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Influence of C-H...O Interactions in the Structural Stability of Single Chain "All-Alpha" Proteins: A Comparative Study with Non-Covalent Interactions

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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 importance of this interaction is revealed by the high degree of conservation observed by them in protein structures. These interactions are mainly formed by long range contacts. Moreover, the study shows that 74% of the stabilizing centers in the single chain all-alpha proteins were involved in C-H…O interactions. Furthermore, the comparison between the non covalent and non conventional interactions clearly depicts the significance of C-H…O interaction in the stability of single chain all-alpha proteins. 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.

Keywords: C-H…O interactions; non-covalent 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 [1-4].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 [7-9] and the geometry of molecular complexes in the gas phase [10-12] 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 protein [17] 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 interactions [17]. They also have their importance in a variety of functional contexts such as macromolecular recognition [18-20], enzymatic action [21] and stabilization of secondary structure [22].

The strength of C-H…O hydrogen bonds has also been an intriguing subject of computational studies [23-30]. The previous ab initio quantum mechanical calculations on C-H…O hydrogenbonds estimated the strength to be 2-3 kcal/mol, about half as strong as the conventional O-H…O hydrogen bonds [25]. 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 structures [31], and are involved in protein-nucleic acid interactions [32]. Recently, we published our results on the influence of C-H...π interactions in single chain all-alpha proteins [33]. 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 co-ordinates of the proteins have been taken from the PDB [34]. 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 SCOP [35] (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).

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

C-H…O interactions

C-H…O interactions were identified, using the program available for this purpose called HBAT [36]. The C-H…O interactions considered here were between all the possible donor C-H groups in single chain all-alpha proteins structures (C_{α}-H, C_{ali}-H and C_{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 Babu [37].

Sequential distance

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 [38-40]. 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 interactions [41].

Computation of stabilization center

Stabilization centers are clusters of residues that are involved in medium or long-range interactions [42]. 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 tetra peptide 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 server [43] 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 server [44]. This server computes the conservation based on the comparison of the sequence of a PDB chain with the proteins deposited in Swiss-Prot [45] 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.

Non-covalent interactions

The non-covalent interactions such as hydrogen bonding, hydrophobic interactions and ionic interactions have been extensively studied for their structural contributions. The hydrogen bond is one of the bedrocks upon which the structure of proteins is constructed. It is the attractive force that arises between donor covalent pair A-H in which hydrogen atom H is bound to a more electronegative atom D, and other non-covalently bound nearest neighbor electronegative acceptor atoms B (A-H…B). The weaker hydrogen bond has a longer H…B interaction with the range of 1.5-3.0 Å; more respectively, 1.5-2.2 Å for moderate hydrogen bonds and 2.2-3.0 Å for weak hydrogen bonds with the angle of 160±20°. To find a (nearly) optimal hydrogen bonding pattern, one needs a force field describing the hydrogen bond interaction energy of any proposed configurations, a definition of the degrees of freedom and the constraints, and a procedure for optimization. Boltzmann's equation was used to derive a good hydrogen bond "force field". Using this force field not only one to one hydrogen bonds but also bifurcated hydrogen bonds can be analyzed. We have computed the optimal hydrogen bonds in the single chain all-alpha proteins data set using WHAT IF, A molecular modeling and drug design program [46]. A method is presented that positions polar hydrogen atoms in protein structures by optimizing the total hydrogen bond energy. Hydrophobic and ionic interactions are another most important non-covalent force that will cause the linear polypeptide to fold into a compact structure. Although the general role of the hydrophobic effect and ionic interactions in protein folding is understood, a more detailed quantitative description of its contributions to structural stability is essential. The information about these hydrophobic and ionic interactions was obtained from PIC server [47]. The knowledge of these non-covalent interactions and their comparison with the non conventional interactions on single chain all-alpha proteins data set probably, is the first such report available in the literature.

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 HBAT [36]. 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 peptides [48]. 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 $C^{\alpha}H$ group and a water molecule [49], as well as the average C^{α}...O distance of 3.31 Å in parallel β-sheets [50]. An oxygen atom approaching a carbon along its covalent bond with hydrogen should not be closer than 3.7Å, 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 Kennard [51]. 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.

