysis, ThTDP binds to Y177A in a one-step mechanism, in contrast to the two-step mechanism observed for ThTDP and ThTTDP binding to parental E1. This result suggests that the conformational change characterizing the two-step binding mechanism is missing in the Y177A variant, i.e. Tyr177 is probably involved in this conformational change. 3) The percent fluorescence quenching by ThTDP of the Y177A (8%) and Y177F (7%) variants is smaller than the 16% observed with parental E1, also indicating that Tyr177 in the E1 subunit may be involved in ThTDP binding. The $K_d$ value for ThTDP binding determined by fluorescence quenching for parental E1 (0.117 $\mu$M) is much greater than the $K_i$ of 0.003 $\mu$M determined by progress curve analysis, indicating that additional step(s)
(intermediates) are present in the inactivation of E1 by ThTDP and that these may contribute to the $K_i$ value (35).

Our studies show that substitutions at Tyr$^{177}$ in E1 do not affect the binding of ThTDP. The $K_i$ values obtained from fluorescence quenching for ThTDP binding to the Y177A (0.052 μm) and Y177F (0.029 μm) variants were similar to that observed with parental E1 (0.113 μm) and also similar to the $K_i$ (0.064 μm) determined by progress curve analysis. The CD signature at 330 nm was similar for the Tyr$^{177}$ variants of E1 and parental E1 and does not contradict the major conclusion that Tyr substitution does not affect ThTDP binding. We had previously reported that cysteine 259 in the E1 subunit is important for ThTDP binding (14) on the basis of inactivation by ThTDP of E1 resolved from the C259N and C259S E1 variants of single-lipoyl domain PDHc. However, as mentioned above, the CD spectra of the C259S and C259N variants of E1 also exhibited the CD band at 330 nm upon addition of ThTDP, clearly indicating that ThTDP binds to these cysteine variants of E1. Further explanations of the observation will have to await solution of the x-ray structure of the E1-inhibitor complexes.

According to the high-resolution structure of the PDHc E1 component from *E. coli*, Tyr$^{177}$ is located in the active site cavity, with the Tyr$^{177}$ phenolic oxygen atom located at 6 Å from the thiazolium C-2 atom, too far to assist in the first essential deprotonation step required to trigger the reaction (Fig. 9). There is a water molecule hydrogen-bonded (3 Å) to the C-2H and N-4' atoms, but too far from the Tyr$^{177}$ side chain oxygen atom (3.7 Å) to form a good hydrogen bond with it. Almost certainly, this water molecule would be replaced by substrate binding. The position of Tyr$^{177}$ in the active site of *E. coli* E1 is different from that observed in the crystal structure of human branched-chain α-ketoacid dehydrogenase (11) and 2-oxoisovalerate dehydrogenase from *P. putida* (12). In the crystal structure of human branched-chain α-ketoacid dehydrogenase, there are three tyrosine residues near ThDP: Tyr$^{113}$, Tyr$^{224}$, and Tyr$^{102}$ (11). Tyr$^{113}$ is in direct contact with the oxygen atom of the β-phosphate group of ThDP; Tyr$^{224}$ is packed against one side of the 4′-aminopyrimidine ring of the ThDP, with Leu$^{164}$ approaching the other side of the ring. It was suggested that these hydrophobic residues maintain the cofactor in the V conformation. Tyr$^{224}$ is coordinated to the Mg$^{2+}$ ion. The Tyr$^{102}$-to-Ala substitution showed that Tyr$^{224}$ is not critical for ThDP binding (10). The Tyr$^{224}$-to-Ala substitution, by contrast, resulted in a markedly decreased ability to bind ThDP and an increased $K_m$ for cofactor binding. The Tyr$^{113}$-to-Ala substitution resulted in a >50–100-fold increase in the $K_m$ for ThDP, indicating that Tyr$^{113}$ is indeed important for ThDP binding (10). In 2-oxoisovalerate dehydrogenase from *P. putida*, Tyr$^{88}$, similar to Tyr$^{102}$ in the human enzyme, is stacked against the 4′-aminopyrimidine ring with other hydrophobic residues (12). Tyr$^{113}$ in the *P. putida* enzyme has a role similar to that of Tyr$^{113}$ in the human enzyme, contributing to the binding of the cofactor through a hydrogen bond with a phosphate oxygen. In the crystal structure of *E. coli* E1, Phe$^{602}$ is stacked against the 4′-aminopyrimidine ring, with a role analogous to that of Tyr$^{88}$ and Tyr$^{102}$ in the two αβε subunit structures. In summary, Tyr$^{177}$ in the *E. coli* E1 enzyme has a different role than the tyrosine residues reported contributing to the binding of the cofactor through a hydrogen bond.

In conclusion, the combination of the x-ray structural evidence on the location of Tyr$^{177}$ with the kinetic and spectroscopic data suggests that Tyr$^{177}$ has a role in stabilizing some transition state(s) in the reaction pathway, starting with the free enzyme and culminating with the first irreversible step, decarboxylation ($k_{\text{cat}}/K_m$-type effects), as well as in some step in reductive acetylation of lipoyl-E2. The results with the Y177F variant suggest that without the hydrogen-bonding ability of the tyrosine, the bulky side chain at position 177 is detrimental to catalysis.

