Communication

Crystal-to-Crystal Synthesis of Triazole-linked Pseudo-proteins via Topochemical Azide-Alkyne Cycloaddition Reaction

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Crystal-to-Crystal Synthesis of Triazole-linked Pseudo-proteins via Topochemical Azide-Alkyne Cycloaddition Reaction

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Supporting Information Placeholder

ABSTRACT: Isosteric replacement of amide bond(s) of peptides with surrogate groups is an important strategy for the synthesis of peptidomimetics (pseudopeptides). Triazole is a well-recognized bioisostere for peptide-bond and peptides with one or more triazole units are of great interest for different applications. We have used a catalyst-free and solvent-free method *viz.* topochemical azide-alkyne cycloaddition (TAAC) reaction to synthesize pseudoproteins with repeating sequences. A designed β-sheet-forming L-Ala-L-Val dipeptide containing azide and alkyne at its termini (N_3-Ala-Val-$NHCH_2C{\equiv}CH$, **1**) was synthesized. The single crystal XRD analysis of the dipeptide **1** showed parallel β-sheet arrangement along 'b' direction and head-to-tail arrangement of such β-sheets along 'c' direction. This head-to-tail arrangement along 'c' direction places the complementary reacting motifs *viz.* azide and alkyne of adjacent molecules at proximity. The crystals of dipeptide **1** upon heating at 85 ˚C underwent crystal-to-crystal polymerization giving 1,4-triazole-linked pseudoproteins. This TAAC polymerization was investigated by various time-dependent techniques such as NMR, IR, DSC and PXRD. The crystal-to-crystal nature of this transformation was revealed from polarizing microscopy and PXRD experiments and the regiospecificity of triazole formation was evidenced from various NMR techniques. MALDI-TOF spectrum showed the presence of pseudoproteins size more than 7 kDa.

Peptides constitute one of the important building block of the biosphere and many peptide based natural biomaterials such as silk, wool *etc*., instilled interests in developement of synthetic peptide-mimics with attractive properties.[1] Peptidomimetics, synthetic mlecules that mimic the structure and often the function of peptides, attract much attention due to their easy synthesis, improved stability, attractive properties over traditional peptides and find use in many fields spanning from medicine to materials.[2] Isosteric replacement of amide bond constitute an important strategy for peptidomimetics synthesis and such peptides with amide-surrogates are called pseudopeptides.[3] Triazole is one of the widely accepted isostere for peptide bond due to its robust and biocompatible nature.[4] Polytriazolylpeptide are attractive polymers in the field of material science. However, the traditional synthesis of such polymers would involve Cu(I) catalyzed click polymerization of monomers having azide and alkyne functionalities.[5] Apart from the unavoidable use of solvents, the purification of oligopeptides is cumbersome as the metal catalyst can bind to the polymer formed. Topochemical reactions, proximity driven reaction in a crystal lattice,[6] represent a plausible alternative that avoid catalysts, solvents and purification. We have developed topochemical azide-alkyne cycloaddition (TAAC) reaction for the synthesis of triazole linked oilgomers/polymers of carbohydrates and nucleosides.[7] We herein report the synthesis of triazole-linked pseudoproteins or pseudopolypeptides.

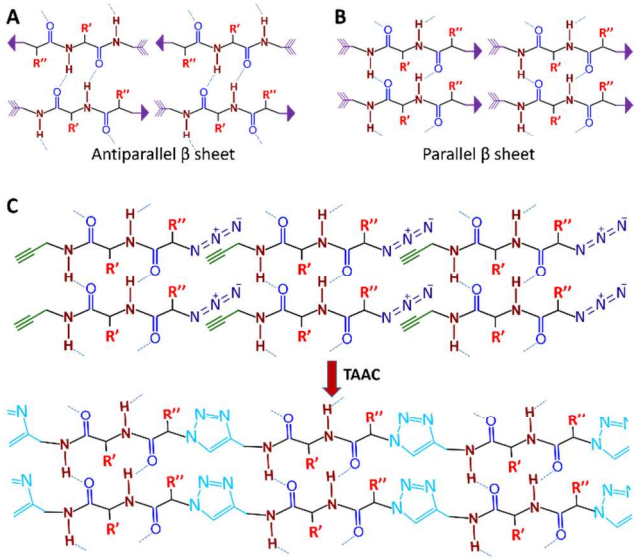

Figure 1. (A) Antiparallel and (B) parallel β-sheet arrangement of peptides. (C) Proposed head-to-tail arrangement of β-sheet forming dipeptide mimic with CRMs and their topochemical reaction.

