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Article

Investigation of the Effect of Turn Residues on Tetrapeptide Aldol Catalysts with β-Turn Propensity

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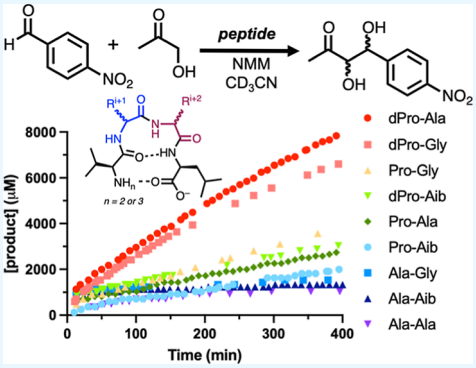
ABSTRACT: Peptide catalysts for a wide diversity of reaction types contain a common motif—residues that bias the sequence toward β-turn secondary structure. In this work, we explore what role that secondary structure plays in the catalysis of aldol reactions for primary amine tetrapeptide aldol catalysts. Using a lead tetrapeptide β-turn catalytic sequence, we varied the *i* + 1 and *i* + 2 residues to amino acids that would affect the β-turn propensity. We then studied the correlation between secondary structure, aldol rate enhancement, and stereoselectivity of the reaction between hydroxyacetone and 4-nitrobenzaldehyde. Using the *i* + 3 amide chemical shift as a measure of β-turn character, we found a rough correlation between the peptide structure and reaction kinetics but minimal effect on stereoselectivity. These trends may help aid the design of future catalytic sequences.



INTRODUCTION

Peptide catalysts are a fruitful middle ground for catalyst development between small-molecule organocatalysts and full-sized enzymes. Among low-molecular-weight peptides in organic solution, the inclusion of a β-turn motif is a remarkably common secondary structural element.[1−5] The ubiquity of this secondary structure motif in catalysts for a wide variety of reaction types[6−9] has driven our motivation to understand its role on rate enhancement and stereoselectivity. We have previously published studies on the role of β-hairpin secondary structure on catalytic activity in peptides that catalyze ester hydrolysis reactions.[10] In this work, we focus on the role of β-turn conformation in peptide catalysts of aldol reactions, a carbon-carbon bond-forming reaction of central importance that has been used in the synthesis of many complex molecules.[11]

Many peptide catalysts for aldol reactions have been reported.[3] Most contain a Pro residue at the N-terminal position using a pyrrolidine catalytic group similar to proline small-molecule aldol organocatalysts. However, there are a few examples of peptide aldol catalysts that use a primary amine as their catalytic group,[8] which is more analogous to the way Type I aldolase enzymes catalyze aldol reactions using Lys side-chain amines.[12] These examples use an N-terminal amine as the active catalytic site, engaging the substrate through enamine catalysis. One such primary amine peptide aldol catalyst reported by Wu and coworkers was the sequence H_2N-Val-dPro-Gly-Leu-OH.[8] The combination of dPro-Gly in *i* + 1 and *i* + 2 positions in a four-residue turn is widely accepted to strongly nucleate a β-turn[13−16] and in this case was intended to bias the sequence toward a β-turn secondary structure, a conformational hypothesis that was given credence by circular dichroism (CD) and NMR data.[8]

Our lab had previously begun investigating analogues of the H_2N-Val-dPro-Gly-Leu-OH lead sequence while developing a colorimetric assay for monitoring aldol reaction progress.[17] In that work, we noticed that the variation of the *i* + 1 and *i* + 2 turn residues had a dramatic impact on the reaction yield of the aldol reaction between hydroxyacetone and 4-nitrobenzaldehyde (as well as other substrates), even though the active catalytic site, the N-terminal amine, was not directly proximal to the residues being varied. That led us to hypothesize that conformational changes were affecting the reaction yield, which would more likely be affected by changes in the two interior residues of the tetrapeptide.

In this work, we sought to embark on a more thorough investigation of the relationship between the interior residues, the peptide conformation, and the resulting characteristics of the peptide catalysts, such as on rate enhancement and stereoselectivity for model aldol reactions, using traditional reaction monitoring techniques such as ^{1}H NMR and chiral chromatography. Based on the β-turn containing Val-dPro-Gly-Leu lead sequence, we varied the internal residues to

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include amino acids that would change the propensity toward β-turn conformation. Then, in the resulting small library of nine sequences, we studied the β-hairpin character of the peptide, rate enhancement measured by the initial reaction velocity, and stereoselectivity for model aldol reactions.

