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STRUCTURAL-FUNCTIONAL ANALYSIS OF BIOPOLYMERS AND THEIR COMPLEXES

UDC 577.150.2

Structure of β-β-Hairpins Closed into Cycles by S-S-Bridges[1]

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Abstract—In the present study, a stereochemical analysis of proteins containing β-β-hairpins closed into cycles by S-S-bridges has been performed. A database of these proteins has been compiled from the Protein Data Bank (total 428 PDB entries). 390 β-β-hairpins closed into cycles by S-S-bridges have been found in non-homologous proteins included into the database. Analysis showed that 118 hairpins contain S-S-bridges formed by cysteins located opposite to each other in the neighboring β-strands. Among them, 110 hairpins are left-turned and 8 β-hairpins are right-turned when viewed from the same side where S-S-bridges are located. In the other group of 272 β-hairpins, the S-S-bridge is formed by two cysteins one of which is located in the β-strand and the other in the loop juxtaposed to the β-hairpin at the N- (84% cases) or C-terminus (16% cases). As shown, in most cases the loop-hairpin region closed into a cycle by the S-S-bridge formed a turn of a left-handed superhelix.

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Key words: proteins, β-strand, β-turn, conformation, stereochemical analysis, handedness

INTRODUCTION

The β-β-hairpin is a structural motif formed by two S-strands adjacent along the chain and folded upon themselves so that to form an antiparallel β-sheet. β-β-Hairpins are widespread in proteins and occur both as isolated, double-strand β-sheets and parts of multiple-strand β-sheets. β-β-Hairpins can be right- or left-turned depending on whether the second β-strand runs on the right (Fig. 1a) or left (Fig. 1b), relative to the first one when viewed from the same side (e.g. as viewed from the hydrophobic core).

In proteins, flat β-β-hairpins are rare, and similar to multiple-strand β-sheets, are almost invariably twisted in a right-handed sense when viewed along the polypeptide chain direction [1]. In many proteins, β-β-hairpins are strongly twisted and coiled so that to form a double-helical structure having both a concave and a convex surface. These double helices are formed by right-turned β-β-hairpins if they are viewed from the concave side [2]. Long β-β-hairpins can fold upon themselves to form the so-called β-β-corners [2] and these β-β-hairpins are also right-turned when viewed from the concave side.

In this study, a stereochemical analysis of β-β-hairpins closed into cycles by S-S-bridges has been performed. It was shown that most β-β-hairpins in which S-S-bridges are formed by cysteins located opposite to each other in the neighboring β-strands, are left-turned when viewed from the side where S-S-bridges are situated. In the other group of β-β-hairpins, the S-S-bridge is formed by two cysteins one of which is located in the β-strand and the other in the loop juxtaposed to the β-hairpin at the N- or C-terminus. As shown, in most cases the loop-hairpin region closed into a cycle by the S-S-bridge forms a turn of a left-handed superhelix. The reasons of this handedness are also discussed.

SUBJECTS AND METHODS

A database of proteins containing β-β-hairpins closed into cycles by S-S-bridges was compiled step by step. At the first step, 13845 protein structures containing S-S-bridges have been selected from the Protein Data Bank (PDB) using the OCA Program (http://www.ebi.ac.uk/). Among them 2199 pro-

[1] The article was translated by the authors.