The essential criterion for topochemical reaction is the proximity of reacting motifs in the crystal lattice and design of molecules that upon crystallization place the reacting partners at distances suitable for their topochemical reaction is one of the major challenges in this field. It is well known that valine and alanine rich small peptides (di or tripeptides) adopt β-sheet packing in their crystals (Figure 1A & 1B, Supporting Information). Also, the adjacent sheets arrange in such a way that peptides form head-to-tail arrangement in the direction perpendicular to the direction of the sheet formation. We envisioned that peptides with L-Ala and L-Val and modified with azide and alkyne, two complementary reacting motifs (CRMs), at their termini would crystallize in β-sheet packing and this would bring azide and alkyne motifs of adjacent molecules in close proximity, which would undergo TAAC reaction between them forming oligo/poly-triazolylpeptides (Figure 1C).

Dipeptide **1** was synthesized from L-valine and L-alanine (see the Supporting Information) and crystallized from DCM/toluene

mixture. FTIR spectrum of crystals of the dipeptide **1** showed the CO stretching band at 1630 cm^{-1} indicating the formation of the β-parallel assembly.[8] Single crystal X-ray analysis revealed that dipeptide **1** crystallizes in the monoclinic C2 space group (Figure 2B). As anticipated, dipeptide **1** adopts parallel β-sheet packing along "b" direction *via* N5-H5…O4 (2.10 Å) and N4-H4…O2 (2.13 Å) hydrogen bonds (Figure 2C). In addition, two C-H…O hydrogen bonds *viz.* C1-H1…O2 (2.31 Å; 150.8°) and C3-H3…O4 (2.44 Å; 149.8°) also help in stabilization of the β-sheet assembly. The shortest distance between azide and alkyne is 3.7 Å (N1…C6) and the distances between the termini of azide and alkyne are 4.1 Å and 4.7 Å. Though these distances satisfy the proximity criterion, the azide and alkyne motifs are not in parallel orientation, an arrangement necessary for their cycloaddition, but at an angle of 72.2°. However, it would be possible to attain the essential parallel geometry by minor conformational changes of the flexible propargyl and the azide groups.

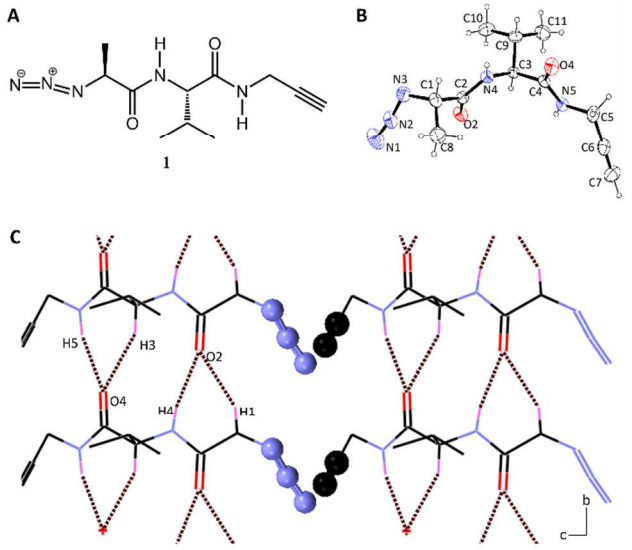

Figure 2. (A) Chemical structure and (B) ORTEP diagram of the dipeptide **1**. (C) Packing arrangement of dipeptide **1** along the "bc" plane, showing the proximal placement of azide and alkyne motifs (highlighted in ball-and-stick model). The brown dotted lines represent the hydrogen bonds, N5-H5…O4 and N4-H4…O2 along the "b" direction.

In order to check the feasibility of topochemical reaction of dipeptide **1**, its crystals were heated. The crystals did not melt even at very high temperature (300 °C) suggesting that the crystals may be undergoing polymerization upon heating. Differential Scanning Calorimetric (DSC) analysis of the crystals of **1** also did not show any endothermic peak that can be ascribed to melting. On the other hand, DSC profile showed an exothermic peak at around 150 °C, which could be due to the exothermic dipolar cycloaddition reaction between azide and alkyne (Figure 3A). The IR spectrum of heated crystals also suggested that the azide and the alkyne reacted during heating; while the signals due to azide and alkynyl group were sharp and strong in the starting crystals, the intensity and sharpness of these peaks reduced to an insignificant level after heating, suggestive of their consumption (Figure 3B). Also the solubility of the heated crystal was very low in common solvents (Supporting Information), ascribable to the formation of large polymers. ^{1}H NMR spectrum of the crystals heated at 153 ˚C for 1 h, showed a complex mixture of products having both 1,4- and 1,5-triazolyl linkages (Figure S2, Supporting Information) suggesting uncontrolled and non-selective polymerization.

