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CH/π Interactions as Demonstrated in the Crystal Structure of Guanine-nucleotide Binding Proteins, Src Homology-2 Domains and Human Growth Hormone in Complex with their Specific Ligands

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Abstract—The CH/π interaction is a weak attractive molecular force occurring between CH groups and π-systems. Possibility has been examined for the role of CH/π interaction, by use of a computer program, in the crystallographic data of several guanine-nucleotide binding proteins, src homology-2 domains and human growth hormone complexed with their specific ligands. Short CH/π contacts have been found in every case where cohesive forces are expected. Comparison of the structures of functionary related proteins has shown that mutation may occur but necessary CH/π interactions are conserved. A considerable part of the non-polar interactions, broadly ascribed in the past to the van der Waals interaction or the so-called hydrophobic effect, has been suggested to be attributed to a more specific attractive force, the CH/π interaction.

Introduction

Evidence has accumulated that an attractive molecular force, the CH/π interaction,[1] plays an important role in various field of chemistry.[2] These include the conformation[3] and chiroptical property[4] of organic compounds,[2a] molecular recognitions,[2b,5] clathrates,[6] host/guest chemistry,[7,8] crystal packing,[9] and coordination chemistry.[10,11] Suggestions have also been made as to the potential role of this attractive force in biochemistry.[1,2,12] Recently we examined the involvement of CH/π interactions in the crystal structures in the Brookhaven Protein Data Bank (PDB); a number of short CH/π contact have been found in a variety of proteins such as haemoglobin, lysozyme, p21 protein, etc. and the complexes with their specific substrates.[2b] To explore the generality of the phenomenon, we analyzed the structures of several types of guanine-nucleotide binding proteins (G proteins), Src homology-2 (SH2) proteins and human growth hormone (hGH) complexed with their specific ligands.

Method

A program (CHPI)[2b] was written in order to search short contacts between CH groups and π systems in protein structures registered in the PDB. Figure 1 illustrates the method. The π-system may be an aromatic group or a double bond. The hydrogen may be a part of an alkyl group, a CH in aromatic rings, N^+H_3, NH_2, NH, OH or SH group. To participate in a XH/π interaction, a hydrogen should be positioned above the π plane, most preferably above the sp^2 atom (region 1 in Figure 1).[13] To cover other possibilities, several kinds of CH/X_{sp2} distance (D_{atm}, D_{pln} and D_{lin}) and angle parameters (θ and ω) were defined and H/π distances within a set of cut-off values were collected. In this paper, the H/π distances shorter than 3.05 Å [= 2.9 Å (1.2 Å for C-H plus 1.7 Å for a half thickness of the aromatic molecule)[14] × 1.05] with stereochemically reasonable angle factors (θ < 62.2° and ω < 127.5°)[2b] were

Key words: CH/π interaction; PDB; G proteins; SH2 domains; human growth hormone.

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considered as relevant for the presence of CH/π interaction. The numbers in the brackets in Tables 1 and 2 correspond to these values.

Table 1 is an output from program CHPI analyzing a protein, ribonuclease A (RNase, PDB code 5RSA, resolution 2.0 Å),[15] using the hydrogen (deuterium) coordinates obtained through the joint refinement of X-ray and neutron diffraction data.

The crystal data in the PDB do not ordinarily contain coordinates of hydrogen atoms. In these cases, the hydrogens were generated on nonhydrogen atoms and their positions optimized. The optimization was carried out by the molecular mechanics energy minimization with program KOPT,[16] the force-field parameters of AMBER version 3.0, revision A[17] were used. Then, atomic contacts shorter than the sum of the conventional van der Waals distances with reasonable angle parameters were collected.[18] Table 2 presents the results obtained by use of the generated hydrogen coordinates for RNase (7RSA, resolution 1.26 Å).[19] The agreement of the results (Table 1 versus Table 2) obtained by the two sets of hydrogen coordinates was satisfactory, at least in view of the present purpose of surveying CH/π interactions in the protein structure. Figure 2 gives a global view of RNase.

The coordinates of the hydrogen (deuterium) atoms from neutron diffraction study are also available for 2-Zn insulin,[20] bovine pancreatic trypsin inhibitor (BPTI)[21] and sperm whale myoglobin.[22] Comparable results were obtained by the use of both sets of coordinates. In view of the limitation to the present methodology and the precision in the crystallographic determination of proteins, however, we do not discuss the interatomic distances in detail. The proton positions calculated on the basis of low-resolution diffraction data (> 2.0 Å) are not enough accurate in discussing the interatomic distances precisely. Discussions regarding the H/π distances with the protein structures by low-resolution studies therefore remain qualitative.