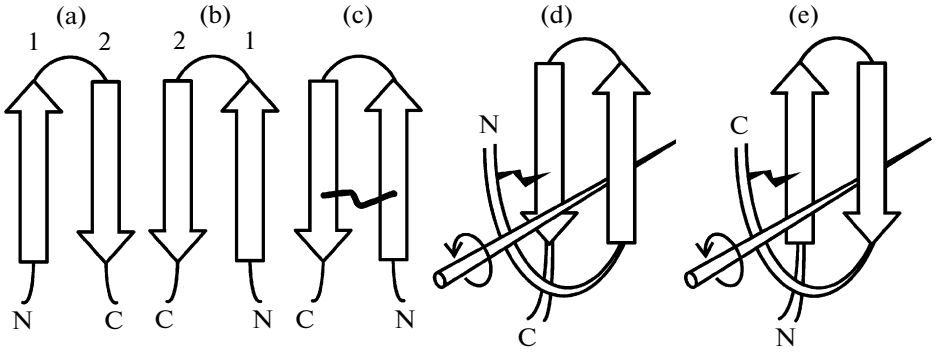

Fig. 1. A schematic representation of a right-turned (a) and left-turned (b) β-hairpin, a left-turned β-hairpin closed into a cycle with an interstrand S-S-bridge (c), and left-handed superhelices formed by β-hairpins and loops juxtaposed to them at the N- (d) or C-terminus (e). β-Strands are shown as arrows directed from the N- to C-ends and imaginary axes of the superhelices as straight bars.

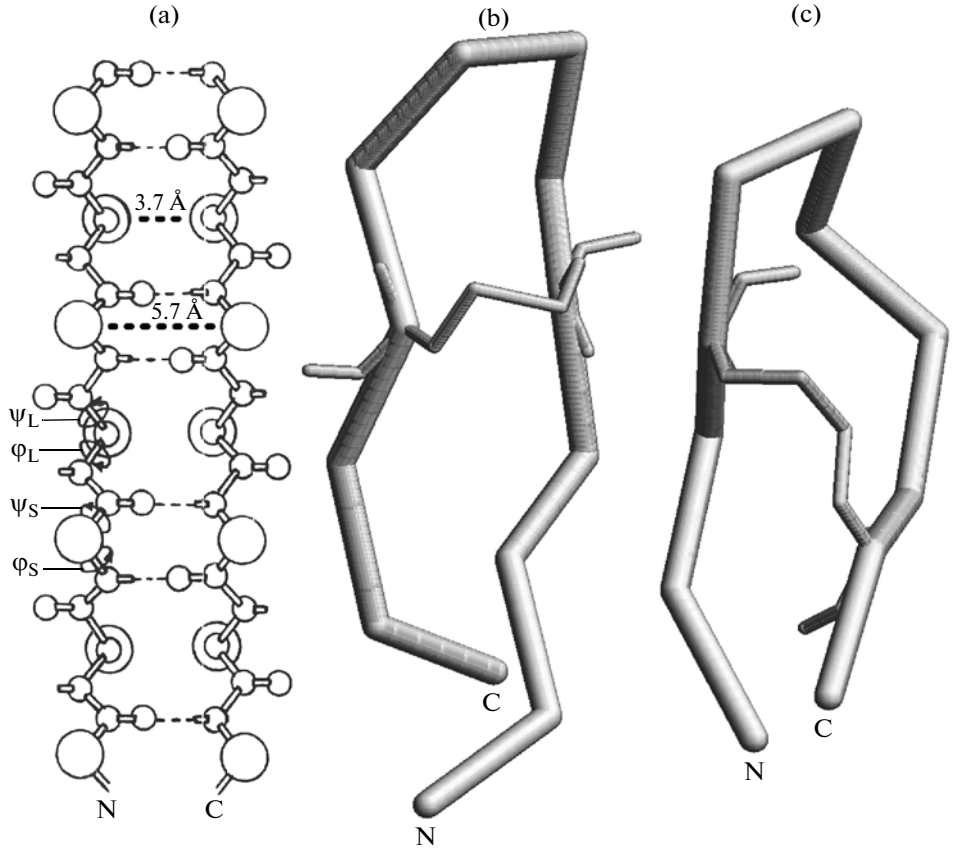

Fig. 2. A fragment of a flat antiparallel double-strand β-sheet (a), and examples of the left-turned (b, region 203–214 in 1PDK) and right-turned (c, region 130–140 in 1LCS) β-hairpins with the interstrand S-S-bridges. Main chain torsion angles are denoted as φL, ψL and φS, ψS for the large and small rings, respectively. The interchain Cβ-atom contact distances are shown with dashed lines and the corresponding values.

teins have 60% of identical amino acid residues in their sequences or less. At the next step, 428 proteins containing β-β-hairpins closed by S-S-bridges were manually selected using Program RasMol [3]. Possible homologies were revealed by the BLAST pairwise alignment (http://www.ncbi.nih.gov/BLAST/) [4]. In total, 390 β-β-hairpins closed into cycles by S-S-bridges have been found in nonhomologous proteins.