In order to do a systematic study of this reaction, we have kept crystals of dipeptide **1** at 85 ˚C and withdrawn small fraction of it at regular intervals for various time-dependent studies. Time-dependent FTIR spectroscopy with these crystals revealed that the azide stretching band at 2103 cm^{-1} gradually decreased with time of heating. This suggests that the azide gets consumed gradually with time, probably by reacting with the alkyne (Figure 3C). As stated earlier, DSC analysis of peptide showed an exothermic peak at 153 ˚C ascribable to the sudden, uncontrolled reaction between azide and alkyne. DSC studies with the crystals kept at 85 ˚C for different durations revealed that the intensity of the exothermic peak due to uncontrolled thermal reaction at around 150 ˚C decreased with increase in duration of pre-heating at 85 ˚C (Figure 3D). This also suggests that some of the azide and alkyne groups are gradually reacting during the preheating thereby reducing the number of azide and alkyne left for their uncontrolled reaction at high temperature. Also the extent of reaction depends on the duration of pre-heating.

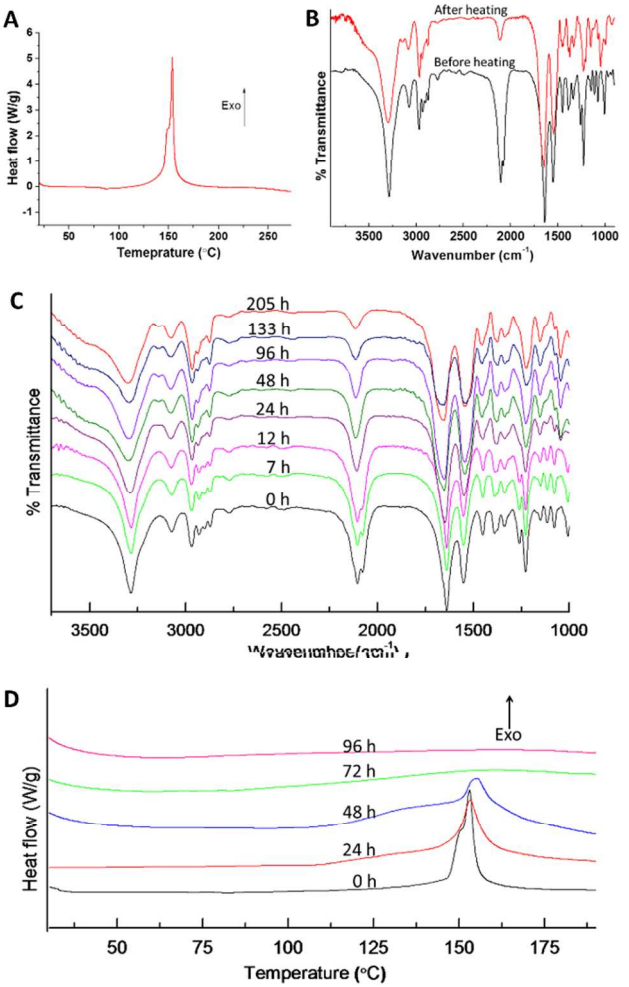

Figure 3. (A) DSC profile of crystals of dipeptide **1**. (B) IR spectra of crystals before and after heating at 153 ˚C. (C) Time-dependent IR spectra during the course of TAAC reaction of dipeptide **1**. (D) Time-dependent DSC analyses during TAAC reaction of dipeptide **1**.

The kinetics of the reaction was studied by time-dependent ^{1}H NMR spectroscopy (Figure 4A). One portion of the pre-heated crystals withdrawn at each time was analyzed by ^{1}H NMR spectroscopy after dissolving in DMSO-d6. It was observed that the reaction started at 7 h of heating as evident from the emergence of new signals notably the ones due to triazolyl proton at δ 7.93,

methyne proton connected to triazole at δ 5.56 and methylene protons as dd at δ 4.42. The intensities of these signals increased gradually with time along with concomitant reduction in the intensities of signals due to the dipeptide **1**. This trend continued till the reaction was 89% complete in 6 days after which the reaction reached a state of stagnancy. A plot of % of the reaction against time revealed that the reaction followed a sigmoidal kinetics, as anticipated for a topochemical reaction (Figure S1B, Supporting Information).