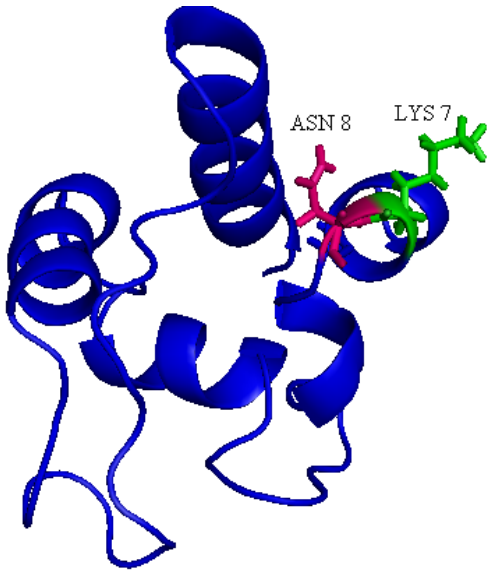

Figure 1: C-H…O interactions in Single Chain all-alpha Proteins [PDB Code 1A56 between Asn 8 and Lys 7].

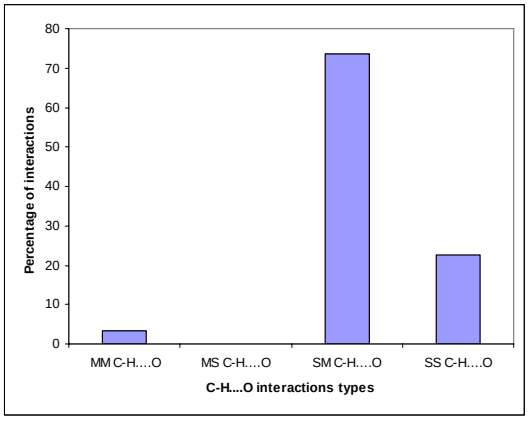

Figure 2: Different types of C-H…O interactions and their contribution in Single Chain all-alpha Proteins data set.

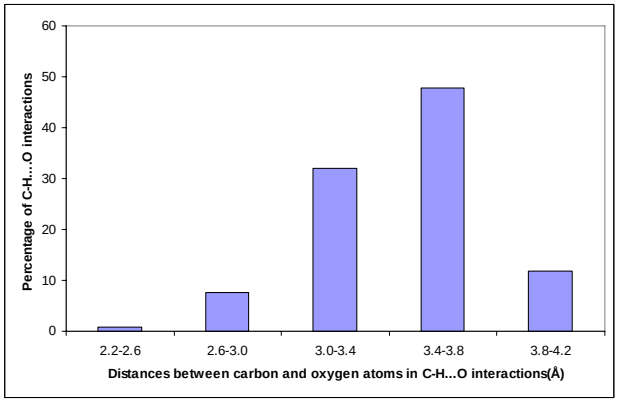

Figure 3: Observed distribution of C-H…O interactions as a function of interatomic (C…O) distance in the Single Chain all-alpha Proteins data set.

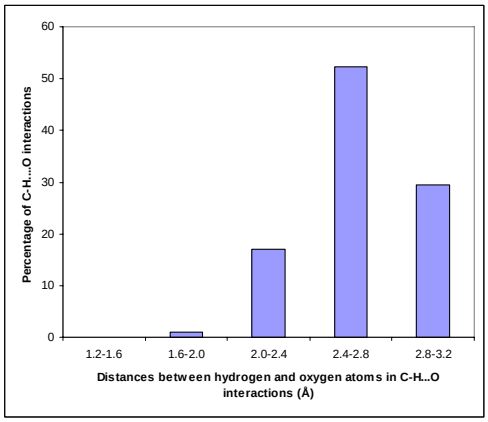

Figure 4: Observed distribution of C-H…O interactions as a function of interatomic (H…O) distance in the Single Chain all-alpha Proteins data set.

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.

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

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.

