

Effect of C-H...O Interactions in the Structural Stability of "All-Alpha" Proteins

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Abstract: A set of single chain all-alpha proteins has been examined to determine the contribution of C-H...O interactions to the protein stability. The study of C-H...O contacts is the prerequisite for comprehending the folding processes. There are number of amino acid residues that can form hydrogen bonds via their side chains in addition to their peptide group. Perhaps, the highest contribution in this category is the aromatic residues such as Phe, Tyr and Trp. A total of 1061 C-H...O interactions were found in a data with a set of 75 single chain all-alpha proteins. The most prominent were the side-chain to main-chain C-H...O interactions (SM- C-H...O). The importance of this interaction is revealed by the high degree of conservation observed by them in protein structures. 74% of the stabilizing centers in the single chain all-alpha proteins were involved in C-H...O interactions. These interactions are mainly formed by long range contacts. Moreover, the study shows that there is an average of one C-H...O interaction observed for every 17 residue in the single chain all-alpha proteins data set. It is thus concluded that the C-H...O interaction can, indeed, be categorized as a true stabilizing force like hydrogen bond in single chain all-alpha proteins.

Key words: C-H...O interactions; single chain all-alpha proteins; conservation score; Interaction range; stabilizing centers.

Introduction

Hydrogen bonds, hydrophobic interactions and salt-bridges are all known for providing stability and specificity to the folded structure of proteins^{1,2,3,4}. Apart from these conventional interactions, it is generally admitted that the weak interactions termed unconventional C-H...O interactions contribute to the structural stability of proteins, which is not limited to small molecules but extended to a biological system. When it was first proposed many years ago^{5,6}, there was some resistance due to the low electro negativity of C which was presumed to make it a weak proton donor. However, this idea was later supported on the basis of the IR data^{7,8,9} and the geometry of molecular complexes in the gas phase^{10,11,12} and in crystalline environment^{13,14}. This C-H...O hydrogen bond

systems share numerous features with the more traditional hydrogen bonds, such as geometric preference, NMR chemical shifts and electron density patterns. It has now gained a wide acceptance as a genuine hydrogen bond^{15,16}.

In another study the occurrence of C-H...O interactions in protein¹⁷ was analyzed by categorizing them into interactions that involve only the main-chain atoms, side-chain atoms, or both. The same study clearly reveals the role of weak C-H...O interactions in conformational flexibility and in facilitating protein-protein interactions¹⁷. They also have their importance in a variety of functional contexts such as macromolecular recognition^{18,19,20}, enzymatic action²¹ and stabilization of secondary structure²².

The strength of C-H...O hydrogen bonds has also been an intriguing subject of computational studies^{23,24,25,26,27,28,29,30}. The previous *ab initio* quantum mechanical calculations on C-H...O hydrogen bonds estimated the strength to be 2-3 kcal/mol, about half as strong as the conventional O-H...O hydrogen bonds²⁵. Even though the C-H...O contributes much less in energetic terms, the observation that they can occur as frequently as, or sometimes more frequently than regular hydrogen bonds, suggests that they could individually contribute less, but cumulatively provide significant energy for the stability of protein.

Possibilities of C-H...O interactions have also been investigated in nucleic acid and carbohydrate structures³¹, and are involved in protein-nucleic acid interactions³². Recently, we published our results on the influence of C-H... π interactions in single chain all-alpha proteins³³. In continuation of that work, we report here our results on the studies of C-H...O interactions on single chain all-alpha proteins, which could be an important and interesting supplement to our earlier communication. In addition to that, a comprehensive comparison of non-covalent interaction with non-conventional hydrogen bond, C-H...O interactions in the single chain all-alpha proteins data set was made. It is the objective of the present paper to carry out just this sort of systematic comparison of the non covalent and non conventional interactions in which single chain all-alpha proteins structures are stabilized.

For all this study, only one chain in the protein structure has been chosen. These represent relatively simpler systems in which all the weaker interactions can be studied in the absence of the effects of a complex quaternary structure and the occurrence of redundancy in the data set. The frequency and extent of conservation presented unequivocally show that the C-H...O interactions cannot and must not be neglected. The consideration of these important interactions might enhance the usefulness of these calculations in general, and further our understanding of protein structures and their functions.