RESULTS AND DISCUSSION

Analysis of 118 β-β-hairpins in which S-S-bridges are formed by cysteins located in the neighboring β-strands shows that most of these hairpins (110 of 118) are left-turned if they are viewed from the side where S-S-bridges are situated (Fig. 1c). The other important feature of these β-β-hairpins is that the cysteins forming the S-S-bridges are located opposite each other, i.e. in register (Figs. 2a and 2b). The third feature is that the cysteins are included in the so-called large hydrogen-bonded rings of β-hairpins [5]. In an ideal flat β-β-hairpin, the interchain C_β-atom contact distances are different for the large and small hydrogen-bonded rings and are equal to 3.7 Å and 5.7 Å (Fig. 2a). If two cysteins are situated opposite to each other in a right-turned β-β-hairpin they should be involved into a small hydrogen-bonded ring. The low frequency of occurrence of the right-turned β-β-hairpins closed by S-S-bridges (8 of 118) suggests that formation of S-S-bridges between cysteins situated in the small hydrogen-bonded rings is sterically constrained because of the larger C_β-atom contact distance (5.7 Å in flat β-hairpins). This appears to be the main reason of that most β-β-hairpins of this type are left-turned. A stereochemical analysis shows that strongly twisted and coiled β-β-hairpins have shorter C_β-atom contact distances in the small rings as compared with those in flat β-β-hairpins. Some right-turned β-β-hairpins with interstrand S-S-bridges found in proteins are strongly twisted and coiled (see, e.g., Fig. 2c).

It should be noted that in amino acid sequences coding for β-β-hairpins closed by S-S-bridges some necessary conditions should be performed. First of all, β-β-hairpins of each type should have a corresponding sequence pattern of the key hydrophobic, hydrophilic and glycine residues [6–8]. β-β-Hairpins with short loops in which β-strands are connected by standard β-β-α_Lβ-, βεγβ-, β$\alpha_L\alpha_L$β-, βαγεβ-, β$\alpha\gamma\alpha_L$β-, and β$\alpha\alpha\gamma\alpha_L$β-turns are better examples. Some examples of amino acid sequences coding for the left-turned β-β-hairpins closed into cycles by S-S-bridges in which the β-strands are connected by β$\alpha_L\alpha_L$β-, βεγβ-, and β$\alpha\alpha\gamma\alpha_L$β-turns are presented in Fig. 3. The sequences are aligned so that each column contains structurally equivalent residues with the conformation shown above. As seen, each type of β-β-hairpins has a corresponding sequence pattern of the key residues [7, 8]. On the other hand, the mutual arrangement of the cysteins and the residues determining the structure of the β-turn (first of all, these are glycines and/or residues with flexible side chains occupying sterically constrained α_L- or ε-positions) is in concert. This means that the sequences code for the left-turned β-β-hairpins and the corresponding Cys residues are located opposite to each other in the large rings.

In the other type of β-β-hairpins studied in this work, the S-S-bridge is formed by two cysteins one of which is located in the β-strand and the other in the loop juxtaposed to the β-β-hairpin at the N- or C-terminus (Figs. 1d and 1e). In total, 272 structures of this type have been found in nonhomologous proteins deposited in the Protein Data Bank (PDB). They can be divided into two groups, 228 structures of the loop-hairpin type, and 44 structures of the hairpin-loop type. In 211 structures of the loop-hairpin type, the polypeptide chain forms a left-handed superhelix and a left-turned β-β-hairpin (Fig. 1d). A right-handed superhelix is found in 17 structures of this type. In 37 structures of the hairpin-loop type (of 44), the polypeptide chain forms a left-handed superhelix and a right-turned β-β-hairpin (Fig. 1e). As seen in most structures of these types (91%), the polypeptide chain forms a left-handed superhelix in three dimensions. The high frequency of occurrence of the left-handed superhelix is determined by the arrangement of secondary structural elements in higher-order structures in which the superhelices are included (see Fig. 4). Most often the superhelices are included into abcd-units [9] having both the reverse (Fig. 4a) and direct (Fig. 4b) polypeptide chain orientation, into 3β-corners [10] (Fig. 4c), and into abCd-units [9, 10] having α-helices in the C-regions (a part of the abCd-unit forming the left-handed superhelix is shown in Fig. 4e; this superhelix often occurs in proteins and can be considered as a structural motif [12]). Fig. 4d shows the left-handed superhelix of the loop-hairpin type for comparison.