RESULTS AND DISCUSSION

Sequence Design. Based on the lead catalytic sequence H_2N-Val-dPro-Gly-Leu-OH, we sought to design analogue sequences that varied the internal $i + 1$ and $i + 2$ residues to alter its β-turn propensity. dPro-Gly is a combination of residues that strongly nucleates a β-turn,[13−16] while the replacement of dPro-Gly with Pro-Gly in short peptides has been reported to disfavor the β-turn conformation.[13,18,19] Therefore, we chose to compare sequences with dPro and Pro in the $i + 1$ position as well as Ala as a less conformationally restricted residue with a low putative propensity toward β-turn nucleation. For the $i + 2$ position, we chose to use Gly, Aib (aminoisobutyric acid), and Ala. Gly was selected because it is frequently found in the $i + 2$ position in turns. Aib was selected because it provides a more conformationally restricted contrast to Gly, yet α,α-disubstituted residues have also been found to promote β-turns when positioned in the $i + 2$ position next to Pro.[18,20]

Peptide Conformation. Short peptides contain a great deal of conformational mobility, and as such, we sought to investigate the secondary structural characteristics of the peptides in solution as opposed to obtaining solid-phase structures. In tetrapeptides, β-hairpins are defined by the presence of two backbone hydrogen bonds (Figure 1a). To analyze longer β-hairpins, CD and NMR techniques such as chemical shift deviation analysis can be useful,[21] but for sequences as short as tetrapeptides, the turn residues consist of such large proportion of residues that these techniques are less diagnostic. Other works that have studied the β-turn conformation of tetrapeptides have used $^3J_{NH-H\alpha}$ values to calculate dihedral angles to see if they match what is expected for β-hairpins or β-turns.[18,22] However, these are extracted from the i and $i + 4$ NH resonances and therefore require tetrapeptides to be acetylated. The peptides we were studying did not have Val NH resonances since they necessitated free N-termini for their catalytic activity. Although NOE signals between nonadjacent residues can be used to provide evidence for hairpin structures, it was not amenable to use as a means of comparison of the degree of β-turn character of the peptides.

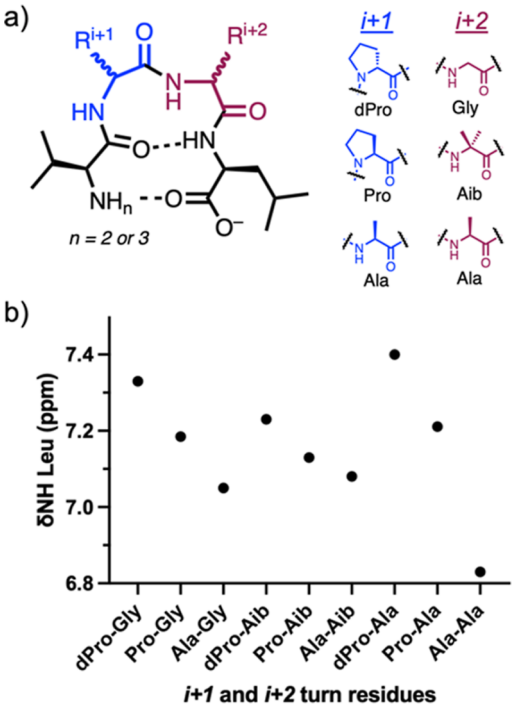

Figure 1. (a) Internal $i + 1$ and $i + 2$ residues were varied of a reported aldol β-turn tetrapeptide catalyst, H_2N-Val-dPro-Gly-Leu-OH. (b) Comparison of the Leu amide chemical shift for each sequence showed variation related to the hydrogen bonding in which this residue is engaged, providing a means to compare the β-turn character of the sequences.

In order to compare the degree of β-turn character of the peptides, we used the $i + 3$ amide chemical shift (Leu residues in all of our sequences), which varies related to the degree of hydrogen bonding this position is engaged in, with more downfield shifts indicative of more hydrogen bonding and thus hairpin formation. Additionally, there is literature precedent for using the terminal amide chemical shifts to provide evidence for β-hairpin formation in very similar tetrapeptide sequences (Ac-Val-dPro-dAla-Leu-NMe_2).[13] Although the Val NH chemical shift was found to exhibit the most variation in that example, in our system, the N-termini are free amines for catalytic activity, necessitating reliance on the relatively smaller variance in the Leu NH chemical shift.