Materials and Methods

Data set

We have selected a set of 75 non-redundant single chain all-alpha proteins, with sequence identity less than 25%, using the sequence analysis package EMBOSS. EMBOSS is an EBI tool that can be used for pair wise alignment of protein sequences. The coordinates of the proteins have been taken from the PDB³⁴. The PDB codes of the proteins used for the analysis are shown in Table 1. The single chain all-alpha proteins have been selected from the following

five folds as classified by the SCOP³⁵ (1) cytochrome c (a.3), (2) DNA/ RNA binding 3-helical bundle (a.4), (3) four helical up and down bundle (a.24), (4) fold/EF hand like (a.39) and (5) alpha-alpha super helix (a.118).

Computational of Unconventional C-H...O interactions

C-H...O interactions were identified, using the program available for this purpose called HBAT³⁶. The C-H...O interactions considered here were between all the possible donor C-H groups in single chain all-alpha proteins structures (C $_{\alpha}$ -H, C $_{\text{ali}}$ -H and C $_{\text{aro}}$ -H) and oxygen containing proton acceptor molecule. The oxygen atoms in proteins are of the hydroxyl, carbonyl and carboxyl type. In terms of their electro negativity, this increases in the order O-H < C=O < C-O-. The distances from C and H to the main chain carbonyl O is 3.8 Å and 3.3 Å and the angles C-H...O and H...O=C is 120° and 90° respectively. The C-H...O interaction types are represented by a two-letter code in which the first letter indicates the donor atom and the second the acceptor: M and S represent the main-chain and side-chain atom respectively. The C-H...O interactions are classified into four types namely, main-chain to main-chain C-H...O interactions (MM- C-H...O), main-chain to side-chain C-H...O interactions (MS- C-H...O), side-chain to main-chain C-H...O interactions (SM- C-H...O) and side-chain to side-chain C-H...O interactions (SS- C-H...O)³⁶. The position and geometry is adapted from the earlier work of Babu³⁷.

Interaction Range

The definition of short, medium and long-range interactions in a protein is based on (i) the amino acid residues and (ii) their respective locations in the sequence. For each residue, the sequential distance of surrounding residues (within a sphere of 8 Å radius) was analyzed^{38,39,40}. The residues within a distance of two residues are considered contributors to short-range interactions, whereas, those within a distance of ± 3 or ± 4 residues contribute to medium-range and those more than four residues apart contribute to long-range interactions. This classification enables us to evaluate the contribution of short range, medium-range and long-range contacts in the formation of C-H...O interactions. The composition of the surrounding residues associated with this residue is calculated for a sphere of radius 8 Å due to the fact that the influence of each residue over the surrounding medium extends effectively only up to 8 Å and is sufficient to characterize the hydrophobic behavior of amino acid residues and to accommodate both the local and non-local interactions⁴¹.

Stabilization centers

Stabilization centers are clusters of residues that are involved in medium or long-range interactions⁴². Residue clusters are identified in protein contact maps where an accumulation of long range interactions is observed. The residues in these cores are called stabilization center (SC) residues, referring to their suspected role in 3D structure stabilization and are identified as follows. The sequence environment of each residue pair involved in a long range interaction is analyzed. Each such residue pair is located in two additional pairs, one in the N-terminal flanking tetrapeptide and the other in the C-terminal tetrapeptide of the original interacting residue pair making the most long range interactions with each other. If the number of interactions of these two triplets, the central interacting residues plus the two additional ones, one on each flanking side is equal to or greater than seven of the possible nine contacts, then the two central residues are accepted as members of an SC. The stabilization centers for the C-H...O interacting amino

acid residues were computed using the SCide program⁴³ for computing the stabilization centers.

Conservation Score

We computed the conservation score of C-H...O interacting amino acid residues in each protein using the ConSurf program⁴⁴. This program computes the conservation based on the comparison of the sequence of a PDB chain with the proteins deposited in Swiss-Prot⁴⁵ and finds the ones that are homologous to the PDB sequence. The number of PSI-BLAST iterations and the E value cutoff used in all similarity searches were 1 and 0.001, respectively. All the sequences that are evolutionarily related to each one of the proteins in the data set were used in the subsequent multiple alignments. Based on these protein sequence alignments, the residues are classified into nine categories from highly variable to highly conserve. Residues with a score of 1 are considered highly variable and those with a score of 9 are highly conserved.