It should be noted that similar left-handed superhelices have been observed in small disulphide-rich proteins containing the so-called "disulphide β-cross" [13]. The authors of this paper have also pointed out the high frequency of occurrence of the superhelices in the abcd-units. In this study,

(a)

ββββββββββ$\alpha_L\alpha_L$βββββββββββ

1CW6 : A	--H**C**------T**K**S**G**--------**C**S--	H8–S15
1J48 : A	--Q**C**AP----VG**G**Q------DA**C**---	Q33 –C43
1MKF : A	---**C**ALQMTSHD**G**VWTSTSSEQ**C**---	C318–C337
1MTZ : A	---**C**IENYAKVN**G**I--YIYYKL**C**---	C5–C22
1QNX : A	EVG**C**GSIKYIQE**K**W--HKHYLV**C**NYG	E167–G190
1U5M : A	-RI**C**VC----DT**G**T------VL**C**DD-	R29–D42
1XKU : A	---**C**QC----HL**R**V------VQ**C**---	C29–C38
1ZRH : A	---**C**LR----DS**G**R------DR**C**---	C223–C232
2COV : D	NYV**C**------LN**D**Y--------**C**LP-	N410–P420
2DQA : A	---**C**RM----DV**G**S------LS**C**---	C27–C36
2HTY : H	--A**C**------VN**G**S--------**C**FT-	A231–T239
2HTY : F	--S**C**YP----NA**G**E------IT**C**V--	S279–V290
Consensus	--C--------G---------C---	

(b)

ββββββββεγβββββββββ

1PDK : A	SFI**C**--N**G**SR--**C**SV--	S204–V214
1U79 : A	-AG**C**--K**G**GS--**C**LI--	A104–T113
1WWL : A	---**C**ELD**E**ESCS**C**N---	C8–N18
1Y9Z : A	--E**C**TVN**G**TSFS**C**----	E259–C269
2A74 : C	-KL**C**--R**D**EL--**C**RC--	K1482–C1491
2GRU : A	KQI**C**--F**A**DR--**C**FNFA	K4–A16
2IFR : A	-TD**C**SVS**G**TTVS**C**SY--	T192–Y205
2CX4 : F	--V**C**--T**K**EL--**C**T---	V48–T55
2NYK : A	RYA**C**LFL**G**FDVI**C**DVYH	R100–H116
Consensus	---**C**---**G**----**C**----	

(c)

ββββββββββββ αα$\gamma\alpha_L$βββββββββββ

1CD9 : B	SNLS**C**LM----HLTT**N**S-----LV**C**QWE	S9–E27
1R4C : A	--AF**C**SFQIYAVPWQ**G**TMTLSKST**C**QD-	A95–D119
1Z4V : A	-RKS**C**SI----STVP**G**G---CMMY**C**FV-	R224–V242
2ID5 : A	----**C**EC----SAQD**R**A-----VL**C**---	C7–C18
8TFV : A	-IIY**C**------NRRT**G**K-------**C**QRM	I8–M21
Consensus	----**C**----------**G**--------**C**---	

Fig. 3. Alignment of the amino acid sequences coding for the left-turned β-β-hairpins closed into cycles with the interstrand S-S-bridges. β-β-Hairpins having the β-α_L-α_Lβ- (a), βεγβ- (b), and βααγα_Lβ-turns (c) are presented. Greek letters α, β, γ, ε and α_L show the conformation of residues in the corresponding columns in accordance with the shorthand nomenclature [6]. PDB codes of the proteins are given on the left and polypeptide chain regions forming the hairpins on the right.