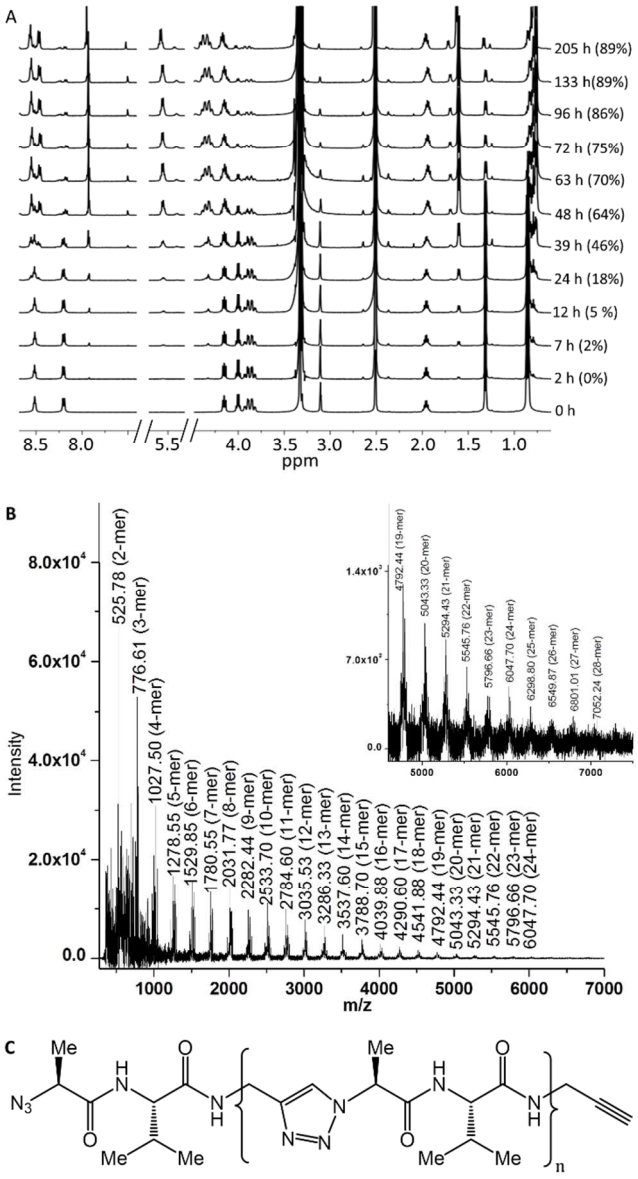

Figure 4. (A) Kinetics of the TAAC reaction at 85 °C monitored by ^{1}H NMR. (B) MALDI-TOF spectrum of polymerized crystals. (C) Chemical structure of oligopeptides obtained by TAAC.

The MALDI-TOF mass spectrum of the soluble fraction of the heated crystals showed peaks corresponding to oligomers from dimer to 28-mers (similar size to a 7 kDa protein) with gradual decrease in their intensities. This decrease in intensities with size suggests that the solubility decreases with increase in size of the polymer (Figure 4B) and this is in line with the trend of other triazole-based polymers.[7a-c] Many natural proteins are as small as 7 kDa.[9] It is interesting to note that we could make pseudopeptides of sizes similar to small functional proteins.

While the reaction under high temperature proceeded non-selectively, distinct and clear ^{1}H NMR signals were observed for each proton throughout the reaction at 85 ˚C suggesting the regiospecific formation of only one kind of triazole linkages (linkage homogeneity) in all the oligomers formed (Figure 4C). In order to know which of the two possible regiomeric triazoles is formed in the reaction, we have chromatographically isolated a few oligomers (trimer and tetramer) and characterized extensively using various spectroscopic techniques (Supporting Information). In both the cases, it was found that 1,4-triazole is formed in the reaction. The very similar NMR pattern of trimer, tetramer and even higher oligomers suggests that in all the oligomers/polymers, the 1,4-triazolyl linkage is conserved.