Table 1. List of PDB codes of single chain ‘all-alpha’ proteins considered for analysis of C-H... O interactions

a.3 Fold	a.4 Fold	a.24 Fold	a.39 Fold	a.118 Fold
1A56	1AOY	1A7D	1CDP	1B89
1C52	1C20	1CGN	1E14	1EYH
1C2N	1D5V	1CPQ	1IG5	1HF8
1CC5	1D8J	1DOV	1IJ5	1HO8
1CCH	1G2H	1G5Z	1K9P	1HU3
1CCR	1GVD	1GS9	1MHO	1HZ4
1CRY	1GXQ	1KTM	1Q80	1IB2
1CYJ	1IG6	1LPE	1RK9	1KLX
1E8E	1JGS	1NFN	1RRO	1LRV
1F1F	1LEA	1NZE	1SRA	1M8Z
1GDV	1LFB	1O3U	1TOP	1OYZ
1GKS	1MIJ	1SR2	2SAS	1PBV
1LS9	1P4W	1TQG	3PAT	1PAQ
1YCC	2EZI	2A0B	5PAL	1TE4
451C	2HTS	2MHR	5TNC	2BCT

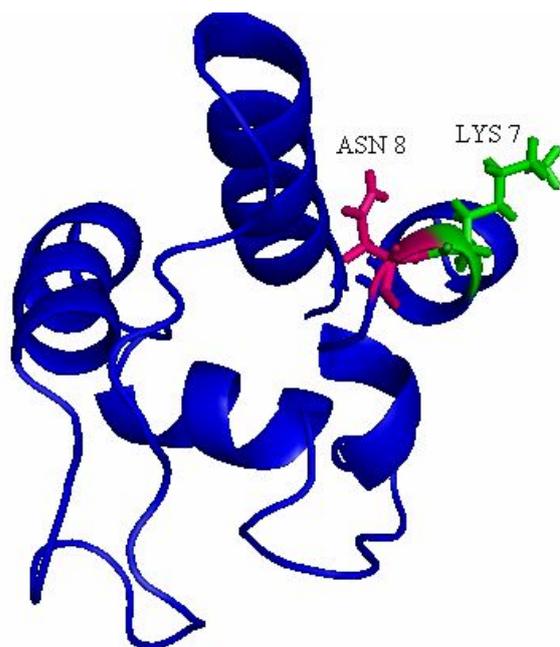


Fig. 1 C-H...O interactions in Single Chain all-alpha Proteins [PDB Code 1A56 between Asn 8 and Lys 7]

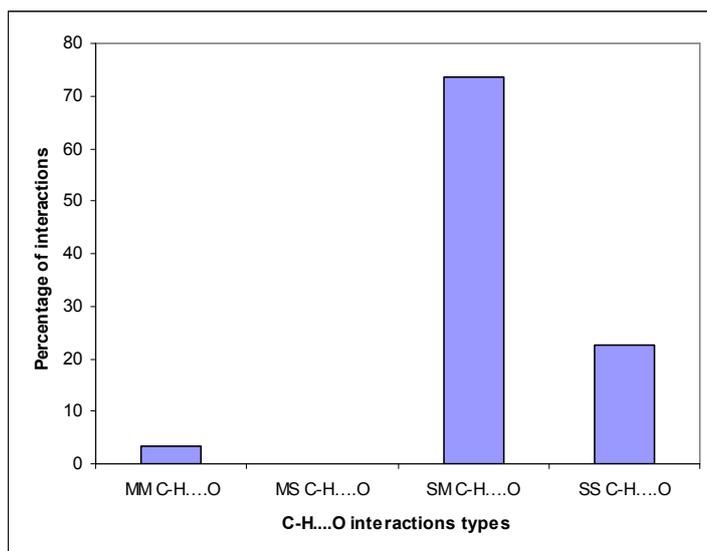


Fig. 2 Different types of C-H...O interactions and their contribution in Single Chain all-alpha Proteins data set.