The Pro-containing sequences were observed to have duplicate sets of peaks, likely arising from proline in the cis and trans rotamers,[18] so the Leu NH chemical shifts from each isomer were averaged according to their abundance. (This was not observed dPro-containing sequences in accordance with prior observations that β-turn tetrapeptides require adoption of the trans rotamer and that heterochiral sequences favor trans rotamers.[13]) As seen in Figure 1b, the variation in Leu NH chemical shifts confirmed many of the hypotheses generated when selecting residues to include in the turn positions. For instance, the dPro-Gly and dPro-Ala containing sequences were the furthest downfield, indicating the greatest degree of β-hairpin character, as expected. Sequences with Ala in the $i + 1$ position had upfield Leu NH chemical shifts, indicating lower degree of hydrogen bonding and β-hairpin character, which also matched expectations. However, the Pro-Gly was not among the sequences with the least hairpin character and had a similar Leu NH chemical shift as Pro-Aib and dPro-Aib despite our expectation that Aib in the $i + 2$ position would be highly turn nucleating.

It should be noted that in the previous report the Leu NH chemical shift was concentration-independent over a wide range of concentrations but did shift upon aggregation at higher concentrations for some sequences,[18] so we carefully controlled the concentration of the peptides to be consistent with the concentration at which they were used in the aldol reaction. Because not all of the peptides were fully soluble in the solvent (acetonitrile-d_3), we determined the amount of peptide that had dissolved fully after sonication and vortexing through the use of an external NMR standard by spiking samples with a known quantity of maleic acid and integrating relative to the external standard. The NMR spectra used to determine the $i + 3$ NH chemical shift were collected without the presence of *N*-methylmorpholine (NMM).

Aldol Reaction Kinetics. In order to monitor the rate enhancement afforded by the peptide, we performed the

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reaction in NMR tubes such that the reaction could be monitored by ^{1}H NMR. We performed the aldol rection in deuterated acetonitrile to match the structural analysis experiment. The reagents were combined in an NMR tube without agitation, and an acquisition was collected at regular time intervals to collect kinetic data on the reaction progress. A peak corresponding to an aromatic 4-nitrobenzaldehyde starting material was compared to a product aromatic peak to determine the percentage conversion (as shown in the Supporting Information). The concentration of the product relative to the reaction time for different peptide catalysts is plotted in Figure 2b. The linear region of the reaction curve (from 0 to 400 min) was used to determine the initial reaction velocity through a linear regression.

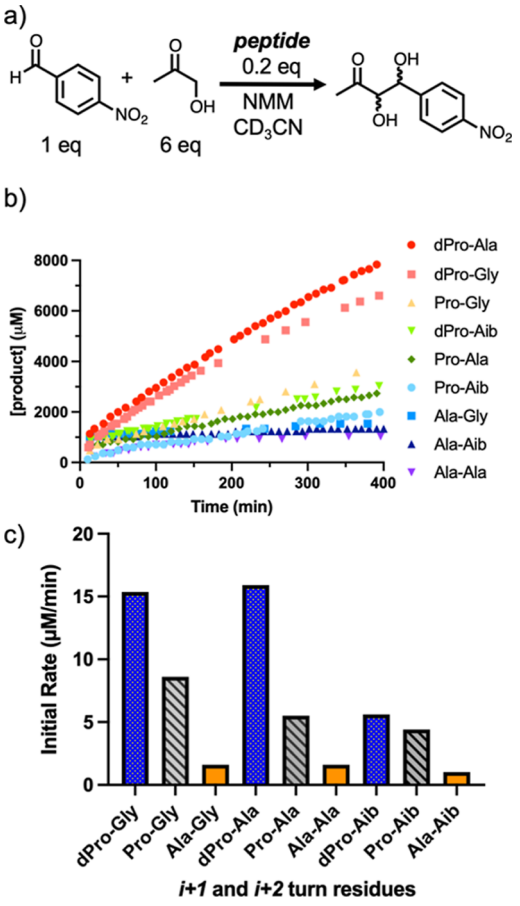

Figure 2. (a) Model aldol reaction studied between 4-nitrobenzaldehyde (1 equiv) and hydroxyacetone (6 equiv) with *N*-methylmorphylene as a base and 20 mol % peptide as the catalyst. (b) Reactions were monitored by ^{1}H NMR to calculate the initial velocity. (c) Trends in the reaction rate based on the residues in the $i + 1$ and $i + 2$ positions. The sequences are listed in order with sequences with the same $i + 2$ residue next to each other. The color of the bar corresponds to the residue in the $i + 1$ position: blue is dPro, gray stripe is Pro, and orange is Ala.

