Dual mechanism of zinc-proline catalyzed aldol reactions in water†

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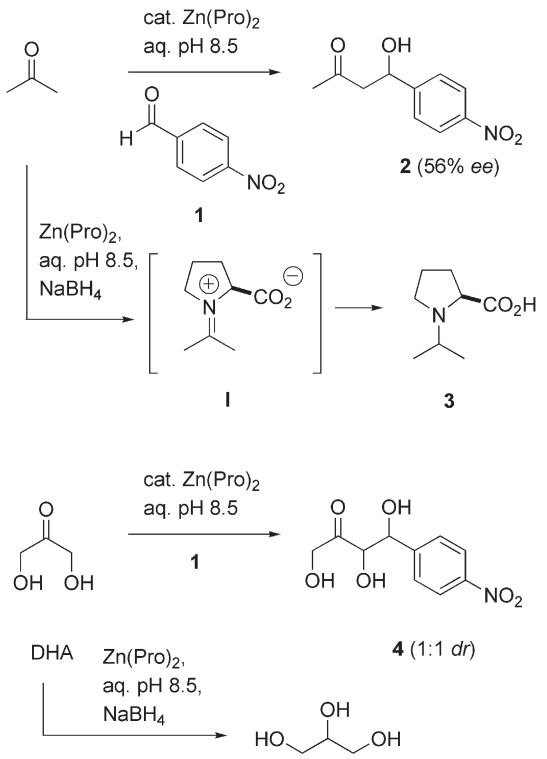
The aldol reaction of acetone with aldehydes in aqueous medium under catalysis by zinc-proline ($Zn(L\text{-}Pro)_2$) and secondary amines such as proline, (2*S*,4*R*)-4-hydroxyproline (Hyp) and (*S*)-(+)-1-(2-pyrrolidinomethyl)pyrrolidine (PMP) is shown to proceed by an enamine mechanism, as evidenced by reductive trapping of the iminium intermediate, while the aldol reaction of dihydroxyacetone (DHA) under catalysis by zinc-proline and by general bases such as *N*-methylmorpholine (NMM) is shown to occur under rate-limiting deprotonation of the α-carbon and formation of an enolate intermediate.

The aldol reaction is a key carbon–carbon bond forming reaction in organic synthesis. The reaction is catalyzed by enzymes using either an enamine (type I) or enolate (type II) mechanism.[1] The type I mechanism has inspired the design of aldolase catalytic antibodies[2] and related biocatalysts,[3] and the development of amino acids as organocatalysts for a variety of aldol and enolate type reactions.[4,5] Recently we showed that zinc-proline catalyzes aldol additions in aqueous medium,[6] including the self-condensation of glycolaldehyde to form carbohydrates,[7] a reaction of possible prebiotic significance.[8] Herein we report a mechanistic study of aqueous aldolization catalysis, showing that zinc-proline uses both enamine and enolate type mechanisms in a substrate dependent manner.

The reactions of acetone and DHA with nitrobenzaldehyde **1** to give the corresponding aldols **2** and **4** are both efficiently catalyzed by zinc-proline and were investigated as model reactions in aqueous buffer at pH 8.5 (Scheme 1). Zinc-proline is a water-soluble complex of zinc stable under these mild basic conditions, featuring both a Lewis-acidic metal center potentially capable of stabilizing an enolate, and two metal-coordinated secondary amines that could engage in enamine catalysis, as suggested earlier.[6b] Formation of the enamine proceeds *via* the iminium intermediate **I**, which should be trapped by reducing agents as has been shown for various aldolase enzymes.[9] Reaction of acetone with zinc-proline in the presence of $NaBH_4$ led to the quantitative formation of *N*-isopropyl proline **3**, implying that an iminium intermediate is formed with zinc-proline and acetone. **3** was also formed when proline was incubated with aqueous acetone and $NaBH_4$,[10] although to a lesser extent, in agreement with the modest aldolase activity of proline in aqueous conditions. On the other hand the reaction of $NaBH_4$ and DHA with zinc-proline or proline itself only gave glycerol without any trace of reductive alkylation at proline. While proline shows only negligible aldolase activity with this ketone, zinc-proline is very active for aldolization of DHA, which suggests that zinc-proline acts by an enolate mechanism in this case.