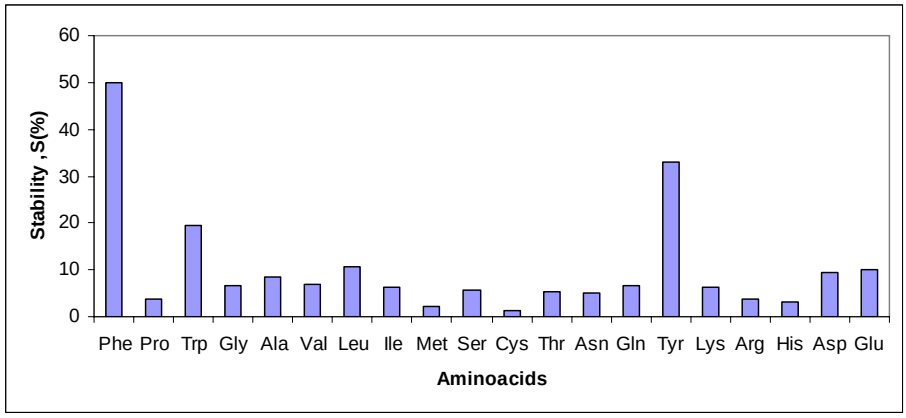

Figure 5: Aminoacids contributions to the stability of Single Chain all-alpha Proteins data set.

Sequential separation

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 [38-40]. 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.

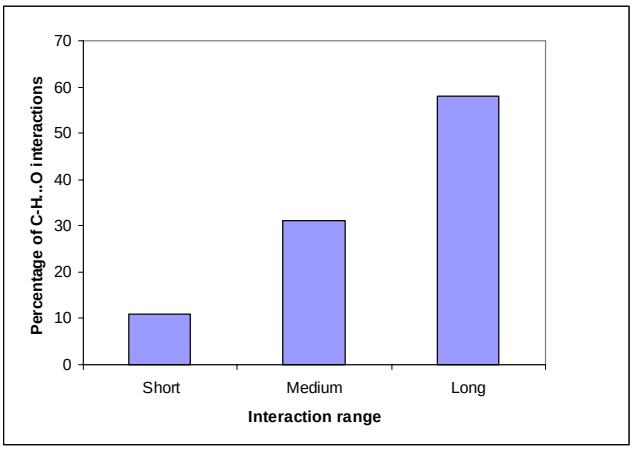

Figure 6: Percentage of Short, Medium and Long range C-H...O interactions in the Single Chain all-alpha Proteins data set.

Stabilization centers

Stabilization centers are clusters of residues that are involved in medium or long range interactions [42]. We have computed the stabilization centers for all single chain all-alpha proteins structure investigated in this work by using the program Scide [43]. 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 server 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.

Non-covalent interactions

The non-covalent interactions such as hydrogen bonding, hydrophobic interactions and ionic interactions have been identified in the set of 75 single chain all-alpha proteins. We undertook these studies to infer the role of non conventional C-H...O interactions by made comparison with conventional stabilizing forces in individual single chain all-alpha proteins and in the whole data set as well. The details of the number of hydrogen bonds, hydrophobic interactions and ionic interactions are listed in Table 2. The hydrogen bond considered here is the best possible hydrogen bonds within the single chain all-alpha proteins structure. We did not include the water molecule in our calculations. A total of 4106 H bonds, 1014 ionic interactions and 7910 hydrophobic interactions were found in a data set of 75 single chain all-alpha proteins. 36% of the single chain all-alpha proteins structure investigated in this study show maximum number of C-H...O interactions than ionic interactions. Nearly 15% of the proteins in the data set show equal number of ionic as well as C-H...O interactions. In contrast the number of hydrophobic and hydrogen bonds were greater than the number of C-H...O interactions in the single chain all-alpha proteins data set. To make this analysis tractable, we have analyzed the energetic contribution resulting from the non-covalent and non conventional interaction in the single chain all-alpha proteins data set. In terms of hydrogen bonding energies, many battles have been fought over the years. The commonly accepted numbers now range from > 10 kcal mol^{-1} for the strongest (e.g. O-H…O-) bonds [1, 52, 53], while the hydrophobic interactions could be −3.0 kcal/mol [54]. The electrostatic magnitude in the data set was found to be the maximum of −9.78 kcal/mol in Arg and Trp interacting pair. Indeed, the C-H...O interactions may contribute 0.5 kcal/mol [55]. Even though the C-H...O interactions is not only comparable in strength to a non covalent interactions but cumulatively can afford a certain degree of stability to the single chain all-alpha proteins structure. Based on the analysis of interplay between non-covalent and non-conventional forces, we emphasize that C-H...O interactions should be considered as an important contributing factor for the structural stability of the set single chain all-alpha proteins studied in this work.

