OXIDATION-REDUCTION PROPERTIES OF GLYCOLATE OXIDASE

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Glycolate oxidase (GAO) catalyzes oxidation of α-hydroxy acids by O_2 to produce α-keto acids and H_2O_2. Previous studies of the isolated enzyme from pig liver have explored its physicochemical and steady-state kinetic properties (1-3). Particular attention was paid to the spectral and kinetic effects of mono- and dianions, e.g. Cl^-, oxalate. These anions are inhibitors except for P_i and arsenate which are activators (3).

The redox potential of GAO has not been reported. It has been suggested that the midpoint potential for native GAO should be more positive than -0.170 V, which is the midpoint potential of GAO in which FMN is replaced by 5-deazaFMN (4). Since the redox potential for the glycolate/glyoxalate couple is -0.086 V at pH 7 (5) and that for free FMN is -0.205 V (6), it seemed of interest to determine the oxidation-reduction properties of GAO and the effects of various anions upon these properties.

The spectroelectrochemical procedures have been described (7,8). Electrochemical reduction of GAO with methyl viologen (MV) as mediator of electron transfer produces the red radical form (9) of GAO (Figure 1). When absorbance data from experiments such as that shown in Figure 1 are plotted at selected wavelengths vs the amount of charge added to the system (inset to Figure 1), the intersections of the linear portions of these plots represent absorbance due to 100% radical formation. There is little difference in spectral characteristics of the radical formed in the presence of P_i or Cl^-. The maximum value obtained for the extinction coefficient at 375 nm is 18,000 M^{-1}-cm^{-1}.

The variation of the reduction potential of GAO as a function of pH is shown in Figure 2. The midpoint potential for the conversion of GAO radical (GAO_1) to fully reduced GAO (GAO_2) is more negative in 0.1 M P_i at

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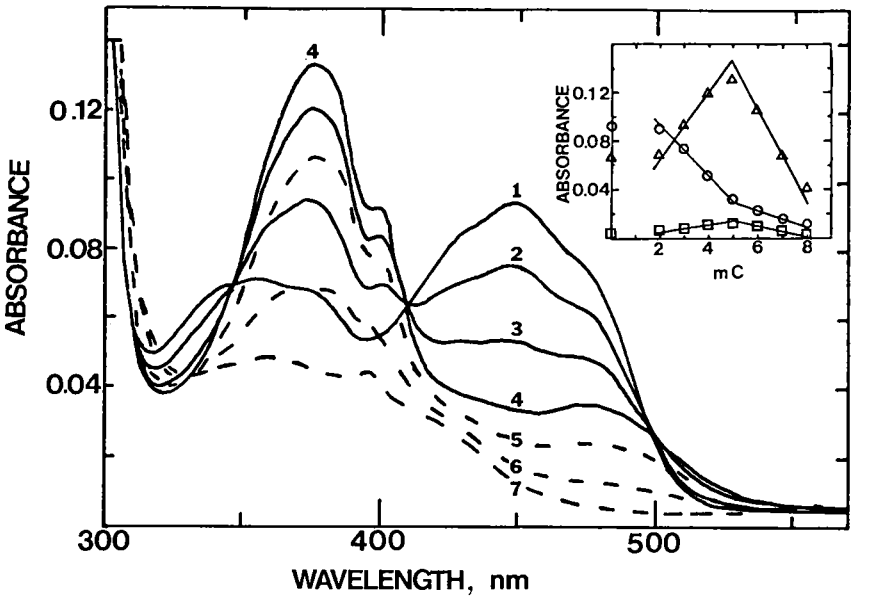

Figure 1. Absorption spectra from reductive coulometric titration of GAO (8.4 μM) at 10°C in 0.26 M triethanolamine-Cl at pH 9.2. Solid lines show radical increasing from the start of the titration (spectrum 1) to a maximum of 80% of the total FMN at 1.1 equivalents added per mole FMN (spectrum 4). Spectrum 2 corresponds to addition of a total of 3 mC. Dashed lines show the decrease in radical concentration which occurred between 1.1 and 2.1 equivalents added per mole FMN.
Inset. Absorbance vs charge at 375 nm (△), 450 nm (○) and 520 nm (□).

pH 7 or pH 8 than in 0.1 M Cl^-, but the change from P_i to Cl^- makes no significant difference in the reduction potentials for the conversion of fully oxidized GAO (GAO_0) to GAO_1. From four experiments in 0.1 M P_i at pH 7.1, the reduction potential for conversion of GAO_0 to GAO_1 is -0.093 ± 0.013 V and that for conversion of GAO_1 to GAO_2 is -0.040 ± 0.009 V. From one experiment in 0.1 M imidazole-Cl, the values are -0.091 V and + 0.020 V, respectively.