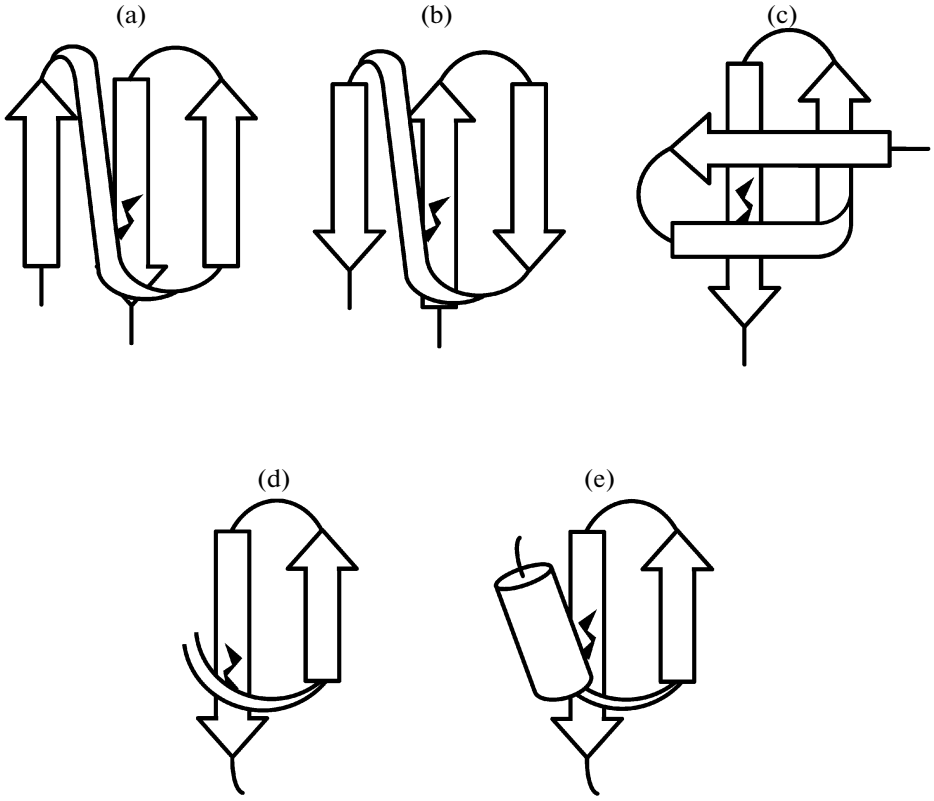

Fig. 4. A schematic representation of some structural motifs having unique folds and a definite handedness that include the left-turned superhelices of the loop-hairpin and hairpin-loop types closed into cycles with S-S-bridges. See also the text.

we have found that the left-handed superhelices occur not only in proteins containing the "disulphide β-crosses", but also in many other protein superfamilies. On the other hand, we have found much more superhelices closed into cycles by S-S-bridges (272 superhelices as compared to 18 examples found in [13]). We have also shown that in proteins the superhelices of both the loop-hairpin and hairpin-loop types occur (all 18 examples found in [13] are of the loop-hairpin type).

The handedness of a superhelix is determined also by the structure of the loop juxtaposed to the β-β-hairpin. For example, if the loop has an arch-like structure with the $\beta\beta_P\beta_P\alpha_L\beta$-conformation [7] the superhelix is always left-handed. An alignment of the amino acid sequences coding for such superhelices in known proteins is presented in Fig. 5. Note that the first β-positions of the $\beta\beta_P\beta_P\alpha_L\beta$-arches are occupied by Cys residues, most the sterically constrained α_L-positions are occupied by glycines, and Pro residues often occur at one of the β_P-positions. All the β-hairpins presented in Fig. 5 are left-turned although the conformation and the length of their loops can be different. The two Cys residues are arranged in the sequence so that in the superhelix they are pointed towards each other and are able to form an S-S-bridge.

As seen, the left-handed β-hairpins closed into cycles by the interstrand S-S-bridges as well as the left-handed superhelices of the loop-hairpin and hairpin-loop types closed into cycles by S-S-bridges are widespread in nonhomologous proteins. These structures have unique folds and predominantly occur in one of the two "mirror-symmetrical" forms. Formation of S-S-bridges appears to play an important role in their folding. The S-S-bridges enhance the stability of the structures and select predominantly one of the two forms for each of them. One may suggest that these structures can fold independently of the remaining part of the polypeptide chain and can act as nuclei and/or "ready building blocks" in protein folding.

