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The electrochemical investigation of the catalytic power of pyruvate decarboxylase and its coenzyme

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Abstract

The change in the energy barriers for the heterogeneous reduction of pyruvate decarboxylase (PDC) relative to its coenzyme, thiamin pyrophosphate (ThPP), was determined experimentally using square wave voltammetry (SWV) to be 5.3 kcal/mol. These results are in agreement with those of reaction rate acceleration provided by thiamin-dependent decarboxylases relative to their coenzyme as determined kinetically based on the pK_a suppression by the enzyme environment.
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Keywords: Pyruvate decarboxylase; Thiamin pyrrophosphate; Square wave voltammetry

1. Introduction

Pyruvate decarboxylase (PDC) is one of the most interesting thiamin-dependent enzymes, which plays an important role in á-keto acid decarboxylation and acyl group transfer reactions [1,2]. It is well known for its vital function in the decarboxylation of pyruvate to form acetaldehyde, an essential reaction step in the alcohol fermentation process [3,4]. PDC requires the metabolically active coenzyme, thiamin pyrophosphate (ThPP), and the cofactor, Mg^{2+}, to convert pyruvate to acetaldehyde, which is further converted to ethanol by alcohol dehydrogenase [2–4]. Of particular interest is the fact that reactions catalyzed by PDC can also be catalyzed by ThPP alone, albeit in a less efficient fashion (Eqs. (1) and (2)) [1,2,4]. This phenomenon provides an effective experimental way of quantifying the change in the thermodynamic driving force provided by protein environments over that afforded by unbound ThPP in solution.

$$\mathrm{PDC} + \mathrm{S} \Leftrightarrow \mathrm{PDC} - \mathrm{S} \Leftrightarrow \mathrm{PDC} + \mathrm{P} \quad \text{(Fast)} \tag{1}$$

$$\mathrm{ThPP} + \mathrm{S} \Leftrightarrow \mathrm{ThPP} - \mathrm{S} \Leftrightarrow \mathrm{ThPP} + \mathrm{P} \quad \text{(Slow)} \tag{2}$$

2. Materials and methods

2.1. Chemicals

Pyruvate decarboxylase from Baker's yeast (EC 4.1.1.1) and thiamin pyrophosphate of analytical grade were obtained from Sigma-Aldrich (St. Louis, MO) and used without any further purification. Potassium chloride purchased from EM Scientific, Inc. (Gibbstown, NJ) and hydrochloric acid and sodium hydroxide purchased from Fisher Scientific (Pittsburgh, PA) were of analytical grade. All solutions were prepared with distilled water filtered through a Millipore Milli-Q system (Bedford, MA).

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2.2. Apparatus

The potentiostat used in this study was the BAS 100B Electrochemical Analyzer (West Lafayette, IN). In the SWV analyses, the working electrode was platinum (MF-2013) obtained from BAS and polished using the BAS polishing alumina (CF-1050). The reference electrode, Ag/AgCl, was obtained from BAS (RE-5B) and a 50 mm (length) × 1 mm (diameter) Pt wire from Aldrich (Milwaukee, WI) was used as the auxiliary electrode. The sensitivity, step potential, amplitude, frequency, samples per points, and the quiet time of the BAS 100B for SWV analyses were set to 10 μA/V, 4 mV, 25 mV, 15 Hz, 256, and 2 s, respectively. The pH of solutions was adjusted with 0.1 M NaOH and 0.1 M HCl solutions using a Fisher Scientific Accumet® model 15 pH meter.

3. Results and discussion

In this investigation, an electrochemical method was used to evaluate the change in the thermodynamic driving force of PDC and ThPP in the absence of substrates and found to be approximately 5.3 kcal/mol. This value corresponded to the change in free energy (5 to 6 kcal/mol) for reactions accelerated by PDC relative to ThPP [5]. In this study, we have demonstrated in detail a unique relationship between the electrochemical behavior of PDC and its catalytic activity.

3.1. Reduction of PDC with square wave voltammetry (SWV)

The reduction potential of ThPP had previously been determined in acidic solutions by means of cyclic voltammetry (CV) at an unmodified platinum electrode (Pt) [6]. Due to a diminutive cathodic peak current generated by the reduction of ThPP in protein environments, no electrochemical behavior of PDC was displayed with our previously developed CV method. Because of its high signal to noise ratio (S/N), square wave voltammetry (SWV) has proven to be a superior technique to CV in the electrochemical investigation of trace amounts of redox active analytes [7]. For this reason, we measured the reduction potentials of both PDC and ThPP using SWV (see Fig. 1, voltammograms "c" and "a", respectively) in similar conditions to those used previously in CV. ThPP was also analyzed in conjunction with PDC (see Fig. 1, voltammogram "b") to establish a baseline condition that served as a reference for the quantitative evaluation of the change in the thermodynamic driving force afforded by PDC over ThPP.