Results and Discussion

C-H...O interactions

The analysis of C-H...O contacts from the total set of 75 single chain all-alpha proteins were first calculated using the program HBAAT³⁶. The selected group of single chain all-alpha proteins shows a total of 1061 interactions and there was an average of one C-H...O interaction for every 17 residue in single chain all-alpha proteins structure investigated in this work. The Fig. 1 shows an example in which the amino acid residue Asn 8 in the single chain all-alpha protein has C-H...O interactions with Lys 7 of the same protein

chain. Among the four types of C-H...O interactions observed, 74 percentages of interactions were from S-M-C-H...O ones. This result was consistent with our earlier reports on antimicrobial peptides⁴⁶. This is shown in Figure 2. The interaction is plotted with respect to C...O distances and H...O distances in Figure 3 and 4, respectively. The distance C...O, representing the donor-acceptor distance; the distance H...O, or the hydrogen bond distance; the crystallographic refinement programs treat close C...O distances as repulsive. Figure 3 shows the frequency of occurrence of these interactions as a function of the

distance value. The mean distance of 3.4-3.8 Å corresponds well with the earlier reports, but does not account for the numerous shorter distances found in all the investigated proteins. This quantity is generally correlated with ΔE , with stronger H-bonds associated with a shorter length. It is, therefore, interesting to find H-bond distance rather uniform in all C-H...O H-bonds, covering a rather narrow range between 3.4 and 3.8 Å. This range agrees quite well with the H-bond length of 3.35 Å measured by neutron diffraction for the interaction between a C ^{α} H group and a water molecule⁴⁷, as well as the average C ^{α} ...O distance of 3.31 Å in parallel β -sheets⁴⁸. An oxygen atom approaching a carbon along its covalent bond with

hydrogen should not be closer than 3.7 Å, unless there is a significant cohesive interaction. Likewise, when the line of approach is away from the hydrogen, the strong repulsive van der Waals' force limits the C...O distance to 3.4 to 3.8 Å. H...O distances between 1.6 and 3.2 Å were analyzed. The maximum interactions were observed in the range of 2.4 to 2.8 Å, below the sum of the van der Waals radii. It is interesting to note that the maximum interactions observed in the range of 2.4-2.8 Å are identical to the earlier reports given by Taylor and Kennard⁴⁹. In each of the cases of study, there is a significant number of short contacts, strongly suggesting a mixed population containing both cohesive interactions and van der Waals' contacts.

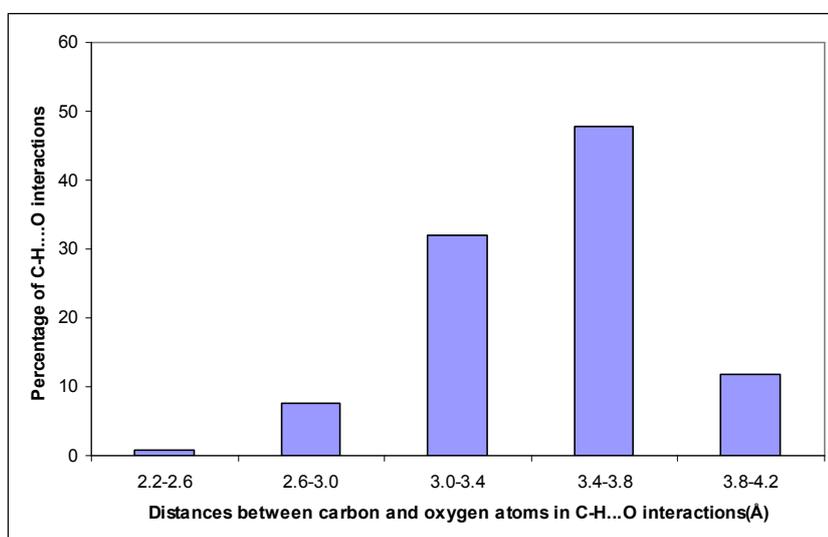


Fig. 3 Observed distribution of C-H...O interactions as a function of interatomic (C...O) distance in the Single Chain all-alpha Proteins data set