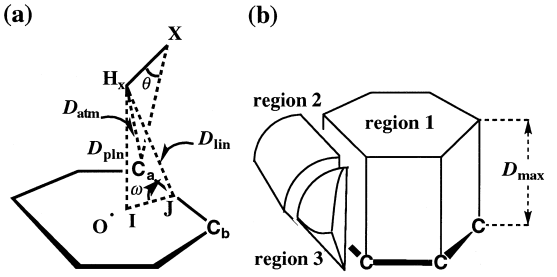

Figure 1. Method for exploring XH/π contacts (X = C, N, O or S). An example is given for a six-membered π-system. (a) O: centre of the π-plane. C_a and C_b: nearest and second nearest sp^2-atoms, respectively, to the H_x hydrogen. ω: dihedral angle defined by C_aOC_b and $H_xC_aC_b$ planes. θ: H_x-X-C_a angle. D_{pln}: perpendicular distance between H_x and the π-plane (H_x/I). D_{atm}: H_x/C_a interatomic distance. D_{lin}: distance between H_x and the line C_a–C_b (H_x/J). (b) Regions to be searched. Region 1: zone where H_x is above the ring. Regions 2 and 3: zones where H_x is out of region 1 but may interact with π-orbitals. The program was run to search for short H_x/π contacts with the following conditions: $D_{max} = 3.05$ Å: (1.2 Å + 1.7 Å)×1.05; $D_{pln} < D_{max}$ (region 1); $D_{lin} < D_{max}$ (region 2); $D_{atm} < D_{max}$ (region 3); $\omega_{max} = 127.5°$; $-\omega_{max} < \omega < \omega_{max}$; $\theta < 62.2°$.

Results and discussion

G proteins

Ras p21 protein. The G proteins constitute a superfamily of guanine-nucleotide binding proteins that serve as transducers of intracellular signaling pathways. They are categorized into three classes: (1) the 21 K products of the oncogenes, (2) the α subunits of the heterotrimeric G proteins and (3) the GTPases used in ribosomal protein synthesis.

Ras p21 is an oncogene product involved in the growth promoting signal transduction system, an important member of the G proteins. Pai et al. determined the crystal structure of Harvey-ras protein p21 in complex with a GTP analogue, GppNp (PDB code 5P21, resolution 1.35 Å).[23] Figure 3 gives a result of our CHPI analysis around the ligand on the crystal structure of the complex.

It is well known that Asp119 and Asn116 (not shown in Figure 3 for clarity) are vital for the high G specificity due to hydrogen bonds with the guanine moiety. Besides Asp119 we see two lysines, Lys117 and Lys147, sandwiching the guanine aromatic ring. Lys117 was reported, in the Ramachandran diagram, to locate itself at a region where few residues other than glycine are found, indicating this to be in an unusual conformation.

Figure 4(a) shows that Lys117 is in CH/π contact at two points with the guanine aromatic ring (Hε/N^9, Hγ/C^5). Lys147 also interacts with guanine (Hβ/N^1 and a hydrogen bond). The guanine aromatic ring is in contact with Phe28 (Hζ/N^3, Hεl/C^5). Importance of this type of interaction is well known and has often been referred to as the edge-to-face aromatic interaction.[24] Phe28 is in turn assisted by two CH/π interactions with Lys147 (Hα/Cγ, Hγ/Cδ2). Low temperature factors were reported for these residues in the crystallographic determination demonstrating that these interactions are strong. The above CH/π interactions certainly contribute to the G specificity of the p21 protein.

Table 1. Computer output from a CHPI analysis of ribonuclease A (PDB code 5RSA, neutron data)

H/π Interaction

	RES	I	VPI	1	2	3	4	5	6
PRTN	HIS	1	FIV	CG	ND1	CE1	NE2	CD2	
PRTN	PHE	1	SIX	CG	CD1	CE1	CZ	CE2	CD2
PRTN	TYR	1	SIX	CG	CD1	CE1	CZ	CE2	CD2
PRTN	TRP	1	FIV1	CG	CD1	NE1	CE2	CD2	
		2	SIX2	CE2	CD2	CE3	CZ3	CH2	CZ2