No.	Codes PDB	ββ$_P$β$_P$α$_L$βββββββββββ–––––βββββββββ	Residue numbers
1.	1AGG:A	**C**CR**G**RPCRC–––––SMIGTNC––––E**C**T–	19–37
2.	1AJJ:A	**C**SA**F**EFH–––––––CLSGE–––––––**C**I–	5–18
3.	1BQF:A	**C**VA**G**YM–––––––RTPDGR––––––**C**KP	7–21
4.	1C3A:A	**C**IP**G**WSA–––––––YDRY––––––––**C**YQ	4–17
5.	1CCV:A	**C**QE**G**FLR–––––––NGEGA–––––––**C**VL	38–52
6.	1CIX:A	**C**CR**G**LTCRS–––––YFPGSTYG–––R**C**QR	23–43
7.	1D2L:A	**C**QP**G**EFA–––––––CANSR–––––––**C**IQ	6–20
8.	1E0F:K	**C**LY**G**QS–––––––CNDGQ–––––––**C**SG	26–39
9.	1EDM:B	**C**LN**G**GSCKD––––––DINS––––––YE**C**WC	56–73
10.	1FF4:A	**C**PA**G**QNVCFKRWH–YVTPKNYDIIKG**C**–	17–42
11.	1HIC:A	**C**GQ**G**NKCI––––––LGSDGEK–––NQ**C**V–	22–40
12.	1IGR:A	**C**PP**N**TY–––––––RFEGWR––––––**C**V–	234–247
13.	1IGR:A	**C**RH**Y**––––––––YYAGV–––––––**C**V–	221–231
14.	1IYC:A	**C**PK**G**KVW–––––––NGFD––––––**C**K–	18–30
15.	1K36:A	**C**LH**G**QCIY––––––LVDMSQ–––NY**C**RC	14–32
16.	1KIG:L	**C**AH**G**YV–––––––LGDDSKS––––**C**VS	411–426
17.	1KLO:A	**C**PG**G**SSCA––––––IVPKTKEV–––V**C**T–	13–31
18.	1LMR:A	**C**CK**G**TTC–––––––MFYANR––––––**C**VG	19–34
19.	1LR7:A	**C**GP**G**KKCR––––––MNKKNKP––––R**C**VC	71–89
20.	1NIX:A	**C**CS**G**YA–––––––CNSRDKW––––**C**KV	16–31
21.	1NXB:A	**C**SP**G**ESSCYHKQWSDFRG––TIIERG**C**–	17–41
22.	1P9G:A	**C**NA**G**LC–––––––CSIYGY––––––**C**G–	11–24
23.	1RFN:B	**C**TE**G**YR–––––––LAENQKS––––**C**EP	111–126
24.	1SKZ:A	**C**PH**G**FQ–––––––RSRYGCE––FCK**C**–	37–53
25.	1WMZ:B	**C**PT**D**WEA–––––––EGDH–––––––**C**YR	3–16
26.	1X5V:A	**C**CR**G**LQC–––––––RYGK–––––––**C**LV	18–31
27.	2DDU:A	**C**DL**G**YTA–––––––AQGT–––––––**C**VS	1430–1443
28.	2E4U:A	**C**GP**G**QW–––––––PTADLSG––––**C**YN	549–564
29.	2F91:B	**C**TP**G**QTKK––––––QDCN––––TCN**C**–	3–19
30.	2HNU:A	**C**GS**G**GRCA––––––AAGI––––––**C**CS	61–75
31.	2JM4:A	**C**SL**G**YF–––––––PCGNITK––––**C**LP	7–22
32.	2UWI:B	**C**GR**G**YGKKG–––––EDEMGNT––––I**C**–	127–146
33.	2UWI:B	**C**EQ**G**VSYY––––––NSQELK––––––**C**CK	22–38
Consensus		**C**–**G**–––––––––––––––––––**C**–	

Fig. 5. Alignment of the amino acid sequences coding for the left-turned superhelices of the loop-hairpin type having short loops with the ββ$_P$β$_P$α$_L$-conformations. Designations are as in Fig. 3.

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