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Antibody-catalyzed decarboxylation and aldol reactions using a primary amine molecule as a functionalized small nonprotein component

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ABSTRACT

Catalytic antibody 27C1 bears binding sites for both a substrate- and a functionalized small nonprotein component in the active site. We investigated the possibility of exploiting imine and enamine intermediates using a primary amine molecule into the active site of antibody 27C1. The antibody catalyzed β-keto acid decarboxylation with a rate enhancement ($k_{cat}/K_m/k_{uncat}$) of 140,000, as well as highly regioselective cross-aldol reactions of ketones and *p*-nitrobenzaldehyde. These studies provide new strategies for the generation of catalytic antibodies possessing binding sites for functionalized components.

1. Introduction

The study of amine-catalyzed decarboxylation and aldol reactions remains a special area of research in synthetic chemistry, bioorganic chemistry and enzymology.[1–4] A number of elegant enzymatic studies elucidated the mechanism by which acetoacetate decarboxylase catalyzes the decarboxylation of acetoacetic acid.[5–14] These studies demonstrated that the reaction proceeds by the formation of a Schiff base between the ε-amino group of a lysine residue in the enzyme and acetoacetate, followed by decarboxylation to form an enamine, which is then tautomerized to a Schiff base and subsequently hydrolyzed to release acetone and the free enzyme. The imine and enamine intermediates developed along the reaction catalyzed by acetoacetate decarboxylase are also found along the reaction coordinate of reactions between aldolases and aldol donors. In fact, natural class I aldolases and the programmed catalytic antibodies 38C2 and 33F12, which both have an active-site lysine residue are bifunctional catalysts. In addition to catalyzing the decarboxylation of β-keto acids, they also catalyze the aldol reaction of aldehydes and ketones.[15–19] Unlike natural enzymes, antibodies 38C2 and 33F12 were found to accept a variety of ketones and aldehydes as aldol donors and acceptors to achieve regio- and enantioselevtive aldol reactions.[20] The application of catalytic antibodies has thus yielded numerous efficient syntheses of stereochemically complex molecules.[21–24]

Recently, we developed catalytic antibody 27C1, which bears an antigen-combining site that functions as an apoprotein for binding functionalized small nonprotein components.[25] This antibody was elicited by immunization with the haptenic phosphonate diester **1**. The *p*-nitrophenyl and *N*-acetylphenyl groups in hapten **1** were designed to elicit binding sites for the substrate and the functionalized component, respectively, in the antigen-combining site (Fig. 1a). With a simple exchange of the functionalized component, antibody 27C1 is capable of catalyzing a wide range of chemical transformations including acyl-transfer, β-elimination, decarboxylation, and aldol reactions. Particularly, antibody 27C1 catalyzed the aldol reaction of acetone and *p*-nitrobenzaldehyde **4** in the presence of the functionalized component **2** (Fig. 1b).[25] Catalysis by 27C1 was efficient compared with the non-catalyzed reaction, showing a rate enhancement $[(k_{cat}/K_m$ **4**$)/k_{uncat}$ of 4.4×10^4 (27C1: $k_{cat} = 25.8\ min^{-1}$, K_m for **4** = 958 μM, K_m for **2** = 67 mM). In the aldol reaction, the direct precursor of the enamine is an imminium ion; the presence of the imminium ion was established by isolation of the reduction product following $NaBH_4$ treatment. Examination of enamine formation with acetone and amine **2** in the presence of $NaBH_4$ showed that antibody 27C1 catalyzed the formation of an isopropylation product, providing evidence for an enamine mechanism for the antibody-catalyzed reaction. This enamine formation was inhibited by addition of hapten **1**, showing that the enamine was formed in the antigen-combining site. In this