RANGE −127.500 < OMEGA < 127.500
RANGE 0.000 < THETA < 62.200
RANGE 2.000 < Dmax < 3.050

π					HX					Geometry					
ID	RES	I	VPI	VATM	N	ID	RES	VATM	N	DATM	DPLN	DLIN	OMEGA	THETA	RG
8	PHE	1	SIX	CD1	2	12	HIS	HD2	16	[2.87]	2.78	****	104.90	36.13	3
9	PHE	1	SIX	CD2	6	117	PRO	HB	9	2.91	[2.73]	2.85	73.15	8.94	1
12	HIS	1	FIV	CD2	5	120	PHE	HB	14	2.80	[2.72]	2.75	82.02	29.02	1
12	HIS	1	FIV	CE1	3	120	PRE	HB	15	2.81	2.70	[2.77]	102.82	44.85	2
25	TYR	1	SIX	CD1	2	20	ALA	HA	7	[2.84]	2.81	****	99.55	21.85	3
25	TYR	1	SIX	CG	1	20	ALA	HB	10	2.82	2.76	[2.79]	97.78	31.06	2
25	TYR	1	SIX	CZ	4	29	MET	HG	14	2.56	[2.54]	2.56	83.33	3.30	1
25	TYR	1	SIX	CZ	4	48	HIS	HE1	17	2.75	2.26	[2.74]	124.54	27.97	2
25	TYR	1	SIX	CD2	6	82	THR	HG2	14	[2.66]	2.16	****	125.70	22.03	3
46	PHE	1	SIX	CD2	6	25	TYR	HE2	20	[2.99]	2.45	****	124.94	22.65	3
46	PHE	1	SIX	CZ	4	29	MET	HB	12	2.84	2.80	[2.84]	99.52	21.73	2
46	PHE	1	SIX	CE2	5	44	ASN	DD2	14	2.62	2.38	[2.58]	112.38	36.44	2
48	HIS	1	FIV	CD2	5	19	ALA	HA	7	2.92	[2.88]	2.88	88.00	25.06	1
73	TYR	1	SIX	CD1	2	62	ASN	HA	10	[2.90]	2.81	****	104.19	38.11	3
73	TYR	1	SIX	CZ	4	115	TYR	HE2	20	2.84	2.41	[2.78]	119.64	25.05	2
76	TYR	1	SIX	CE2	5	60	GLN	HA	11	2.84	2.58	[2.84]	114.90	34.27	2
76	TYR	1	SIX	CD2	6	60	GLN	HB	13	2.88	[2.79]	2.85	78.24	36.62	1
76	TYR	1	SIX	CE2	5	60	GLN	HG	14	2.96	2.31	[2.89]	126.75	37.04	2
76	TYR	1	SIX	CE1	3	61	LYS	HB	12	[2.67]	2.12	****	127.26	8.49	3
92	TYR	1	SIX	CD1	2	39	ARG	HA	13	[2.61]	2.35	****	115.83	34.22	3
92	TYR	1	SIX	CD1	2	93	PRO	HA	8	2.69	[2.60]	2.61	86.57	13.56	1
92	TYR	1	SIX	CE2	5	93	PRO	HB	10	2.90	[2.87]	2.87	89.17	24.06	1
97	TYR	1	SIX	CD2	6	30	MET	HG	14	2.89	[2.77]	2.83	78.47	23.59	1
97	TYR	1	SIX	CZ	4	30	MET	HE	15	2.58	2.47	[2.58]	106.12	8.00	2
97	TYR	1	SIX	CD1	2	86	GLU	HA	11	[2.98]	2.86	****	106.65	41.27	3
105	HIS	1	FIV	CD2	5	78	THR	HG2	14	2.81	2.70	[2.78]	103.95	29.92	2
115	TYR	1	SIX	CZ	4	110	CYS	HB	9	2.82	2.74	[2.77]	98.86	33.59	2
119	HIS	1	FIV	NE2	4	121	ASP	HA	10	2.86	2.68	[2.86]	110.90	35.66	2
120	PHE	1	SIX	CZ	4	45	THR	HG2	13	[2.80]	2.55	****	114.51	28.69	3

[ ]:H/π distance

Number of H/π interactions: 29

Transducin α. Sigler et al. studied the crystal structure of transducin α, a subunit of a heterotrimeric G protein, in complex with a GTP analogue, GTPγS (PDB code 1TND, resolution 2.2 Å).[25] Figure 4(b) illustrates interactions involved in the ligand-binding pocket of the complex analyzed by CHPI. Lys266 (Lys117 in p21) seems to be in CH/π contact at two points (Hγ/C^6, Hε/C^8) with the guanine aromatic ring. Lys147 in p21 is replaced in transducin α by threonine (Thr323). Nevertheless, the necessary CH/π (and OH/π) interactions (Hγ2/C^5 and HOγl/N^1) still remain there.

EF-Tu. Elongation factor Tu (EF-Tu) is a GTP-binding protein that is essential for protein biosynthesis. Kjeldgaard et al. determined the crystal structure of EF-Tu from *Thermus aquaticus* in the GTP-bound conformation (PDB code 1EFT, resolution 2.5 Å).[26] Figure 4(c) is a schematic representation of the ligand-binding site of

Table 2. Computer output from a CHPI analysis of ribonuclease A (PDB code 7RSA, generated H)

H/π interaction

	RES	I	VPI	1	2	3	4	5	6
PRTN	HIS	1	FIV	CG	NDI	CE1	NE2	CD2	
PRTN	PHE	1	SIX	CG	CD1	CE1	CZ	CE2	CD2
PRTN	TYR	1	SIX	CG	CD1	CE1	CZ	CE2	CD2
PRTN	TRP	1	FIV1	CG	CD1	NE1	CE2	CD2	
		2	SIX2	CE2	CD2	CE3	CZ3	CH2	CZ2