Table 2: Non covalent and unconventional C-H...O interactions in single chain all alpha proteins data set.

PDB code	Number of Hydrophobic interactions.	Number of Ionic interactions.	Number of H-bond	Number of C-H...O interactions	PDB code	Number of Hydrophobic interactions.	Number of Ionic interactions.	Number of H-bond	Number of C-H...O interactions
1a7d	86	30	44	-	1jgs	273	28	57	4
1a56	43	7	27	103	1k9p	56	10	32	5
1aoy	50	5	22	7	1klx	68	10	49	14
1b89	30	4	123	31	1ktm	112	3	60	1
1c2n	76	13	41	13	1lea	52	10	24	5
1c20	105	8	47	8	1lfb	29	4	28	6
1c52	106	9	43	15	1lpe	93	24	63	9
1cc5	28	5	28	3	1lrv	196	43	80	14
1cch	42	7	26	3	1ls9	51	-	31	7
1ccr	71	4	32	9	1m8z	264	42	128	29

1cdp 85	12	35	11	1mho 50	14	31	7
1cgn 88	5	52	13	1nfn 86	22	58	9
1cpq 81	11	53	12	1nze 78	12	47	6
1cry 66	8	31	12	1o3u 88	13	46	11
1cyj 62	5	30	9	1oyz 191	29	100	15
1d5v 73	1	25	11	1p4w 58	5	26	5
1d8j 51	9	28	3	1paq 143	17	62	26
1dov 106	17	87	2	1pbv 178	18	70	20
1e8e 63	8	39	11	1q80 441	31	63	31
1el4 132	24	107	133	1rk9 76	12	37	13
1eyh 88	19	57	14	1rro 89	10	34	10
1f1f 59	3	31	6	1sr2 89	11	41	5
1g2h 52	3	19	6	1sra 114	14	51	19
1g5z 112	23	65	5	1te4 72	10	40	4
1gdv 40	4	30	4	1top 110	18	57	10
1gks 46	6	27	6	1tqg 104	9	94	5
1gs9 100	21	59	5	1ycc 65	7	32	10
1gvd 26	5	16	3	2a0b 202	29	50	7
1gxq 82	6	40	13	2bct 345	9	209	11
1hf8 219	24	97	24	2ezi 42	3	24	5
1ho8 417	48	196	32	2hts 106	6	29	11
1hu3 118	21	74	11	2mhr 83	28	43	19
1hz4 306	38	156	38	2sas 140	15	68	33
1ib2 92	10	121	27	3pat 71	9	34	9
1ig5 75	16	23	5	5pal 87	13	35	9
1ig6 68	10	36	9	5tnc 108	19	56	11
1ij5 109	3	123	25	451c 47	5	27	4

Conclusions

Quantitative evaluation of 75 different single chain 'all-alpha' protein reveals the fundamental nature of this important non covalent 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. The comparative analysis between the non-covalent interactions and C-H...O interaction obtained from consensus approach will be helpful to understand the stability of single chain all alpha proteins structures by means of non conventional interactions. It is worth stressing that C-H...O interactions can be categorized as important stabilizing force in single chain all alpha proteins, although they of course

tend to be weaker due to the normally lesser proton donating ability of C as compared to that of O. Even though the C-H...O bond is not only comparable in strength to a known stabilizing forces such as hydrogen bond, hydrophobic and ionic interactions but can actually make a quantitatively greater energetic contribution to single chain all alpha proteins structural stability.