Catalysis by various amines was investigated to provide further evidence for type I and type II mechanisms (Fig. 1). The aldolization of acetone with **1** to form **2** in aqueous buffer pH 8.5 was catalyzed by zinc-proline, proline, Hyp and PMP, all of which are suitable for enamine formation. Hyp showed the highest activity and was efficiently converted to the *N*-isopropyl derivative in the presence of $NaBH_4$. These data suggest that acetone aldolization in water occurs only by an enamine (type I) mechanism, in agreement with an earlier study of enamine catalysis in water.[11] By contrast, the reaction with DHA was catalyzed by most of the amines, including tertiary amines such as NMM not capable of enamine formation, consistent with an enolate mechanism for DHA under general base catalysis. The rate of aldolization was correlated with the pK_a values of the amines, giving an apparent β-value of 0.45 (Fig. 2). This β-value indicates that a proton transfer step is rate-limiting in the reaction, presumably the enolate forming deprotonation at the α-carbon.



Scheme 1 Aqueous aldol reactions of zinc-proline and reductive trapping of the iminium intermediate.

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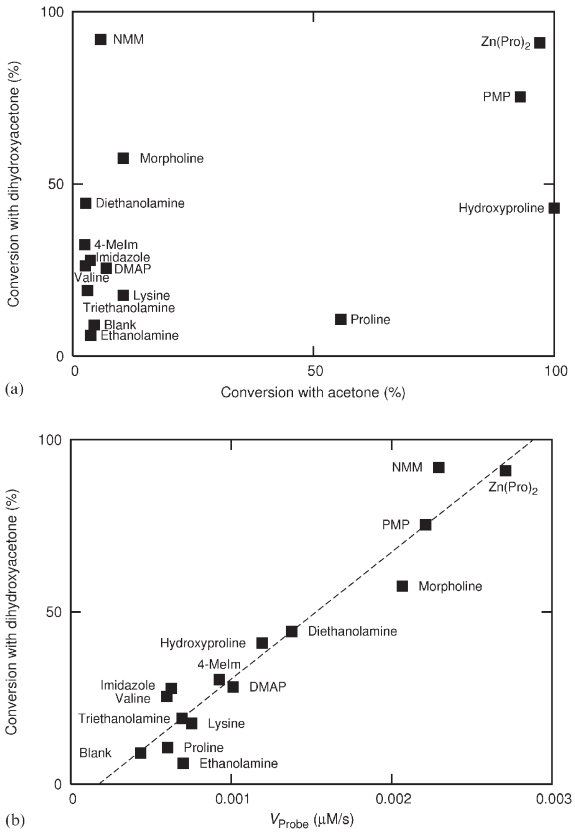


Fig. 1 Reactivity of zinc-proline and amines as catalysts for aldol reactions of nitrobenzaldehyde **1** and deprotonation of probe **5** in aqueous buffer pH 8.5. A) Aldol reaction with DHA (*y*-axis) and acetone (*x*-axis). B) Aldol reaction with DHA (*y*-axis) as function of the rate with probe **5** (*x*-axis). Conditions for aldol reactions: 100 mM **1**, 10 mM catalyst[a], 50% v/v aq. 20 mM bicine buffer pH 8.5, and 50% v/v 2 M DHA in MeOH, or 50% v/v acetone. Formation of aldols **4** (t = 36 h) or **2** (t = 3 h) was determined by RP-HPLC. Fluorogenic reaction of probe **5**: 200 μL assays in polypropylene 96-well plates were initiated by mixing 180 μL 20 mM catalyst in 20 mM aq. bicine buffer pH = 8.5 with 20 μL 1.0 mM **5** in buffer with 10% CH_3CN. The fluorescence increase was measured at λ_{em} = 460 ± 20 nm (λ_{ex} = 360 ± 20 nm) using a Cytofluor II plate reader from Perseptive Biosystems. [a]The pH of all stock solutions were adjusted to pH 8.5 with 0.1 M NaOH/HCl.