The redox potential for conversion of GAO_0 to GAO_1 is more negative than that for conversion of GAO_1 to GAO_2 under all conditions tested. Radical stability increases with increasing pH (Figure 2), as indicated by the difference in potentials for the two one-electron steps (5). However, less than 30% radical is stable, even at pH 9. Higher percentage radical

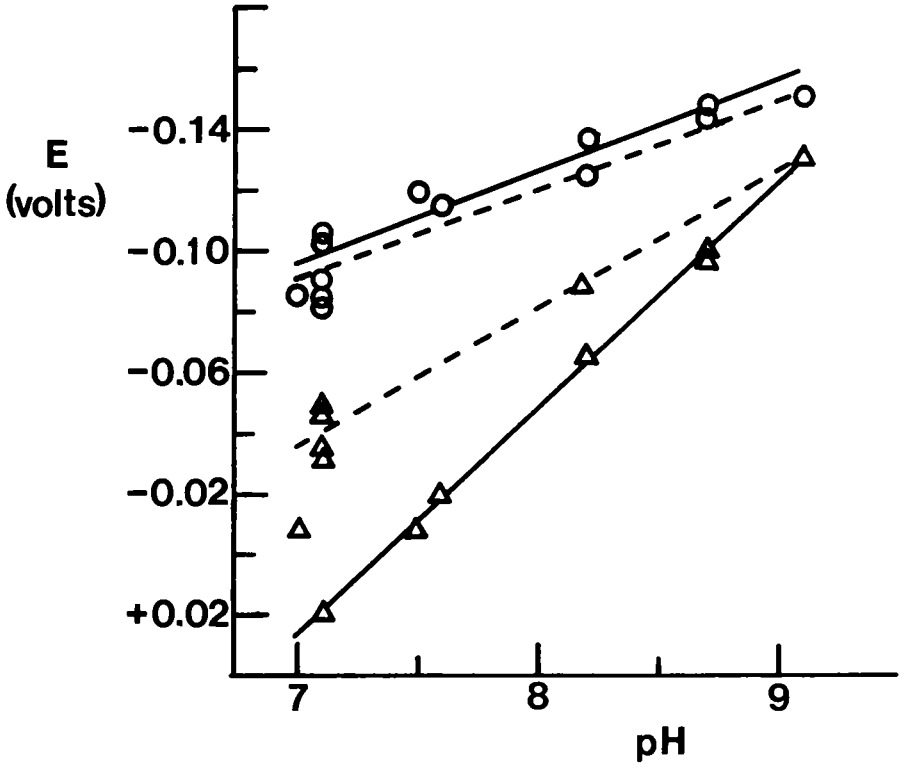

Figure 2. Reduction potentials for conversion of fully oxidized GAO to GAO radical (O) or GAO radical to fully reduced GAO (Δ) vs pH at 10°C. Lines are from least squares analysis of data from experiments in 0.1 M P_i (continuous lines) or 0.1 M Cl^- (dashed lines). The slopes of these lines in V/pH unit are 0.029 in P_i and 0.031 in Cl^- for the conversion of fully oxidized GAO to GAO radical. For the conversion of GAO radical to fully reduced GAO, they are 0.046 V/pH unit in P_i and 0.074 V/pH unit in Cl^-.

during coulometric titration is a kinetic phenomenon.

Both electron transfers in reduction of GAO are pH dependent. In 0.1 M P_i, the slope of the least squares plot indicates a stoichiometry for conversion of GAO_0 to GAO_1 of 0.52 proton per electron. In 0.1 M Cl^-, it is 0.54 proton per electron. For conversion of GAO_1 to GAO_2, the apparent stoichiometry is 0.82 proton per electron in 0.1 M P_i and 1.34 proton per electron in 0.1 M Cl^-. Transfer of one proton per electron during conversion of radical to fully reduced enzyme has been observed in this laboratory for L-amino acid oxidase (10) and D-amino acid oxidase (11). However

the conversion of fully oxidized enzyme to radical was independent of pH for both amino acid oxidases. Variation of redox potential with pH is expected for interconversion of GAO_0 and GAO_1, especially at the alkaline end of the pH range examined here. The pK for dissociation of the 3-imino group of FMN is shifted from 10.35 for free FMN (6) to 8.0 for GAO (2). However, upward curvature of the pH dependence for interconversion of GAO_0 and GAO_1 is not apparent from the data in Figure 2.

Conclusions:

1. Upon binding to GAO, the redox potentials for FMN are shifted near that of the glycolate/glyoxalate couple and transfer of electrons from glycolate to FMN becomes thermodynamically feasible.
2. From pH 7 to pH 9, the reduction potential for conversion of GAO_0 to GAO_1 is more negative than that for conversion of GAO_1 to GAO_2 and the thermodynamic stabilization of radical is low.
3. Reduction of GAO in 0.1 M P_i exhibits no thermodynamic advantage over reduction of GAO in 0.1 M Cl^-.
4. Both electron transfers to GAO are pH dependent.

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