Abstract: Three peptides from two unique collagen sequences (α1 and α2 chains) of two dinosaur species i.e. Tyrannosaurus rex and Brachylophosaurus canadensis had previously been found to overlap with the integrin binding site on the rat collagen microfibril structure and was reported to be a significant non-random distribution suggesting mechanisms of protein survival over long geologic periods. The sequences of these peptides featuring certain conserved residues as was revealed by analyzing multiple alignments from different organisms, functional importance of recognition motif and the nature of collagen itself favoring conservation, were used to computationally model the triple helix structure and the resultant structure was docked with the crystal structure of α1 (I) Integrin domain (PDB Code-2M32). Similar patterns of interaction were observed between the receptor i.e. α1 (I) Integrin domain and each of the different ligands i.e. modelled collagen structure of the two dinosaur species and the synthetic collagen mimetic peptide (present in the original complex of 2M32).

Keywords: Dinosaur, collagen, docking, integrin, modelling

1. Introduction

The retrieval of bio-molecular information of evolutionary value from fossils is a coveted yet often elusive goal. Although both proteins and DNA are components of the tissues of all organisms, they undergo degradation over geological time. The time range over which meaningful protein and DNA sequence information is preserved depends on a variety of variables such as the environmental temperature, humidity and the type of matrix in which the molecules are contained [1]. It is widely accepted that proteins have the potential to survive significantly longer periods of time than DNA [2]. Asara et al. (2007) [3] reported the sequencing of proteins from 68-million-year-old Tyrannosaurus rex (Museum of the Rockies-MOR 1125) fossil and established similarities between dinosaur and chicken genomes. The authors generated seven T. rex peptides by matching mass spectra against collagen proteins. Collagen peptide sequences were subsequently derived from a second dinosaur, Brachylophosaurus canadensis (MOR 2598) [4], and included many of the earlier lines of supporting evidence as well as independent replication of data in multiple labs.

The dinosaur collagen peptide sequences that have been determined by Mass spectrometry were mapped onto the 3 dimensional structure of rat collagen micro-fibril and a non-random localization to specific regions within the structure was reported [5]. Out of the many features of peptide distribution reported by them two features were concluded by the workers as statistically significant. One of the feature was a non-random co-localization of three peptides (from two unique sequences of α1 and α2 chains) belonging to the two species i.e., T. rex and B. canadensis, to a region on the collagen monomer identified to be the integrin binding site that promotes cell- collagen interactions, angiogenesis and osteoblast differentiation.

In the present study the structures for the dinosaur collagen peptides have been computationally modelled and docked with an Integrin structure available from PDB. The primary structure of the collagen itself with its repeating GXY triplets along its entire sequence favors computational structure prediction and analysis possible. An analysis of the docked protein-protein complex, is carried out for identifying any similarities in the patterns of interacting residues.

2. Literature Survey

Eleven fossil derived peptide sequences from the two dinosaurs, T. rex and B. canadensis[3],[4] were mapped on molecular models of extant human and rat collagens [5] and it was found that they were non-randomly distributed in several respects. In particular, fossil sequences mapped to regions of the protein partly shielded by tight molecular packing [6], which may physically stabilize and protect them from enzymatic degradation, thus contributing to their preservation. In fact, the only position where alpha 1 chain peptides [i.e., Peptide „GVQGPPGQPGP” of both T. rex and B. canadensis] co-localize with an alpha 2 chain peptide [i.e., Peptide GLPESGAVGPA (I*)GR of B. canadensis/T. rex*] mapped to the integrin binding site that promotes cell-collagen interactions, angiogenesis, and osteoblast differentiation; its fibril location and association with severe mutations also suggest its crucial nature [7] and hence strong selective pressure for conservation of sequence.

Integrins α1β1 and α2β1 are the major integrin collagen receptors [8]. The α1, α2, and α10 subunits each contain an “inserted” (I) domain near the N terminus. The „I” domains

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have been shown to contain a ligand-binding site and a MIDAS motif, which needs to be occupied by an appropriate cation for effective ligand binding by the integrin. Recombinant proteins duplicating these small (approximately 200 amino acids) I domain polypeptide segments effectively bind collagen; presumably it is these regions that are responsible for the integrin’s binding to collagens [9].

A specific collagen motif recognized by the α2-I domain has recently been identified as the hexapeptide GFOGER (O-5 hydroxyproline) within the context of a collagen triple helix [10]. Analysis of published data suggests a critical role for the central integrin binding site, GFPGER, forming the critical recognition motif for the integrins on collagen present in a majority of species as evident from the alignment was performed using COBALT and the results were submitted to THeBuScr along with setting of parameters to build the structure of collagen and the output was obtained in pdb format. The modelled collagen structures were assessed using CCP4MG [21]-[23]. The structures of dinosaur collagen peptides modelled using THeBuScr ver. 1.07 are presented (Figs 3a and 3b). The collagen peptides are of type I, consisting of two alpha 1 and one alpha 2 chains. Evaluation of the modelled structures of dinosaur collagen peptides by „RAMPAGE” (Ramachandran Plot Assessment) indicated that 100.0% of the residues lied in the favoured region which is greater than the expected value (~98.0%) suggesting the reliability and the quality of structure prediction. Also the result obtained from performing a pair-wise structure alignment of the dinosaur collagen micro-fibril with rat collagen micro-fibril (PDB code 3HQV) using „RaptorX” showed how the modelled structures exhibited a similar fold as that of the rat micro-fibril structure. This is reflected in the computed TM scores of the top solutions i.e., 0.4420 (>0.3) for the modeled collagen structures of both the dinosaur species. Visual inspection of the top ranking refined solution structures showed that the dinosaur collagen peptides binds to a trench shaped groove of the α1 (I) Integrin domain (Figs 4a and 4b) which is comparable to the binding of collagen mimetic peptide to the α1 (I) Integrin domain in the PDB structure 2M32.