To compare the tetrapeptides as catalysts, we used the simple and widely used model substrates 4-nitrobenzaldehyde and hydroxyacetone. NMM was used as a base for consistency because the peptides were supplied as TFA salts. To investigate the role of the NMM and the peptide, three control experiments were performed (data in the Supporting Information). In the first, the reaction was set up under identical reaction conditions with the reagents and NMM but no peptide catalyst. This experiment showed no product formation over the time period of observation (10,000 min), indicating the peptide to be the catalytically active species. The second control reaction was set up with all reagents including a peptide catalyst with a sequence analogous to the lead catalytic sequence with an acylated N-terminus (Ac-Val-dPro-Gly-Leu-OH). This reaction also resulted in no observable reaction, confirming the hypothesis that the N-terminus of the peptide was the active catalytic species. The third control reaction was set up with a peptide catalyst (Val-dPro-Ala-Leu-OH) but no NMM base. The resulting initial rate was about half of the initial rate of the reaction carried out with the NMM base.

Figure 2c compares the initial rate of the aldol reaction catalyzed by each peptide sequence. The peptide sequences with dPro-Gly and dPro-Ala in the turn positions resulted in the most rate enhancement. Sequences with Ala in the $i + 1$ position and Aib in the $i + 2$ position resulted in lower initial rates. Consistently, the sequence resulting in the highest initial rates was the one with dPro in the $i + 1$ position, followed by Pro, followed by Ala, no matter what residue was in the $i + 2$ position. The trend for the $i + 2$ position was not as consistent.

Aldol Reaction Stereoselectivity. Chiral chromatography was used to separate the product isomers and examine the diastereo- and enantioselectivities of the catalysts. Although moderate diastereoselectivity was observed favoring the syn product, there was not much variation between the catalysts (we were unable to obtain data for three of the sequences). Minimal enantioselectivity was observed, and there were no discernible trends between catalysts (Figure 3b). Although the initial report of the lead catalytic sequence H_2N-Val-dPro-Gly-Leu-OH used chiral agents to achieve greater enantioselectivity,[8] we did not use these in our reactions as we sought to isolate the role of the peptide secondary structure.

Correlation of Peptide Conformation and Kinetics. Based on our findings that varying the internal $i + 1$ and $i + 2$ turn residues in a primary amine tetrapeptide aldol catalyst does influence both the degree of β-hairpin conformation in solution and the aldol reaction rate, we wondered what type of correlation, if any, existed between these two features. Plotting the rate enhancement versus the Leu NH chemical shift, as seen in Figure 3c, showed a crudely linear trend. The sequences with downfield Leu NH reflecting higher relative degrees of hairpin formation are the sequences that resulted in higher initial rates for the aldol reaction. This observation of the relationship between the peptide secondary structure and rate enhancement will aid in the design of future catalytic sequences.

CONCLUSIONS

Much of the interest in peptide catalysts is for their diastereo- and enantioselectivities,[4,5,23] with notable examples of peptide catalysts capable of regioselective catalysis.[24] A great deal of the work on peptide catalysts seeks to use secondary structural elements, such as β-turns, to enhance the catalysis. Our recent work has sought to better understand the specific role that the peptide conformation plays on catalysis. For ester hydrolysis catalysts, we previously found that non-hairpin analogues resulted in higher initial reaction velocities than otherwise identical catalytic sequences with β-hairpin conformation.[10] In this paper, we focused on the role of β-turns in primary amine tetrapeptide aldol catalysts. However, we did find the β-turn character of the catalyst to correlate with rate enhancement.

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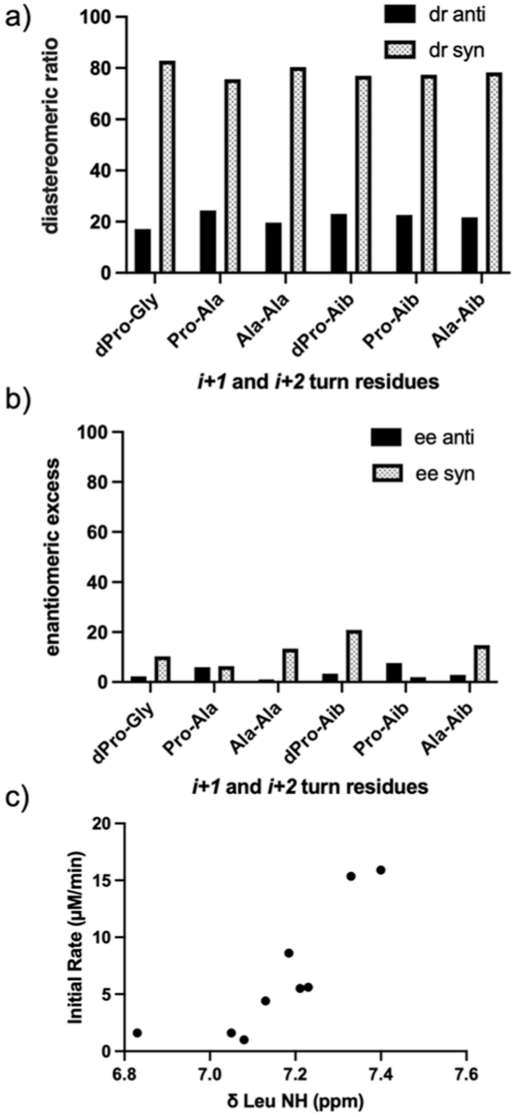