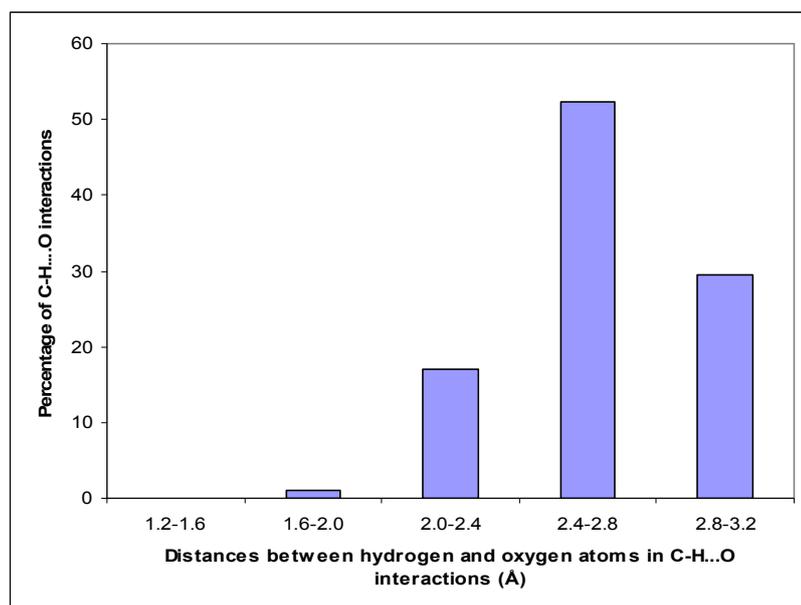


Fig. 4 Observed distribution of C-H...O interactions as a function of interatomic

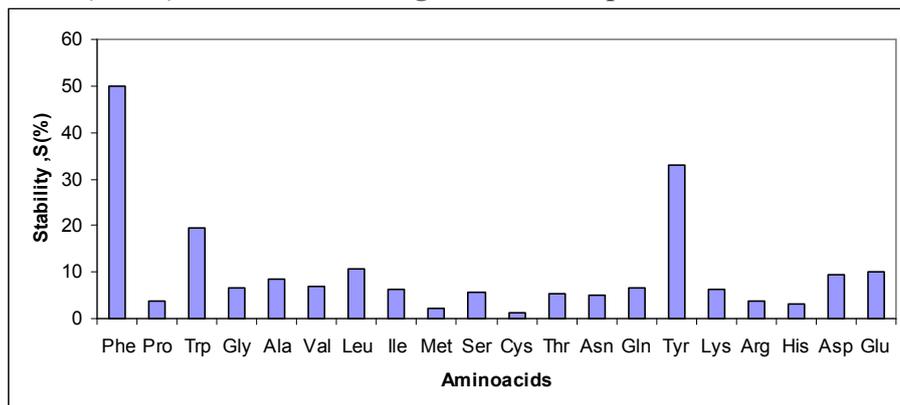
(H...O) distance in the Single Chain all-alpha Proteins data set

Fig. 5 Aminoacids contributions to the stability of Single Chain all-alpha Proteins data set.

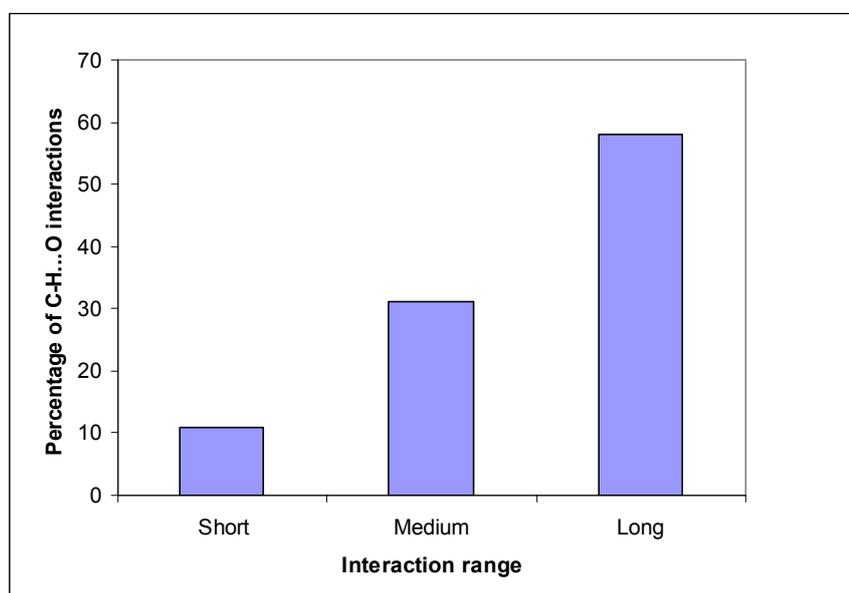


Fig. 6 Percentage of Short, Medium and Long range C-H...O interactions in the Single Chain all-alpha Proteins data set