RANGE −127.500 < OMEGA < 127.500
RANGE 0.000 < THETA < 62.200
RANGE 2.000 < Dmax < 3.050

π					HX				Geometry					
ID	RES	I	VPI	VATM	N	ID	RES	VATM	N	DATM	DPLN	DLIN	OMEGA	THETA	RG
8	PHE	1	SIX	CD1	2	12	HIS	HD2	14	[2.77]	2.69	****	104.15	34.01	3
8	PHE	1	SIX	CE2	5	117	PRO	HB	8	2.95	[2.77]	2.86	75.30	29.12	1
12	HIS	1	FIV	CD2	5	11	GLN	HB	9	2.87	2.83	[2.86]	99.45	28.22	2
12	HIS	1	FIV	CE1	3	120	PHE	HB	8	2.94	2.82	[2.91]	104.73	49.34	2
12	HIS	1	FIV	CD2	5	120	PHE	HB	9	2.92	[2.83]	2.86	82.30	30.61	1
25	TYR	1	SIX	CD1	2	20	ALA	HA	4	3.00	2.96	[2.99]	98.12	22.18	2
25	TYR	1	SIX	CZ	4	29	MET	HG	11	2.52	[2.52]	2.52	99.66	12.04	1
25	TYR	1	SIX	CZ	4	48	HIS	HE1	16	2.72	2.32	[2.72]	121.44	23.59	2
46	PHE	1	SIX	CD2	6	25	TYR	HE2	18	[2.95]	2.51	****	121.72	20.32	3
46	PHE	1	SIX	CG	1	25	TYR	HH	21	[2.92]	2.76	****	109.03	9.98	3
46	PHE	1	SIX	CZ	4	29	MET	HB	8	[2.87]	2.79	****	103.16	29.40	3
46	PHE	1	SIX	CE2	5	44	ASN	HD2	13	2.64	2.53	[2.60]	102.80	27.01	2
48	HIS	1	FIV	CD2	5	19	ALA	HA	4	2.86	[2.85]	2.85	89.47	18.69	1
73	TYR	1	SIX	CD1	2	62	ASN	HA	4	[2.93]	2.86	****	102.85	38.27	3
73	TYR	1	SIX	CZ	4	62	ASN	HD2	13	3.05	[3.00]	3.02	83.42	47.21	1
73	TYR	1	SIX	CZ	4	115	TYR	HE2	18	3.04	2.65	[2.98]	117.26	25.98	2
76	TYR	1	SIX	CE2	5	60	GLN	HA	4	3.00	2.58	[2.95]	118.92	35.26	2
76	TYR	1	SIX	CE2	5	60	GLN	HB	8	3.14	[2.97]	3.07	75.59	52.85	1
76	TYR	1	SIX	CE2	5	60	GLN	HG	12	2.76	2.25	[2.74]	124.79	34.19	2
92	TYR	1	SIX	CD1	2	39	ARG	HA	4	2.78	2.57	[2.77]	111.75	34.20	2
92	TYR	1	SIX	CD1	2	93	PRO	HA	3	2.61	[2.56]	2.56	89.60	15.85	1
92	TYR	1	SIX	CE2	5	93	PRO	HB	7	3.05	[3.02]	3.02	87.07	25.90	1
97	TYR	1	SIX	CD2	6	30	MET	HG	11	2.87	[2.74]	2.80	78.12	22.99	1
97	TYR	1	SIX	CE1	3	30	MET	HE	19	2.82	2.62	[2.79]	109.87	15.92	2
105	HIS	1	FIV	CD2	5	78	THR	HG2	14	2.95	2.86	[2.89]	98.12	28.06	2
115	TYR	1	SIX	CZ	4	110	CYS	HB	9	2.88	2.78	[2.84]	102.19	38.84	2
119	HIS	1	FIV	NE2	4	121	ASP	HA	4	[2.69]	2.24	****	123.46	34.10	3
120	PHE	1	SIX	CZ	4	45	THR	HG2	13	[2.82]	2.45	****	119.88	36.90	3

[ ]:H/π distance

Number of H/π interactions: 28

this protein. We see Lys137 (Lys117 in p21) and Leu176 (Lys147 in p21) sandwiching the ligand; they seem to be in CH/π contact at two and three points with the guanine aromatic ring, respectively.

Asp119 (in ras p21) is invariant throughout the superfamily. Lys117 is well conserved (67 out of 68 proteins).[27] In 43 small G proteins,[28] Lys147 (p21) is replaced by leucine (12/43), threonine (2/43) or methionine (1/43). Inspection of the molecular structures suggests that CH/π interaction is possible with the residues indicated. To summarize, substitution may occur but the necessary CH/π interactions are conserved, together with essential hydrogen bonds.

SH2 proteins. The SH2 domains are modules of approximately 100 amino acid residues which were first identified from sequence similarities in the noncatalytic regions of Src family tyrosine kinases. The SH2 domains serve to localize the proteins to specific receptors and

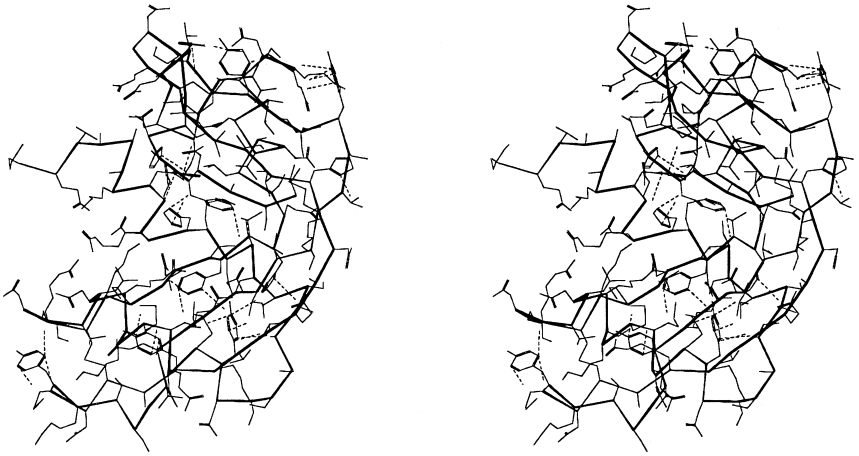

Figure 2. Global view of ribonuclease A showing CH/π interactions (stereo). Thick lines indicate the α carbon plot (hydrogens are omitted).