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References

[1] Jeffrey, G. A., Saenger, W., 1991, "Hydrogen Bonding in Biological Structures," Springer-Verlag, NY.

[2] Bordo, D., and Argos, P., 1994, "The role of side-chain hydrogen bonds in the formation and stabilization of secondary structure in soluble proteins," J. Mol. Biol., 243, pp.504–519.

[3] Dill, K. A., 1990, "Dominant forces in protein folding," Biochemistry, 29, pp. 7133–7155.

[4] Fersht, A. R., and Serrano, L., 1993, "Principles of protein stability derived from protein engineering experiments," Curr. Opin. Struct.Biol., 3, pp. 75–83.

[5] Glasstone, S., 1937, "The structure of some molecular complexes in the liquid Phase," Trans. Faraday Soc., 33, pp. 200–214.

[6] Dippy, J. F. J., 1939, "The dissociation constants of monocarboxylic acids; their measurement and their significance in theoretical organic chemistry," Chem. Rev., 25,

[7] pp. 151–211.

[8] Allerhand, A., and Schleyer, P. V. R., 1963, "A survey of C-H groups as proton donors in hydrogen bonding," J. Am. Chem. Soc., 85, pp. 1715–1723.

[9] DeLaat , A. M., and Ault, B. S., 1987, " Infrared matrix isolation study of hydrogen bonds involving carbonhydrogen bonds: alkynes with oxygen bases," J. Am. Chem. Soc., 109, pp. 4232– 4236.

[10] Kariuki, B. M., Harris, K. D. M., Philp, D., and Robinson, J. M. A., 1997, "A triphenylphoshine oxide-water aggregate facilitates an exceptionally short C-H. . .O hydrogen bond," J. Am. Chem. Soc., 119, pp. 12679–12680.

[11] Astrup, E. E., and Aomar, A. M., 1975, "The Molecular Structure of 2, 2-methoxypropane, CH_3-O-C(CH_3)$_2$-O-CH_3, in the Gas Phase," Acta. Chem. Scand., A29, pp. 794–798.

[12] Peterson, K. I., and Klemperer, W., 1984, "Water hydrocarbon interactions: Rotational spectroscopy and structure of the water-acetylene complex," J. Chem. Phys., 81, pp. 3842–3845.

[13] Fujii, A., Fujimaki, E., Ebata, T., and Mikamiet, N., 1998, "A new type of intra molecular hydrogen bonding: hydroxyl? methyl interactions in the o-cresol cation," J. Am. Chem. Soc., 120, pp. 13256–13257.
[14] Sutor, D. J., 1962, "The C-H. . .O hydrogen bonds in crystals," Nature, 195, pp. 68–69.
[15] Kaufmann, R., Knochel, A., Kopf, J., Oehler, J., and Rudolph, G., 1977, "Anion activation, IV. Crystal and Molecular Structure of the Malononitrile Adduct of 18-Crown-6 (1,4,7,10,13,16-Hexaoxacyclooctadecane)," Chemische Berichte, 110, pp. 2249–2253.
[16] Wahl, M. C., and Sundaralingam, M., 1997, "C-H...O hydrogen bonding in biology," Trends Biochem. Sci., 22, pp. 97–102.
[17] Desiraju, G. R., and Steiner, T., 1999, "The weak hydrogen bond in structural chemistry and biology," Oxford University Press New York, pp. 29–121.
[18] Jiang, L., and Lai, L., 2002., "C-H. . .O hydrogen bonds at protein-protein interfaces," J. Biol. Chem., 277, pp. 37732–37740.
[19] Bella, J., and Humphries, M. J., 2005, "Cα-H···O=C hydrogen bonds contribute to the specificity of RGD cell-adhesion interactions," BMC Struct. Biol., pp. 5-4.
[20] Kryger, G., Silman, I., and Sussman, J.L., 1999, "Structure of acetylcholinesterase complexed with E2020 (Aricept): implications for the design of new anti-Alzheimer drugs," Structure, 7, pp. 297–307.
[21] Parkinson, G., Gunasekera, A., Vojtechovsky, J., Zhang, X., Kunkel, T. A, Berman, H., and Ebright, R. H., 1996, "Aromatic hydrogen bond in sequence specific protein DNA recognition," Nature Struct. Biol., 3, pp. 837–841.
[22] Derewenda, Z. S., Derewenda, U., and Kobos, P.M., 1999, "(His) C-H...O=C hydrogen bond in the active sites of serine hydrolases," J. Mol. Biol., 241, pp. 83–93.
[23] Armstrong, K. M., Fairman, R., and Baldwin, R. L., 1993, "The (i, i+4) Phe-His interaction studied in an alanine-based alpha-helix," J. Mol. Biol., 230, pp. 284–291.
[24] Gu, Y., Kar, T., and Scheiner, S., 1999, "Fundamental Properties of the C-H...O Interaction: Is It a True Hydrogen Bond?," J. Am. Chem. Soc., 121, pp. 9411-9422.
[25] Scheiner, S., Kar, T., and Gu, Y., 2001, "Strength of the C-H...O hydrogen bond of amino acid residues," J. Biol. Chem., 276, pp. 9832-9837.
[26] Vargas, R., Garza, J., Dixon, D. A., and Hay, B. P., 2000, "How strong is the C-H...O=C hydrogen bond?," J. Am. Chem. Soc., 122, pp. 4750-4755.
[27] Scheiner, S., Grabowski, S. J., and Kar, T., 2001, "Influence of hybridization and substitution on the properties of the C-H...O hydrogen bond," J. Phys. Chem., A105, pp. 10607-10612.
[28] Hartmann, M., Wetmore, S. D., Radom, L., 2001, "C-H...X Hydrogen Bonds of Acetylene, Ethylene and Ethane with First-and Second-Row Hydrides," J. Phys. Chem., A105, pp. 4470-4479.