If deprotonation at the α-carbon is rate-limiting for the aldol reaction of DHA, the aldolase reactivity of the different amine

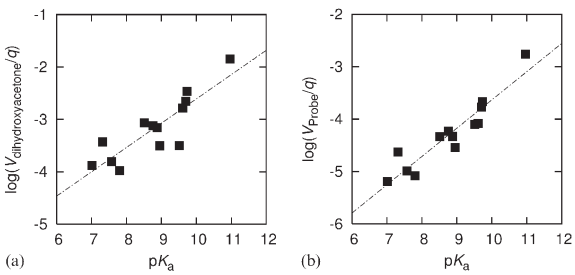
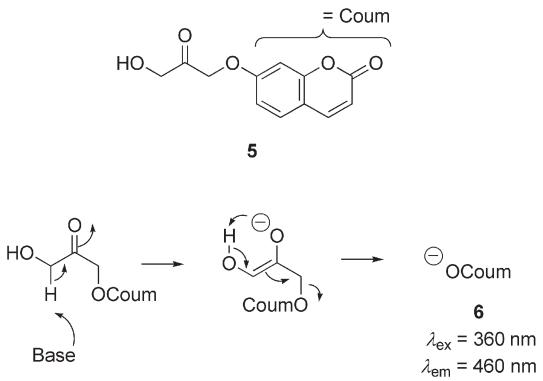


Fig. 2 Brønsted plots: A) Rate of aldol reaction between DHA and nitrobenzaldehyde (**1**) as a function of pK_a values of the different amines (β = 0.45, r^2 = 0.75). B) Rate of reaction of probe **5** as a function of pK_a values of the different amines (β = 0.55, r^2 = 0.88). Assay conditions are as in Fig. 1.



Scheme 2 The umbelliferyl ether of DHA **5** is a fluorogenic probe for enolization.

catalysts should also be apparent by measuring this deprotonation directly. The umbelliferyl ether of DHA **5** should allow measurement of the reaction by fluorescence since enolate formation should be followed by β-elimination of umbelliferone **6** due to the strong leaving group ability of this fluorophore (Scheme 2).[12] Ketone **5** indeed liberated umbelliferone under the action of zinc-proline and the various amine catalysts with an apparent β-value of 0.55, also consistent with rate-limiting α-CH deprotonation (Fig. 2B). The reactivity of the various catalysts with ketone **5** was proportional to their aldolization reactivity with DHA, providing further support for rate-limiting enolate formation in the aldol reaction (Fig. 1B).

The reaction of ketone **5** with $NaBH_4$ and zinc-proline, proline, or hydroxyproline only gave umbelliferone and the reduced 1,2-diol, with no detectable *N*-alkylation product as analyzed by HPLC, suggesting that enolization of **5** occurs *via* the enolate only. Nevertheless, the fluorogenic reaction of **5** was catalyzed by aldolase antibody 38C2 (K_m = 81 μM, k_{cat} = 0.16 min^{-1}, k_{cat}/k_{uncat} = 1570). The reaction was completely inhibited by acetyl acetone, showing that the active site lysine residue was responsible for the reaction. Antibody 38C2 also catalyzes aldol reactions with α-hydroxy ketones very efficiently,[13] and is probably using an enamine mechanism with these substrates as well as with **5**.

In summary, the formation of *N*-alkylation products with acetone and zinc-proline, proline or Hyp together with the absence of aldolization catalysis with tertiary amines, supports an enamine (type I) mechanism of aldolization with acetone as substrate. By contrast, the absence of *N*-alkylation of the same catalysts using DHA and the occurrence of aldolization under general base catalysis by various amines including tertiary amines indicate an enolate (type II) mechanism. The mechanistic switch from type I to type II from acetone to DHA is presumably caused by the OH groups, which lower the pK_a of the α-CH by inductive effect and probably stabilize the enolate by intramolecular hydrogen bonding, thereby facilitating enolate formation. Inductive effects by the OH groups should also destabilize the iminium cation, rendering enamine formation more difficult.