Figure 3. (a) Diastereomeric ratio favored syn products but did not vary much between catalysts. (b) Enantiomeric excess was low and also did not show notable trends between peptide catalysts. (c) Plot showing the relationship between the initial rate from each catalyst versus the Leu amide chemical shift corresponding to the degree of β-turn character of the sequence.

While the secondary structure often plays a role in the stereoselectivity of a catalyst, unexpectedly, we did not find that to be the case in this system. These findings show that a single NMR shift can roughly correlate with catalytic activity, providing a potential predictive tool for selecting new catalytic sequences. Our results help isolate the specific role of the β-turn secondary structure on catalytic activity for this type of primary amine tetrapeptide aldol catalyst and serve as a basis for predicting and designing catalytic sequences. Future work may focus on mechanistic studies to further understand this phenomenon.

EXPERIMENTAL METHODS

Peptide Preparation. The peptides were purchased as HPLC purified (>95% purity) TFA salts from the company Biomatik. The mass and purity were verified before use by LC-MS.

Determination of Peptide Concentration. In an NMR tube, 0.8 mg of peptide dissolved in 200 μL of acetonitrile-d_3 was combined with 60 μL of 0.07 M maleic acid solution (in acetonitrile-d_3), and the volume was adjusted to 600 μL with acetonitrile-d_3 such that the final concentration of maleic acid was 0.007 M. In the resulting ^{1}H NMR spectrum, the singlet peak at 6.32 ppm corresponding to the maleic acid and the Leu H_α resonance near 4.5 ppm were integrated to calculate the peptide concentration.

Monitoring Aldol Reaction Kinetics. Stock solutions of 4-nitrobenzaldehyde (75 mM), hydroxyacetone (450 mM), and *N*-methylmorpholine (75 mM) were made in acetonitrile-d_3 in dram vials. 100 μL of each of these stock solutions was added to an NMR tube for a final concentration of 12.5 mM 4-nitrobenzaldehyde (1 equiv), 75 mM hydroxyacetone (6 equiv), and 12.5 mM *N*-methylmorpholine (1 equiv). The peptide was added to a final concentration of 2.5 mM (0.2 equiv, variable volume depending on the concentration of the peptide stock solution as determined in the external standard experiment). The reaction mixture was diluted to a final volume of 600 μL with acetonitrile-d_3. Upon mixture of all reactants, the time was recorded and ^{1}H NMR spectra were acquired using a Bruker Advance III 400 MHz NMR spectrometer every 10 min within the first 2 h after the reaction started, every hour for the next 12 h, and daily for the next week. At each time point, the peaks at 8.4 and 8.2 ppm, corresponding to aromatic protons from the reactant and product, respectively, were integrated, and the percent conversion was calculated.

Chiral Chromatography. Each crude reaction mixture was dried using a BioChromato Spiral Plug Smart Evaporator. After evaporation, the solid crude reaction mixture was dissolved in 40 μL of hexane and 60 μL of isopropanol and then syringe filtered prior to chiral chromatography on a Waters Aquity Arc UHPLC with UV/Vis detector using a Chiralpak IA-3 column (Daicel Chiral Technologies). A gradient of 2−44% isopropanol over 44 min was run. In the resulting chromatograms, the four peaks with elution time between 11 and 17 min were integrated to calculate the stereoselectivity. The chromatograms of the crude reaction mixtures were compared to that of the product purified according to published procedures (data in the Supporting Information).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c05921.

Additional details on the materials and instrumentation used, characterization data including ^{1}H, TOCSY, and NOESY NMR spectra of peptides, proton assignments, data for the control reactions, data from chiral chromatography, and details of calculation of the kinetic data (PDF)

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Notes

The authors declare no competing financial interest.

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