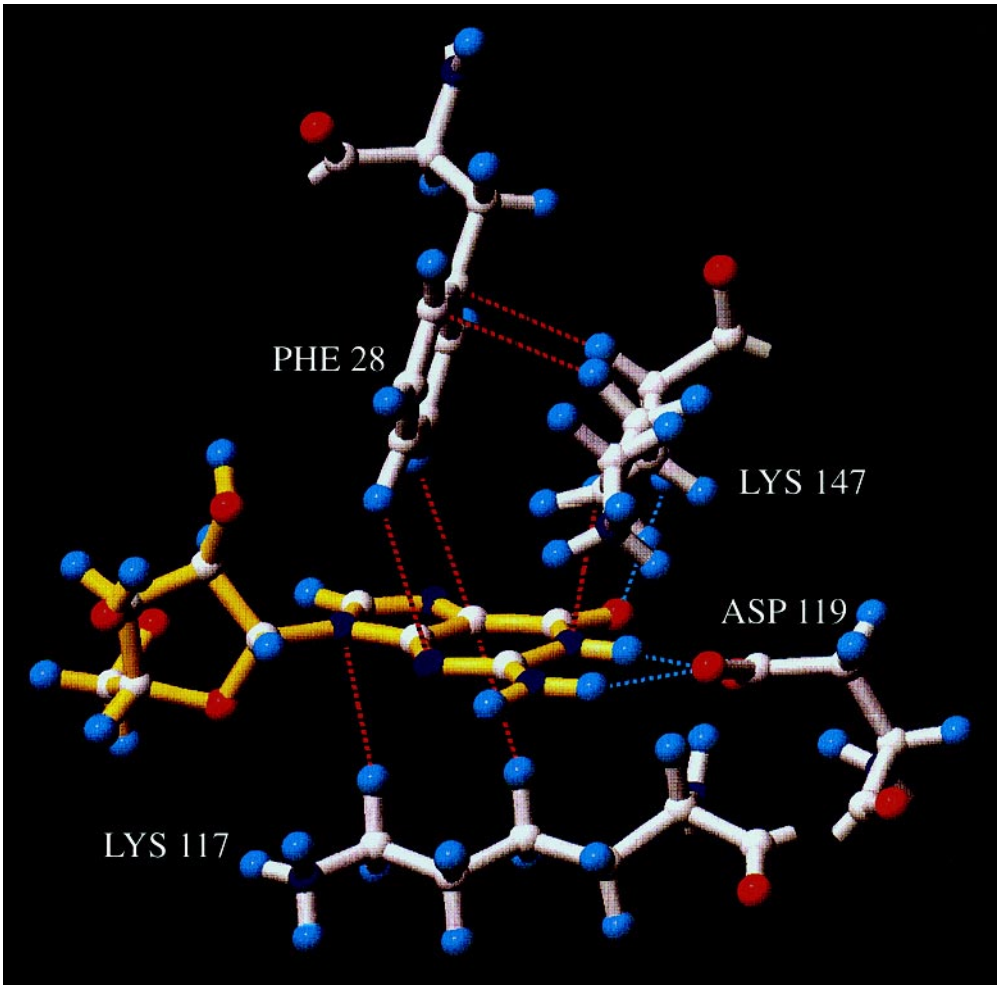

Figure 3. The guanine-binding region of Ras p21/GppNp complex (5P21) analyzed by CHPI. Gold sticks: ligand. White sticks: protein. Red and blue dotted lines indicate CH/π contacts and hydrogen-bonds, respectively.

are implicated in the modulation of the signals. They bind phosphotyrosine (pTyr) containing peptides with high affinity, recognizing pTyr in the context of the adjacent polypeptide sequences.[29] A number of cytoplasmic proteins in signaling pathway, such as phosphatidylinositol-3′-kinase (PI3K), ras-GTPase-activating protein (GAP) and phospholipase C–γ (PLCγ) have since been found to contain the SH2 motif(s).

Src SH2 domain complexed with a phosphorylated tyrosine peptide. The crystal structure of the SH2 domain was studied by Kuriyan et al.[30] for v-Src protein in complex with a tyrosine phosphorylated peptide, pTyr-Val^{+1}-Pro-Met-Leu (PDB code 1SHA, resolution 1.5 Å; residues are referred to by their position relative to pTyr such as Val^{+1} etc., when necessary).

LysβD6 (residues are labeled according to the stereochemical notations by Eck et al.)[31] was reported to form van der Waals interactions with pTyr. Our analysis (Figure 5) shows that CHs of LysβD6 participate in CH/π interactions with sp^2 carbons of the pTyr aromatic ring (2.9 Å for Hβ/Cδ1, 2.9 Å for Hε/Cδ2); NHs in the ω-amino group are remote even from the nearest C_{SP^2}. The guanidino group in ArgαA2 was reported to be close and locate itself above the aromatic ring of pTyr. In Figure 5 we see one of the terminal amino groups of ArgαA2 interacting with the tyrosine aromatic ring by two NH/π interactions (2.6 Å for HNη2/Cζ and 3.0 Å for HNη2/Cδ2). ArgαA2 is hydrogen-bonded with the phosphoryl group. HisβD4 is in CH/π contact with ArgαA2 (Hγ/Cε1, 2.9 Å) and ValβD2 (Hγ1/Nε2, 2.9 Å). Val^{+1} in the ligand peptide is CH/π bonded with TyrβD5 at two points (3.0 Å for Hβ/Cδ1 and 2.9 Å for Hγ2/Cε1).