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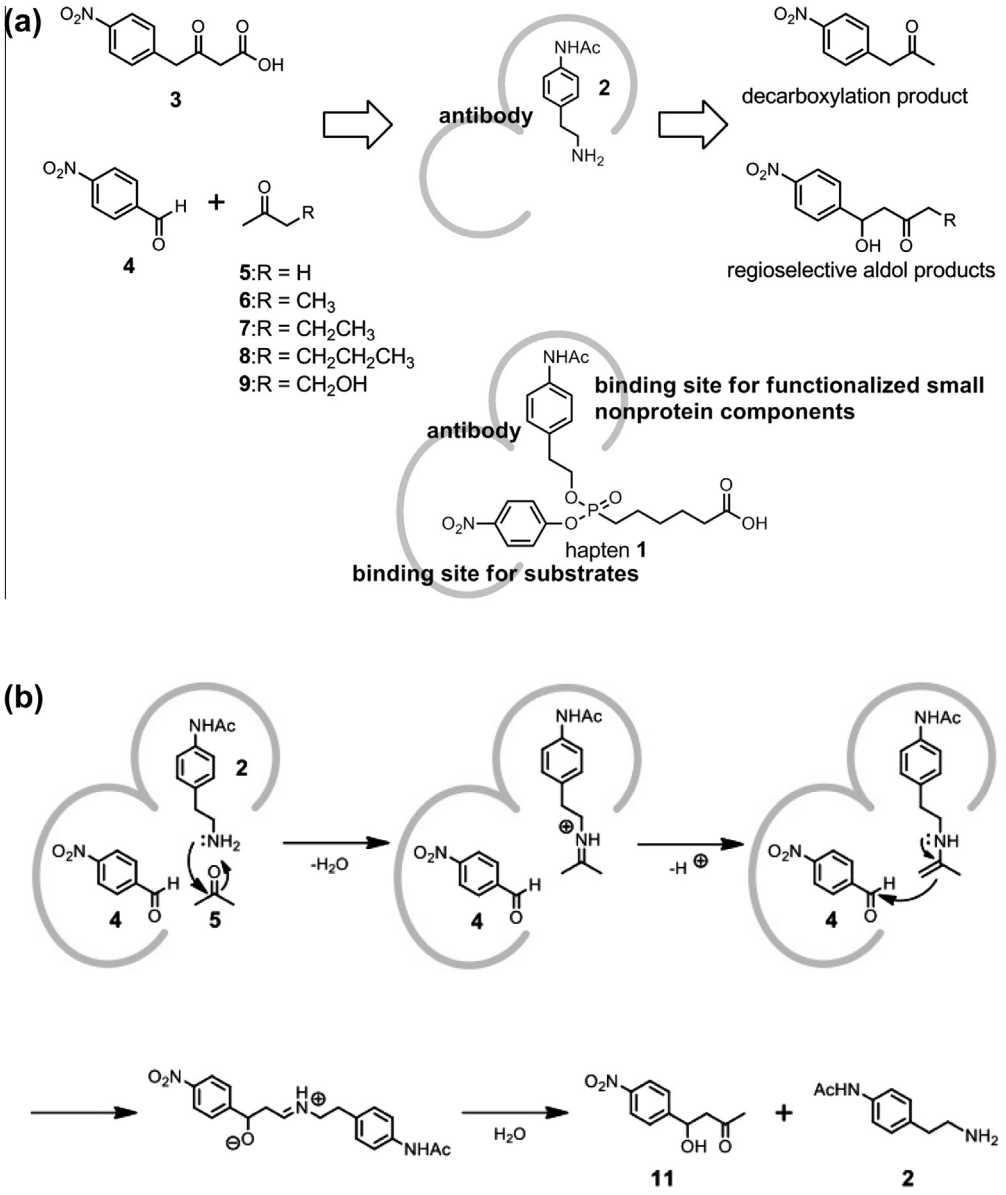

Figure 1. (a) Decarboxylation and aldol reactions catalyzed by antibody 27C1 and structure of the immunized hapten **1**. (b) The mechanism of the antibody-catalyzed aldol reaction.

work, we have investigated the possibility of exploiting the common imine and enamine intermediates using a primary amine molecule in the antibody-combining site. The primary amine mimics a lysine residue correctly aligned in the active sites of natural enzymes and the catalytic antibodies 38C2 and 33F12. Here, we demonstrate the utility of the amine molecule for catalyzing the decarboxylation of a β-keto acid, as well as aldol reactions using structurally related aldol donors (Fig. 1a).

2. Results

2.1. Decarboxylation reaction

Antibody 27C1 catalyzed the decarboxylation reaction of β-keto acid **3** to afford (*p*-nitrophenyl)acetone. Lineweaver–Burk plots were constructed by holding the either the substrate or the functionalized component constant while varying the concentration of the other (Fig. 2). The slopes and *y*-intercepts obtained from this analysis were replotted as a function of substrate or functionalized component concentration to provide the true maximum rate V_{max} (12.9 μM min^{-1}) and the Michaelis constant, K_m (Fig. 2). The uncatalyzed reaction of β-keto acid **3** with amine **2** was monitored by high performance liquid chromatography (HPLC) as a pseudo-first-order reaction. Product formation was assayed from 0 to 540 min and the second-order rate constant was calculated ($k_{uncat} = 4.55 \times 10^{-6}$ mM^{-1} min^{-1}). The k_{uncat} can be considered as k_{amine}, amine **2**-catalyzed decarboxylation of β-keto acid **3**. Catalysis by 27C1 was remarkably efficient compared with the non-catalyzed reaction, showing a large rate enhancement [(k_{cat}/K_m **3**)/k_{uncat} (the relative efficiencies over amine catalysis)] of 140,000; (k_{cat} (per binding site) = 1.29 min^{-1}, K_m (for **3**) = 2.1 mM, K_m (for **2**) = 6.7 mM, pH 8.0) (Fig. 2).

2.2. Cross-aldol reactions

To demonstrate the versatility and define the scope of antibody 27C1 for the cross-aldol reactions, we tested a variety of commercially available ketones **5**–**10** as donors and *p*-nitrobenzaldehyde