[29] George, L., Sanchez-Garcia, and E., Sander, W., 2004, "Matrix isolation infrared and ab initio study of formic acid–acetylene interaction: example of H…π and C–H…O interaction," J. Phys. Chem., A107, pp. 6850-6858.

[30] Donati, A., Ristori, S., Bonechi, C., Panza, L., Martini, G., and Rossi, C., 2002, "Evidences of strong C-H...O bond in an o-carboranyl beta-lactoside in solution," J. Am. Chem. Soc., 124, pp. 8778-8779.

[31] Wang, B., Hinton, J. F., and Pulay, P., 2003, "C-H···O Hydrogen Bond between N-Methyl Maleimide and Dimethyl Sulfoxide: A Combined NMR and ab initio Study," J. Phys. Chem. A 107, pp. 4683-4687.

[32] Steiner, T., and Saenger, W., 1992, "The ordered water cluster in vitamin B_{12} coenzyme at 15 K is stabilized C – H...O hydrogen bonds," J. Am. Chem. Soc., 114, pp. 10146–10154.

[33] Mandel-Gutfreund, Y., Margalit, H., Jernigan, R. L., and Zhurkin, V. B., 1998, "A role for C-H. . .O interactions in protein-DNA recognition," J. Mol. Biol., 277, pp. 1129–1140.

[34] Shanthi, V., Ramanathan, K., and Sethumadhavan, R., 2010, "Exploring the Role of C– H….π Interactions on the Structural Stability of Single Chain "All-Alpha" Proteins," Appl. Biochem. Biotechnol., 160, pp. 1473–1483.

[35] Berman, H. M., Westbrook, J. Z., Feng, G., Gillilandm, T. N., Bhat, H., Weissig, I. N., Shindyalov, N. I., and Bourne, E. P., 2000, "The Protein Data Bank," Nucleic Acids Research, 28, pp. 235–242.

[36] Conte, L. L., Ailey, B., Hubbard, T. J. P., Brenner, S. E., Murzin, A. G., and Chothia, C., 2000, "SCOP: a structural classification of proteins database," Nucleic Acids Research, 28, pp. 257–259.

[37] Tiwari, A., and Panigrahi, S. K., 2007, "HBAT: A complete package for analyzing strong and weak hydrogen bonds in macromolecular crystal structures," In Silico Biology 7, pp. 0057.