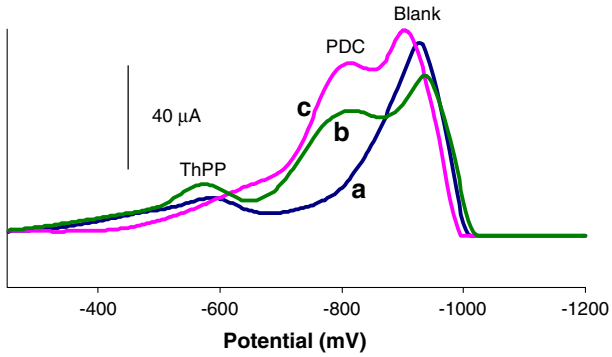

Fig. 1. Square wave voltammograms of a) 2 mM ThPP (pH 4.6), b) 4 mM ThPP and 1 unit PDC in 16 mL of 1 M KCl solutions (pH 5.3) and c) 1 unit of PDC in 10 mL of 1 M KCl solutions (pH 6.3) obtained at Pt Vs. Ag/AgCl reference electrodes.

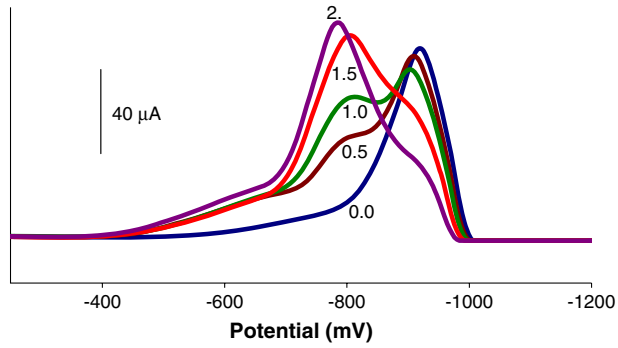

Fig. 2. Square wave voltammograms of various amounts of PDC (0.0, 0.5, 1.0, 1.5, and 2.0 units) in 10 mL of 1 M KCl (pH 6.3) obtained at Pt vs. Ag/AgCl reference electrodes.

A careful background study was performed to rule out contributions from the non-enzyme components such as EDTA, buffer, and Mg^{2+} (stabilizers in commercial enzyme samples) and to avoid the erroneous assignment of electrochemical features to PDC. Due to the lack of commercially available apo-PDC, selected thiamin complex enzymes (pyruvate dehydrogenase and α-ketoglutarate) with low thiamin content were analyzed by SWV to observe the effect of various amino acids or peptide chains within our potential range. Amino acids in the investigated enzymes did not display any redox behavior under our experimental conditions and potential range. Furthermore, the peak currents associated with PDC (−0.82 V) increased with an increase in PDC concentration up to 2 units of PDC in a 10 mL solution, confirming the attribution of the observed redox activity to PDC (see Fig. 2). At higher PDC concentrations (>2 units), the peak currents were nearly the same (plateau). However, the addition of PDC to ThPP at lower pH (<6) caused a gradual decrease in ThPP peak currents (−0.59 V) (figure not shown). This phenomenon may be due to PDC saturation with ThPP. As the pH was increased from 6.3 to about 10.6, the current peak of PDC gradually decreased and diminished at about pH 10.6 (Fig. 3). This observation is attributable to the unfolding of PDC, accompanied by a release of ThPP into solution (pH>7) [8], and thereby undetectable under our experimental conditions (pH).

Furthermore, the peak at approximately −0.92 V that appears in all voltammograms originated from the blank and

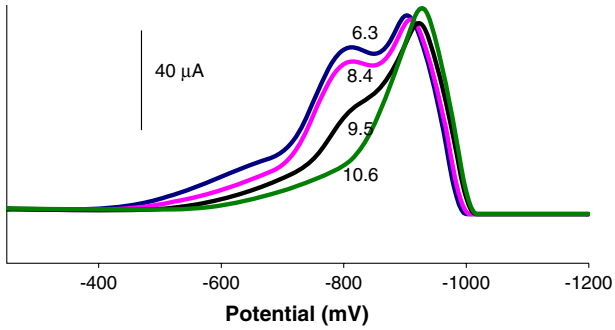

Fig. 3. Square wave voltammograms of 1 unit PDC in 10 mL of 1 M KCl solutions at various pHs (6.3, 8.4, 9.5,10.6) obtained at Pt vs. Ag/AgCl reference electrodes.

might be associated with the reduction of water. It is therefore assumed, but not proven, that the redox behavior of PDC observed in this investigation originated from its coenzyme (ThPP) moiety.