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**REFERENCES**

1. Bieswanger, H. (1974) *Eur. J. Biochem.* 48, 377–387
2. Yi, J., Nemeria, N. S., McNally, A., Jordan, F., Mashado, R. S., and Guest, J. R. (1996) *J. Biol. Chem.* 271, 33192–33200
3. Stephenson, P. E., Darlison, M. G., Lewis, H. M., and Guest, J. R. (1983) *Eur. J. Biochem.* 134, 155–162
4. Gutowski, J. A., and Lienhard, G. E. (1976) *J. Biol. Chem.* 251, 2863–2866
5. Shreve, D. S., Holloway, M. P., Haggerty, J. C., III, and Sable, H. Z. (1983) *J. Biol. Chem.* 258, 12405–12408
6. Kluger, R., Gish, G., and Kaufman, G. (1984) *J. Biol. Chem.* 259, 8860–8865
7. O’Brien, T. A., and Gennis, R. B. (1980) *J. Biol. Chem.* 255, 3302–3307
8. Jordan, F., Akinoyeoye, O., Dikdan, G., Kudzin, Z., and Kuo, D. (1988) *Thiamin Pyrophosphate Biochemistry*, pp. 79–92, CRC Press, Inc., Boca Raton, FL
9. Tripatara, A., Korotchkina, L. G., and Patel, M. S. (1999) *Arch. Biochem. Biophys.* 367, 39–50
10. Wynn, R. M., Ho, R., Chuang, J. L., and Chuang, D. T. (2001) *J. Biol. Chem.* 276, 4168–4174
11. Evarsson, A., Chuang, J. L., Wynn, R. M., Turley, S., Chuang, D. T., and Hol, W. G. J. (2000) *Structure* 8, 277–291
12. Evarsson, A., Seger, K., Turley, S., Sokatch, J. R., and Hol, W. G. J. (1999) *Nat. Struct. Biol.* 6, 785–792
13. Hasson, M. S., Mascate, A., McLeish, M. J., Poluvnikova, L. S., Gerlt, J. A., Kenyon, G. L., Petko, G. A., and Ringe, D. (1998) *Biochemistry* 37, 9918–9930
14. Nemeria, N., Volkov, A., Brown, A., Yi, J., Zipper, L., Guest, J. R., and Jordan, F. (1998) *Biochemistry* 37, 911–922
15. Gutowski, J. A. (1979) *Methods Enzymol.* 62, 120–125
16. Lakowicz, J. R. (1983) *Principles of Fluorescence Spectroscopy*, Chapter 10, Plenum Publishing Corp., New York
17. Liu, R., and Sharam, F. J. (1996) *Biochemistry* 35, 11865–11873
18. Segel, I. H. (1993) *Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems*, pp. 72–74, John Wiley & Sons, Inc., New York
19. McNally, A. J., Motter, K., and Jordan, F. (1995) *J. Biol. Chem.* 270, 19736–19743
20. Tittmann, R., Golik, R., Ghiha, S., and Hubner, G. (2000) *Biochemistry* 39, 10747–10754
21. Hajipour, G., Schowen, K. B., and Schowen, R. L. (1999) *Bioorg. Med. Chem.* 7, 887–894
22. Cha, S. (1975) *Biochem. Pharmacol.* 24, 2177–2185
23. Williams, J. W., and Morrison, J. F. (1979) *Methods Enzymol.* 63, 437–467
24. Bieth, J. G. (1995) *Methods Enzymol.* 248, 59–84
25. Morrison, J. F. (1982) *Trends Biochem. Sci.* 7, 102–105
26. Khailova, L. S., Kurochkin, L. G., and Severin, S. E. (1989) *Ann. N. Y. Acad. Sci.* 573, 36–54
27. Korotchkina, L. G., Tucker, M. M., Thakkumkara, T. J., Madhusudhan, K. T., Pons, G., Kim, H., and Patel, M. S. (1995) *Protein Expression Purif.* 6, 79–90
28. Li, H., Furey, W., and Jordan, F. (1999) *Biochemistry* **38**, 9992–10003
29. Henning, J., Kern, G., Neef, H., Bisswanger, H., and Hüblner, G. (1996) in *Biochemistry and Physiology of Thiamin Diphosphate Enzymes* (Intemann, A. U. C., ed) pp. 255–242, Wissenschaftliche Verlagsgesellschaft, Prien, Germany
30. Jordan, F., Nemeria, N., Gub, F., Baburina, L., Gao, Y., Kahyaoglu, A., Li, H., Wang, J., Yi, J., Guest, J. R., and Furey, W. (1998) *Biochim. Biophys. Acta* **1385**, 187–306
31. Chen, Y., and Barkley, M. D. (1998) *Biochemistry* **37**, 9976–9982
32. Kochetov, G. A., and Usmanov, R. A. (1970) *Biochim. Biophys. Res. Commun.* **41**, 1134–1140
33. Meshalkina, L. G., and Kochetov, G. A. (1979) *Biochim. Biophys. Acta* **57**, 218–223
34. Shin, W., Fletcher, J., Blank, G., and Sax, M. (1977) *J. Am. Chem. Soc.* **99**, 3491–3499
35. Fersht, A. (1999) *Structure and Mechanism in Protein Science*, W. H. Freeman & Co., pp. 106–107, New York
36. Henderson, P. J. F. (1972) *Biochem. J.* **127**, 321–333
Inhibition of the *Escherichia coli* Pyruvate Dehydrogenase Complex E1 Subunit and Its Tyrosine 177 Variants by Thiamin 2-Thiazolone and Thiamin 2-Thiothiazolone Diphosphates: EVIDENCE FOR REVERSIBLE TIGHT-BINDING INHIBITION

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