Src SH2 complexed with a pTyr peptide of higher affinity. The above peptide binds rather weakly the Src SH2 domain; millimolar concentration is required to compete

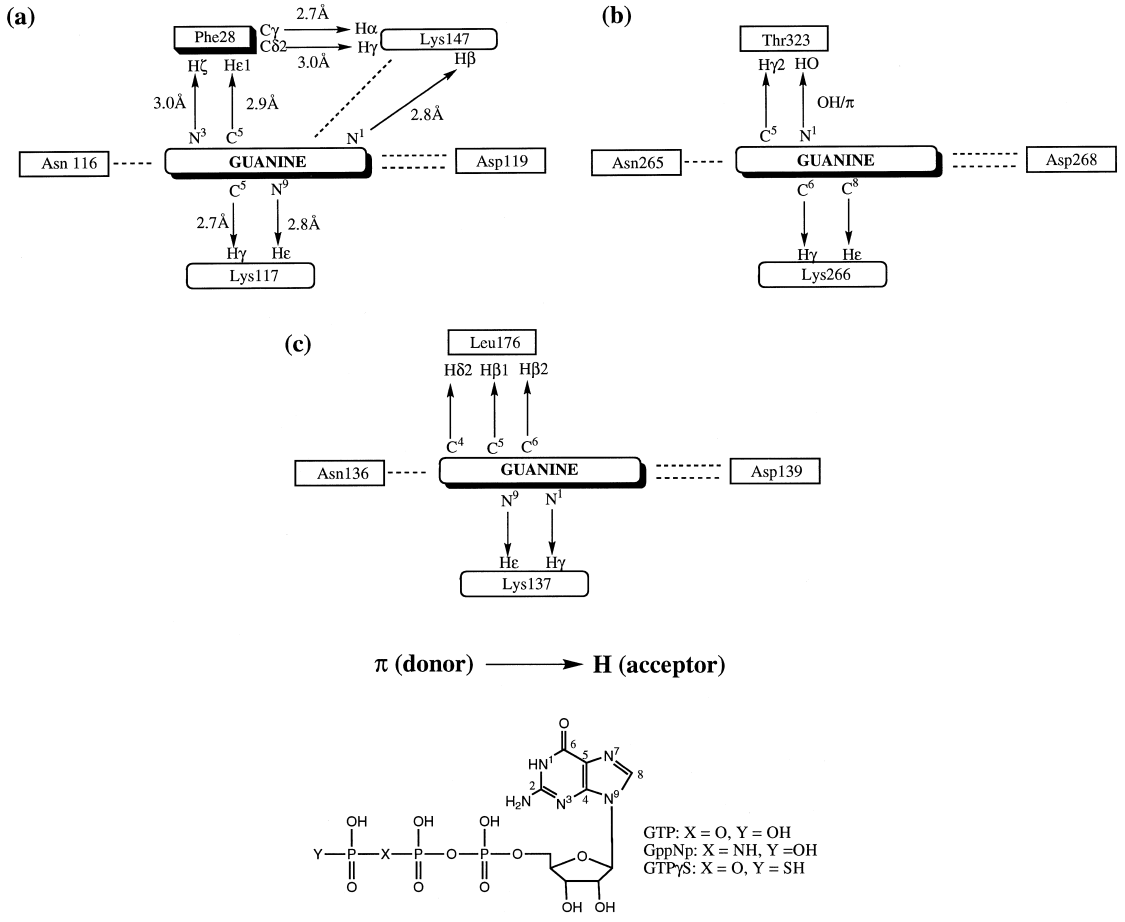

Figure 4. Schematic illustrations of the guanine-binding region of G proteins analyzed by CHPI. Arrows and dashed lines indicate CH/π contacts and hydrogen-bonds, respectively. (a) Ras p21/GppNp complex. (b) Transducin α/GTPγS complex. (c) EF-Tu/GppNp complex.

for SH2 binding with phosphorylated epidermal growth factor receptor. Kuriyan et al. studied the structure of Src SH2 protein complexed with a 11-residue phosphopeptide of higher affinity (*K*d ca. 3–6 nM, Glu-Pro-Gln-pTyr-Glu^{+1}-Glu-Ile^{+3}-Pro-Ile-Tyr-Leu: a sequence found in hamster middle T antigen: PDB code 1SPS, resolution 2.7 Å).[32] Three different structures (A/D, B/E, C/F) were found in a unit cell. No appreciable difference was found among these structures. Figure 6 gives a stereo view of the pTyr binding site of the complex (molecule A/D, thick lines). Interactions LysβD6/pTyr (two×CH/π), ArgαA2/pTyr (two×NH/π), ValβD2/HisβD4 (CH/π), and ArgαA2/HisβD4 (CH/π) are in common with those found in the complex of the low affinity peptide. TyrβD5 is in CH/π contact with Glu^{+1} [Hβ(1)/Cε2 and Hβ(2)/Cε2]. TyrβD5 is in turn supported, from the rear side of the ring, by a CH/π contact with LeuBG4 (Hδ1/Cζ, not shown).

Difference in the binding constants noted between the high and low affinity complexes was reported to be due to the presence of Ile^{+3} in the former. It was argued that the isoleucine side-chain is bound tightly in a pocket lined with a number of lipophilic residues. We sought for interactions around Ile^{+3} by CHPI. The isoleucine secondary butyl group, however, was not found in close proximity (< 3.5 Å) to an aromatic residue.