4. Results and Discussion

The results that were obtained by submitting the multiple sequence alignment from COBALT to „ConSurf” it was seen that certain residues of the peptides were identical in different organisms included in the alignment, and coloured according to ConSurf’s conservation score (Fig 1). As can be seen from the ConSurf coloured structure of 2M32 (Fig 2a) the region where the collagen triple helix (displayed as a grey backbone) binds features highly conserved residues which are involved in ligand binding and thus implies their functional importance. The same can be attributed to the ConSurf coloured structures of modelled dinosaur collagen docked with the α1 integrin I domain in which the region where the collagen binds i.e. the trench shaped groove contains residues that have been conserved (Figs 2b and 2c).

A comparative analysis of the α1(I) Integrin domain bound with collagen mimetic peptide complex (PDB code 2M32) and the refined solution structures of dinosaur collagen docked with the α1(I) Integrin domain to identify the residues involved in the protein-protein interaction was done using SPPIDER. The SPPIDER analysis revealed that the residues involved in ligand binding and thus implies their functional importance. The same can be attributed to the ConSurf coloured structures of modelled dinosaur collagen docked with the α1 integrin I domain in which the region where the collagen binds i.e. the trench shaped groove contains residues that have been conserved (Figs 2b and 2c).

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A comparative analysis of the α1 (I) Integrin domain bound with collagen mimetic peptide complex (PDB code 2M32) and the refined solution structures of dinosaur collagen docked with the α1 (I) Integrin domain to identify the residues involved in the protein-protein interaction was done using SPPIDER. The SPPIDER analysis revealed that the following residues i.e., N80, S81, Y116, G77, Q86, D114, G115, E116, S117, H118, S145, R148, F156, E159 of α1 (I) Integrin domain i.e., of the receptor molecule were involved in the protein-protein interaction and was common to all the complexes analyzed- 2M32, T. rex and B. canadensis collagen docked with α1 (I) Integrin domain.

Visualization and analysis in CCP4MG showed that the residues Lys131, Glu132 and Thr133, Glu136 of the receptor formed H-bond interaction with Arg6 of leading strand and...
Gly$^{115}$ of the receptor formed H-bond interaction with Gln$^9$ and Arg$^6$ of the middle strand in the refined model of T. rex collagen docked with α1 (I) Integrin domain. Similarly the residues Ile$^{16}$, Ser$^{13}$ and Arg$^76$ of the receptor formed H-bonds with Arg$^6$ of middle strand of collagen and Ser$^{15}$ of the receptor formed H-bond interaction with Gln$^9$ of middle strand of collagen ligand in the refined model of B. canadensis collagen docked with α1 (I) Integrin domain. The different residues involved in interaction between the docked dinosaur collagen peptides and the α1 (I) Integrin domain is presented in Fig 5.

Figure 1: Multiple sequence alignment of peptides (a) GVQGPQGPGPR, (b) GLPGESGAVGPIGR and (c) GLPGESGAVGPPGSR color coded by ConSurf. [TRBC-collagen peptide from the alpha 1 (I) chain of both Tyrannosaurus rex and Brachylophosaurus canadensis; TR-Tyrannosaurus rex; GG- Gallus gallus; MA- Mammut americanum; HS- Homo sapiens; MM-Mus musculus; AP-Anas platyrhynchos; OH- Ophiophagus Hannah; FD-Fukomys damarensis; RN- Rattus norvegicus; MB- Myotis brandti; HG- Heterocephalus glaber; PA- Pteropus alecto] "GFPGER" critical motif highlighted.

Figure 2: Visualization of evolutionarily conserved residues (colored spheres) of 2M32 α 1 (I) integrin bound with (a) collagen present in the original structure (b) T. rex collagen and (c) B. canadensis collagen (shown as grey backbone in foreground). The residues in the region where the collagen triple helix binds, are conserved showing its functional significance (ConSurf output- coloration according to conservation score and viewed in Rasmol)

Figure 3: T. rex(a) and B. canadensis(b) collagen modelled using THeBuScr and visualized in Rasmol [Chain A-Blue; Chain B- Green; Chain C- Red] (Display type- Space fill; Color- by chain type)
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References


Figure 4: Interface representation of modeled T. rex (a) and B. canadensis (b) collagen docked with α1 (I) Integrin domain. The collagen triple helix binds across a surface trench in the α1 (I) Integrin domain (displayed as a grey molecular surface). The leading, middle and trailing strands of the collagen triple helix shown here in cyan, magenta and green respectively.

Figure 5: CCP4MG analysis of the refined solution structure of modelled (a) T. rex collagen and (b) B. canadensis collagen docked with α1 (I) Integrin domain displaying the residues (labeled) involved in the interaction. [Receptor chain residues Ser (13), Ser (15) and Thr (81) coordinating with the Mg2+ ion (green sphere) displayed in blue dashed lines; H-bonds between the residues of receptor and ligand displayed in red dashed lines]


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