Even after 89% of the reaction was over, the crystals were morphologically intact. Polarizing microscopic images of dipeptide **1** before and after the polymerization reaction show birefringence, suggesting that the crystallinity is maintained throughout the course of the reaction (Figure 5A & 5B).[6ad] This was also evident from the time-dependent PXRD analysis. A comparison of the PXRD spectra of the crystals pre-heated for different durations suggested that the reaction occurred in a crystal-to-crystal fashion (Figure 5C). Diffraction pattern of polypeptide (a heated single crystal of dipeptide **1**) also suggested its crystalline nature. However we failed in solving its crystal structure (Figure S15, Supporting Information). It is clear that the crystals of the dipeptide **1** undergo crystal-to-crystal topochemical azide-alkyne cycloaddition upon mild heating giving oligomers/polymers having only 1,4-triazolyl linkages. The regiospecificity is very interesting as the parent crystal did not have any orientation of azide and alkyne that favor any of the two possible regioisomers. However, from a close look at the crystal structure, it is clear that the both propargyl and azide groups can rotate around C-N single bond, without significant steric hindrance, to adopt a conformation, wherein the azide and the alkyne orient in anti-parallel arrangement and favors 1,4-triazole formation. Also in this conformation, the azide and alkyne are at much more favorable distances for their easy topochemical reaction (Figure 5D).

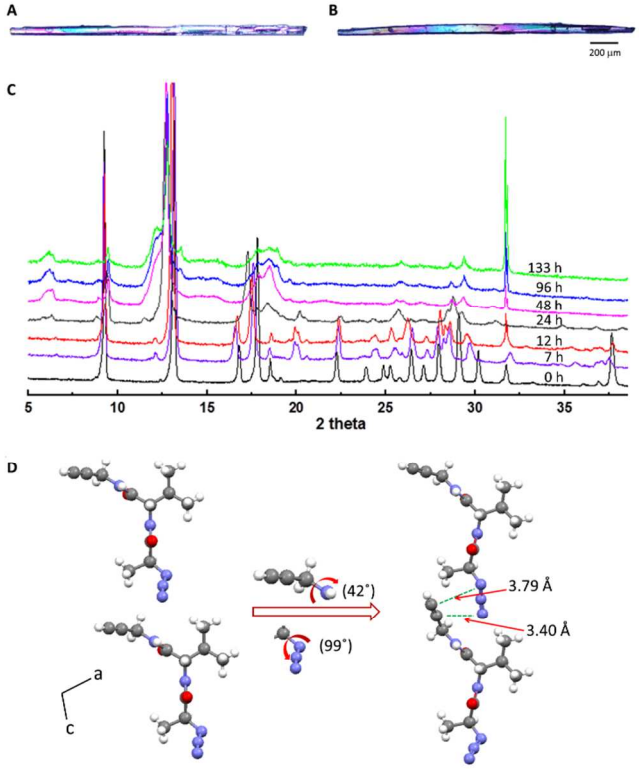

Figure 5. (A) Polarizing microscopic image of the crystals of dipeptide **1** (A) before and (B) after TAAC. (C) Time-dependent PXRD spectra of crystals of dipeptide **1** kept at 85 °C for various durations. (D) Plausible rotation of alkyne and azide giving TS-like-arrangement for 1,4-regioisomer.

In summary, we have designed a dipeptide, decorated with two complementary reacting motifs *viz.* azide and alkyne, which can self-assemble to form β-sheet structures in its crystal with proximally placed azide and alkyne from adjacent sheets. The crystals of this modified dipeptide underwent smooth topochemical azide-alkyne cycloaddition reaction upon heating yielding triazole-linked polypeptides, which are otherwise difficult to synthesize using conventional solution phase reactions. Though the azide and alkyne were not properly oriented, for the topochemical reaction, in the crystal, the flexibility of the propargyl and azide groups and the empty space around them allowed their rotation, which lead to a transition state-like anti-parallel arrangement of azide and alkyne at short distance and this lead to the regiospecific formation of 1,4-triazolyl-linked polypeptide in a crystal-to-crystal fashion. This is the first synthesis of pseudoprotein or pseudopolypeptide in the solid state. This proof-of-concept would generate interests in topochemical synthesis of several pseudopolypeptides with repeating sequences for various applications.

ASSOCIATED CONTENT

Supporting Information

Details of synthesis and characterization of dipeptide **1** and oligomer (trimer **9** and tetramer **10**), DSC, PXRD, SXRD, IR, NMR, TGA and MALDI-TOF. This material is available free of charge via the internet at http://pubs.acs.org.

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