Lck SH2 domain complexed with a peptide fragment from hamster middle T antigen. Eck et al. studied the complex structure of the above same peptide with a SH2 domain of tyrosine kinase Lck p56 (PDB code 1LCJ, resolution 2.2 Å). This protein is a member of the Src family tyrosine kinase associated with the T cell receptor. Figure 6 gives a stereo view of the pTyr binding site (thin lines: superimposed on that of 1SPS). Overall, interactions of pTyr with LysβD6 and ArgαA2, those

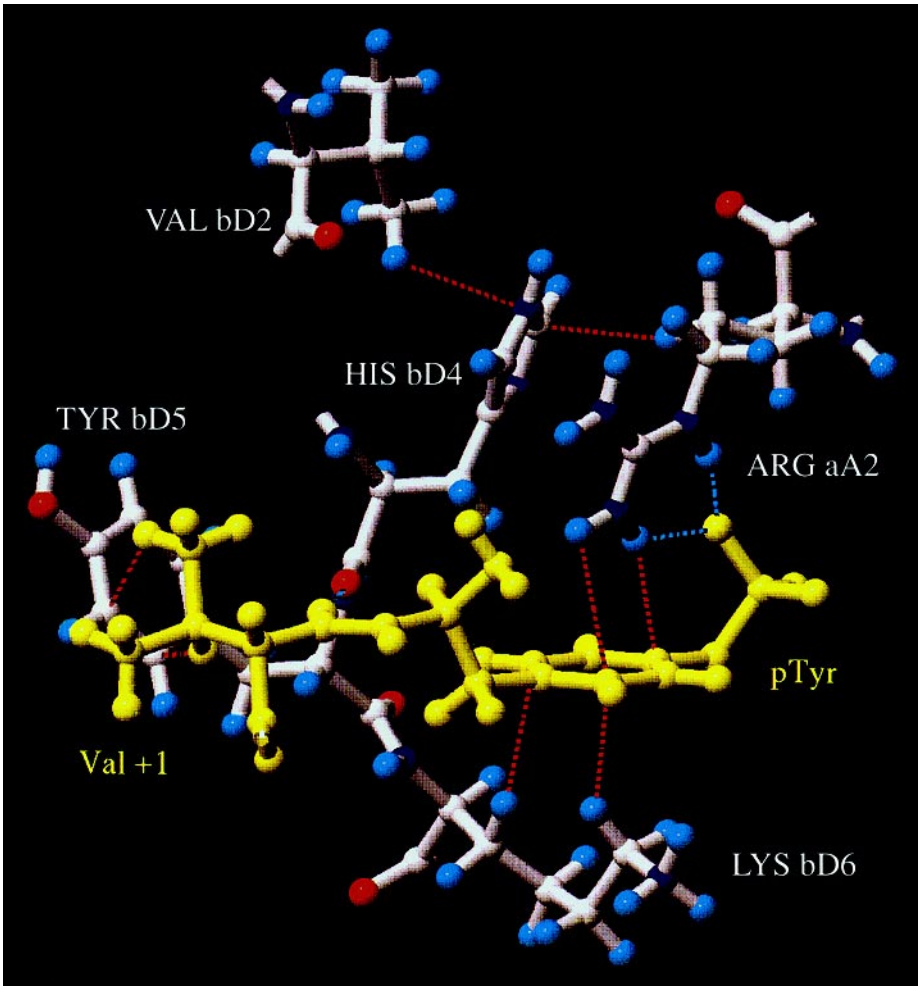

Figure 5. The phosphotyrosine (pTyr) binding region of a protein/peptide complex. Src SH2l/pTyr-Val-Pro-Met-Leu (1SHA). Yellow: ligand. White sticks: protein. Red and blue dotted lines indicate CH/π contacts and hydrogen-bonds, respectively.

between HisβD4 with ValβD2 and ArgαA2, and Glu^{+1} with TyrβD5 are in common with those observed in 1SPS.

Syp SH2 domain complexed with a peptide fragment from PDGF receptor. Kuriyan et al. studied the crystal structures of N terminal SH2 domain of Syp tyrosine phosphatase (SypN) in complex with peptide fragments from platelet derived growth factor (PDGF) receptor,[33] a high affinity peptide PDGFR-1009 (Ser-Val^{-2}-Leu-pTyr-Thr-Ala-Val-Gln-Pro-Asn-Glu: PDB code 1AYA, resolution 2.05 Å) and a low affinity one PDGFR-740 (Asp-Gly-Gly-pTyr-Met-Asp-Met-Ser-Lys-Gly-Ser: 1AYC, 2.3 Å). The mode of binding of SypN with these peptides, as a whole, was reported to be similar to that shown in the Src and Lck structures.

Two molecules were found in the asymmetric unit in crystals of the SypN/PDGFR-1009 complex (1AYA): molecule A/P and molecule B/Q. Figure 7 illustrates schematically the pTyr binding site of these molecules. Interactions of pTyr with ArgβB5 (bidentate hydrogen bonds) and LysβD6 (CH/π bonds) are conserved. ArgαA2 in Src and Lck SH2 is replaced by glycine in