In order to identify the percentage contribution by an amino acid to the stability, the ratio between the numbers of interactions involving a particular amino acid to the total number of interactions involving all the amino acids was calculated and denoted as S.

$$S = \frac{\text{Interactions involving a particular amino acid}}{\text{Total number of interactions}} \times 100$$

The values of S obtained for all the amino acids were plotted in Fig. 5. Out of the 20 residues, Phe is the most common amino acid involved in such interactions. This is likely because Phe side-chain is found more suited for the C-H...O interaction than the other residues. It is imperative to note that the highest

occurrence of Phe among the amino acid in single chain all-alpha proteins.

Interaction Range

The contribution of C-H...O interactions in the set of single chain all-alpha proteins could be defined as either the local or the global stability of the proteins. Therefore, there is a need to evaluate the contribution of inter-residual C-H...O interactions. The residues that are within a distance of two residues are considered to contribute to short-range interactions, whereas those within a distance of ± 3 or ± 4 residues contribute to medium-range and those more than four residues to long-range interactions^{38,39,40}. This classification enables us to evaluate the contribution of short-range, medium-range and long-range contacts in the formation of C-H...O interactions. The sequential distance between residues that contributed to C-H...O

interactions were calculated and results depicted in Fig. 6. About 11% of the C-H...O interactions were observed as short range interactions, 31% and 58% of C-H...O interactions were found to be medium-range and long range interactions respectively. Long-range C-H...O interactions are the predominant type of interactions in the set of single chain all-alpha proteins studied. These results indicate that the long-range C-H...O interactions contribute significantly to the global conformational stability of single chain all-alpha proteins structure.

Stabilization centers

Stabilization centers are clusters of residues that are involved in medium or long range interactions⁴². We have computed the stabilization centers for all single chain all-alpha proteins structure investigated in this work by using the program Scide⁴³. We observed a total of 309 residues as stabilization centers in the set of 75 single chain all-alpha proteins studied. It is worth stressing that 92% of the stabilizing centers residues in the single chain all alpha proteins structure take part in the C-H...O interactions either as a donor or as an acceptor.

Conservation Score

We used the ConSurf program to compute the conservation score of amino acid residues involved in C-H...O interactions in single chain all-alpha proteins. 23% of the amino acid residues that contributed donor atoms in C-H...O interactions had the highest conservation score of 9, while 54% of the amino acid residues had a conservation score in the range of 6-8. Thus, 77% of the donor amino acid residues had a high conservation score. In the case of amino acid residues that contributed the acceptor atoms in C-H...O interactions, 14% of the acceptor amino acid residues had the highest conservation score of 9, while 55% of the amino acid residues had a conservation score, in the range of 6-8. Thus, 69% of the acceptor amino acid

residues had a high conservation score. From these observations, we were able to infer that most of the amino acid residues involved in C-H...O interactions might be conserved in single chain all alpha proteins. The conservation of amino acid residues may in some cases be linked to their involvement in C-H...O interactions and to the stability or the function of the protein.

Conclusions

Quantitative evaluation of 75 different single chain 'all-alpha' protein reveals the fundamental nature of this important noncovalent interactions. We find that C-H...O interactions are common-one favorable interaction can be expected for every 17 residues of protein length. In addition, our finding indicates that almost 70 percentage of C-H...O interacting residues are highly conserved. This analysis clearly brings out that the weaker interactions that contribute to the stability of single chain all alpha proteins cannot be ignored. Albeit weak, they are numerous and therefore, might help explain the well-known problem that protein stabilities, interaction energies and folding energies cannot be calculated very accurately. To sum up, the analysis reported here support the notion that C-H...O interactions can be categorized as true H-bonds, although they, of course, tend to be weak due to the normally lesser proton donating ability of C as compared to that of O. These results make a compelling case that C-H...O interactions should be considered alongside the more conventional hydrogen bonds, salt bridges and hydrophobic effects in any analysis of protein structure.

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