3.2. Thermodynamic relevance

Our data obtained using SWV in the absence of a substrate measures the electron energy, which is the driving force for reactions catalyzed by enzymes or their corresponding coenzymes. In this paper we relate the thermodynamic driving force of PDC (evaluated electrochemically) to its increased catalytic activity or reaction rate over its coenzyme in biological assays. Our previous study on the electrochemical behavior of ThPP in acidic media at a platinum electrode established that the protonated thiamin (A^{2+}) at the N1′ position in the pyrimidine ring was the electroactive species (see Fig. 4) [6]. A^{2+} was reduced in a one-electron transfer mechanism at a potential of −0.59 V (measured against Ag/AgCl) to produce a very reactive radical cation, $A^{\bullet+}$, which underwent a fast dimerization $[AA]^{2+}$ as shown below [6].

$$A^{2+} + e^- \rightarrow A^{\bullet+} \quad (3)$$

$$2A^{\bullet+} \rightarrow [AA]^{2+} \text{(fast dimerization)} \quad (4)$$

In protein environments, the active or most efficient forms of thiamin-dependent enzymes are also known to be protonated at the N1′ position in the pyrimidine ring of ThPP, thereby forming a hydrogen bond with the Glu51 residue of the enzyme (see Fig. 5) [4,5,9–15]. This effect also makes the pyrimidine ring of thiamin-dependent

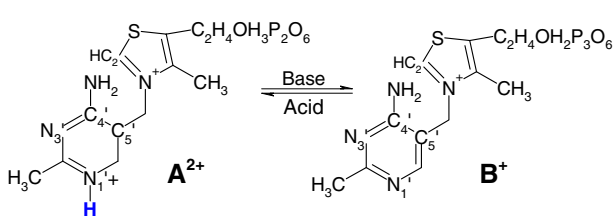

Fig. 4. ThPP structures in acidic (A^{2+}) and neutral (B^+) solutions.

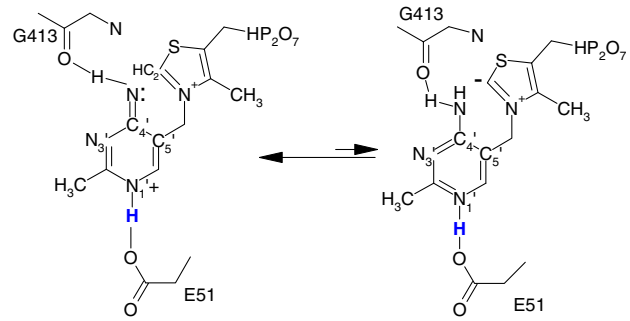

Fig. 5. ThPP bound to E51 via hydrogen bond in PDC (from Ref. [4]).

enzymes a much more electron withdrawing group, which is susceptible to an electrochemical reduction at a potential of −0.82 V (measured against Ag/AgCl). The fact that ThPP is covalently bound to PDC (or is structurally constrained by the protein) suggests that no free ThPP is available in the active site to allow dimerization upon an electrochemical reduction. Thus the radical formed might be expected to extract protons from water molecules present in the vicinity of the active site [4]. The change in the reduction potentials of PDC and ThPP of 0.23 V is equivalent to an increase of 5.3 kcal/mol in the thermodynamic driving force of PDC relative to that of ThPP [16].

The protonation at the N1′position in the pyrimidine ring of ThPP and its interaction with Glu51 is known to generally influence the catalytic activity of PDC, and particularly the deprotonation of C2 in ThPP-dependent enzymes, which is the key intermediate in PDC catalyzed reactions [4,5,9–15]. The pK_a suppression of approximately 9 units for the dissociation of the C2 proton of ThPP in water relative to that in protein environments observed by Jordan et al. [5] accounts for a >109-fold rate acceleration in thiamin-dependent enzymes [5]. In these enzymes, the change in free energy for the C2 proton transfer to N4′(in PDC compared to ThPP) at distances of 4 Å and 3.2 Å has been estimated to vary from 5 kcal/mol to 6 kcal/mol, respectively [5]. These values are in agreement with the change in the thermodynamic driving force obtained electrochemically (5.3 kcal/mol) based on the reduction potentials of ThPP and PDC in which C2 is located at 3.5 Å from the N4′[4].

4. Conclusion

In summary, our electrochemical studies as presented in this communication measure the change in the thermodynamic driving force of PDC relative to ThPP in the absence of a substrate. We have proven that there is a unique relationship between the redox behavior of enzymes and their increased catalytic activities relative to their coenzymes. This research is a stepping-stone toward efficiently relating redox factors that affect enzyme's increased activities relative to their coenzymes.

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[16] The free energy in kJ/mol was calculated by using the equation $\Delta G = -nF\Delta E$, where n (1) represents the number of electrons transferred in the reaction, F is the Faraday constant (96.5 kJ/V mol) and ΔE is the change in reduction potentials of ThPP and PDC.