[38] Babu, M. M., 2003. "NCI: A server to identify noncanonical interactions in protein structures," Nucleic Acid Res., 31, pp. 3345–3348.

[39] Gromiha, M. M., and Selvaraj, S., 1997, "Influence of medium and long range interactions in different structural classes of globular proteins," J. Biol. Phys., 23, pp. 151–162.

[40] Gromiha, M. M., and Selvaraj, S., 2004, "Inter-residue interactions in protein folding and stability," Prog. Biophys. Mol. Biol., 86, pp. 235–277.

[41] Gromiha, M. M., Santhosh, C., and Shandar, A., 2004, "Structural Analysis of Cation-π Interactions in DNA Binding Proteins," Int. J. Biol. Macromol., 34, pp. 203–211.

[42] Selvaraj, S., and Gromiha, M.M., 2003, "Role of hydrophobic clusters and long-range contact networks in the folding of (alpha/beta) 8 barrel proteins," Biophys. J., 84, pp.1919– 1925.

[43] Dosztanyi, Z., Fiser, A., and Simon, I., 1997, "Stabilization centers in proteins: identification, Characterization and predictions," J. Mol. Biol., 272, pp. 597–612.

[44] Dosztanyi, Z., Magyar, C., Tusnady, G. E., and Simon, I., 2003, "Scide: Indentification of stabilization centers in proteins," Bioinformatics, 19, pp. 899–900.

[45] Glaser, F., Pupko, T., Paz, I., Bell, R. E., Bechor, D., Martz, E., and Ben-Tal, N., 2003, "ConSurf: identification of functional regions in proteins by surface mapping of phylogenetic information," Bioinformatics, 19, pp. 163–164.

[46] Boeckman, B., Bairoch, A., Apweiler, R, Blatter, M. C., Estreicher, A., Gasteiger, E., Martin, M. J., Michoud, K., O'Donovan, C., Phan, I., Pilbout, S., and Schneider, M., 2003. "The swiss prot protein knoweldge base and its supplement TrEMBL in 2003," Nucleic Acids Research, 31, pp. 365–370.

[47] Vriend, G., 1990, "WHAT IF: A molecular modeling and drug design program," J. Mol. Graph, 8, pp. 52–56.

[48] Tina, K. G., Bhadra, R., and Srinivasan, N., 2007, "PIC: Protein Interactions Calculator," Nucleic Acid Res., 5 pp. W473–W476.

[49] Ramanathan, K., Shanthi, V., and Sethumadhavan, R., 2009, "Contribution of Unconventional C-H. . .O Bonds to the Structural Stability of Antimicrobial Peptides," Interdiscip. Sci. Comput. Life Sci., 1, pp. 263–271.

[50] Steiner, T., 1995, "Weak hydrogen bonding. Part 1. Neutron diffraction data of amino acid C_{α}–H suggest lengthening of the covalent C–H bond in C–H...O interactions," J. Chem. Soc. Perkin Trans., 2, pp. 1315–1319.

[51] Fabiola, G. F., Krishnaswamy, S., Nagarajan, V., and Pattabhi, V., 1997, "C-H...O hydrogen bonds in Beta-sheets," Acta Crystallogr, D53, pp. 316–320.

[52] Taylor, R., and Kennard, O., 1982, "Crystallographic evidence for the existence of C-H. ..O, C-H. . .N and CH. .Cl hydrogen bonds," J. Am. Chem. Soc., 104, pp. 5063–5070.

[53] Baker, E. N., and Hubbard, R. E., 1984, "Hydrogen bonding in globular proteins," Prog. Biophys. Mol. Biol. 44, pp. 97–179.

[54] Branden, C., and Tooze, J., 1991, "Introduction to protein structure," New York Garland.

[55] Kellis Jr, J. T., Nyberg, K., Sali, D., and Fersht, A.R., 1988, "Contribution of hydrophobic interactions to protein stability," Nature, 333, pp. 784–7.

[56] Desiraju, G. R., 1991, "The C-H...O hydrogen bond in crystals: What is it?," Acc. Chem. Res., 24, pp. 290–296.