Zinc-proline appears to be a particularly efficient catalyst for both enamine and enolate type catalysis. This dual reactivity is shared by PMP and in part by Hyp, both of which feature a pyrrolidine with a relatively low pK_a (Hyp: 9.73, PMP: 7.31, 10.94) which can act as an enamine catalyst in one case and as a general base in the other case. Addition of base to zinc-proline induces

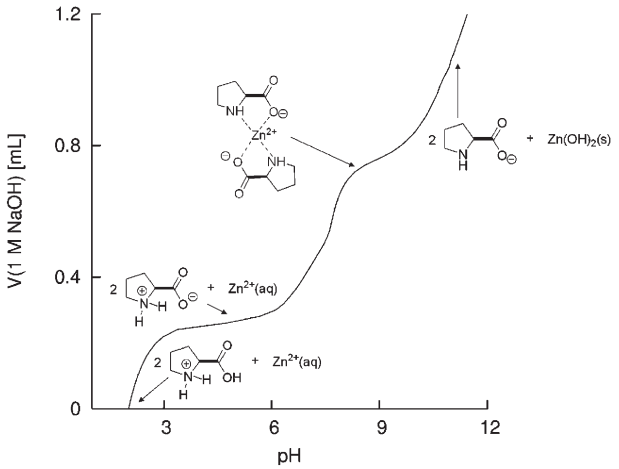
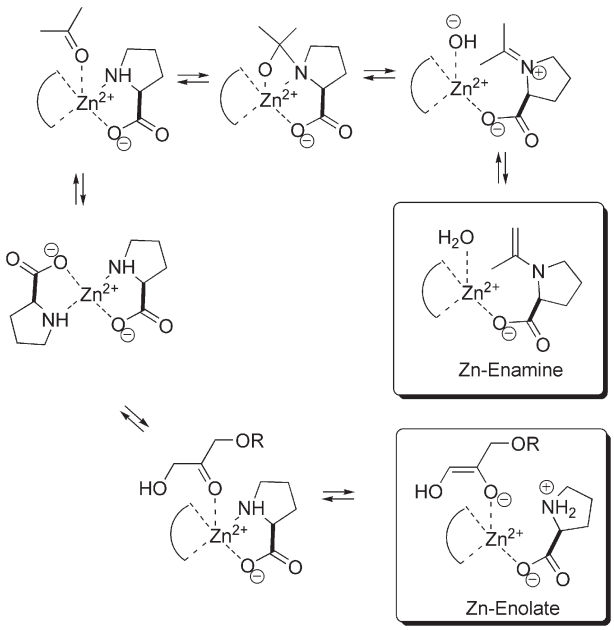


Fig. 3 Titration of 25 mL 10 mM Zn-Pro$_2$(aq) (acidified to pH ~2 with 1 N HCl) with 20 μL aliquots of 1 N NaOH to pH ~12.



Scheme 3 Mechanisms of zinc-proline catalysis.

precipitation of $Zn(OH)_2$ above pH 9, implying that the conjugate base $Zn(OH)(L\text{-Pro})_2$ is not available as a general base for deprotonating DHA. On the other hand the pH curve indicates that proline is readily decomplexed from zinc upon protonation from pH 8 to pH 6 (Fig. 3). We propose that zinc-proline catalysis involves coordination of the carbonyl oxygen to the acidic metal center, followed either by hemi-aminal, iminium and enamine formation with acetone, or deprotonation of the α-CH of DHA and probe **5** by the secondary amine to form a zinc-enolate (Scheme 3). Coordination of the aldehyde to the metal center is not necessary in kinetic terms since enamine formation is sufficient to explain catalysis,[11] but might explain why the reaction of acetone with **1** is enantioselective for (*S*)-**2** with zinc-proline but not at all with only proline in water.

The fluorogenic probe **5** reported here offers a simple means for measuring catalysis of α-carbon deprotonation, and provides a useful high-throughput screening assay for this process. The fact that the reactivity with probe **5** correlates with aldolase reactivity for a variety of catalysts suggests that it might be useful for high-throughput screening of aldolases under aqueous conditions.[14] Applications of this screening method to discover aldolases from combinatorial libraries of peptide dendrimers[15] will be reported in due course.

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