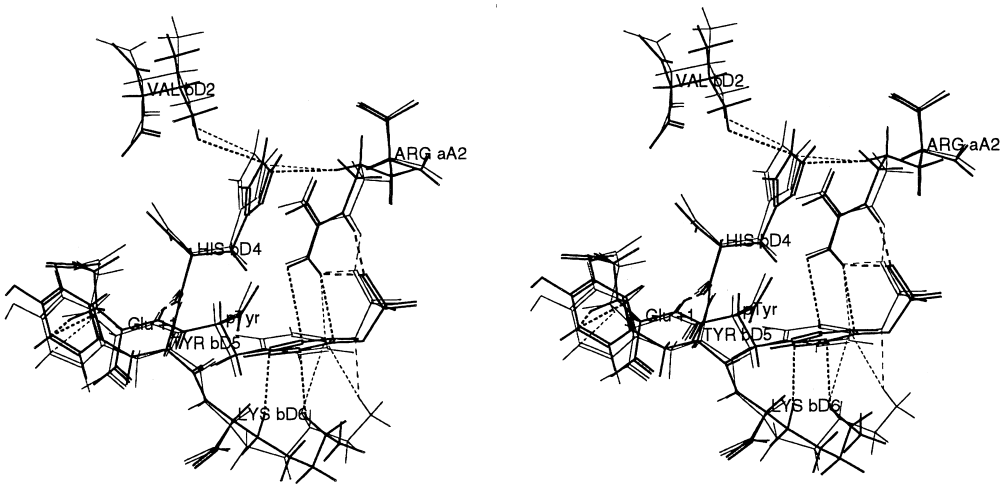

Figure 6. Superimpose of stereo views of Src SH2 protein complexed with a phosphopeptide, Glu-Pro-Gln-pTyr-Glu-Glu-Ile-Pro-Ile-Tyr-Leu (1SPS: thick lines) and Lck SH2 complexed with the same peptide (1LCJ: thin lines). Dotted and dashed lines indicate CH/π contacts and hydrogen-bonds, respectively.

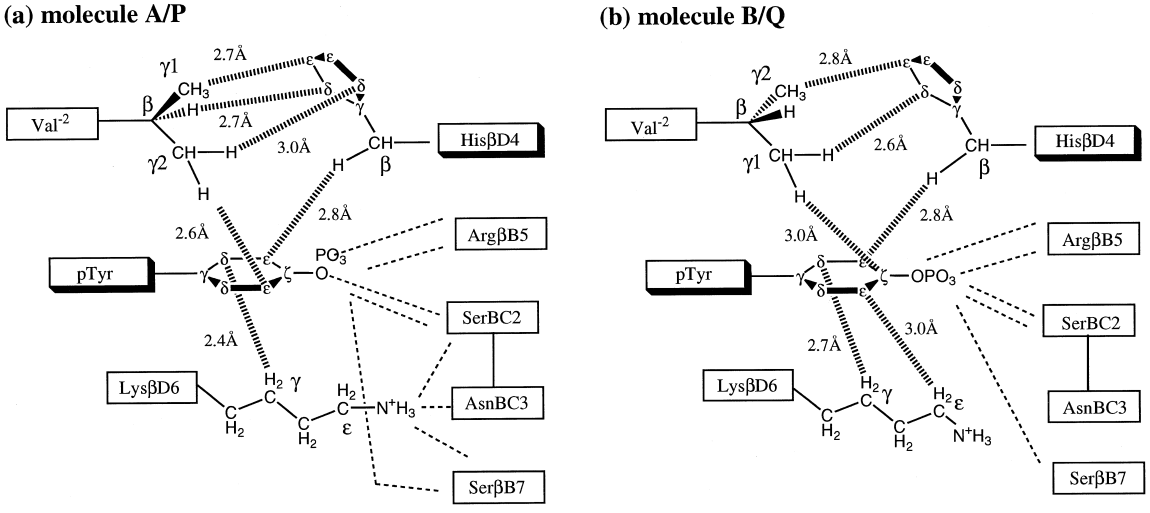

Figure 7. Schematic illustrations of the tyrosine-binding region of SypN SH2 domain complexed with a high affinity phosphotyrosyl peptide PDGFR-1009 (Ser-Val-Leu-pTyr-Thr-Ala-Val-Gln-Pro-Asn- Glu (1AYA). (a) Molecule A/P. (b) Molecule B/Q. Hashed and dashed lines denote CH/π bonds and hydrogen bonds, respectively. Aromatic residues are shaded.

Syp proteins (SypN and SypC) but the necessary CH/π interaction with pTyr is supplied by the use of Hβ of HisβD4; the aromatic ring of the histidine in turn binds the side-chain of Val^{-2} at three points in molecule A/P [Figure 7(a)] and at two points in molecule B/Q [Figure 7(b)]. The latter interactions are absent in the PDGFR-740 complex; Val^{-2} in PDGFR-1009 is replaced by glycine in PDGFR-740.

Hydrogen bondings of ArgβB5, SerBC2, and AsnBC3 with the phosphoryl group remain practically unaltered between the molecules A/P and B/Q. The mode of bindings with regard to the ligand aromatic part, however, is quite different from each other. In molecule A/P [Figure 7(a)], LysβD6 is hydrogen-bonded to three residues (SerBC2, AsnBC3 and SerβB7) with the use of ω-amino group but has only one CH/π contact (Hγ/Cδ2) with pTyr. On the other hand, in molecule B/Q [Figure 7(b)], LysβD6 binds the tyrosine aromatic ring, by two CH/π interactions (Hγ/Cδ1, Hε/Cε2), but its amino group is free from hydrogen bond. A seesaw like switching of the binding forces takes place. This suggests that the CH/π interaction is at least equally important (if not stronger) as compared with the hydrogen bond in stabilizing the structure of the complexes.

In molecule A/P, three hydrogens (Hγ1, Hγ2 and Hβ) in Val^{-2} are involved simultaneously in interactions with the aromatic ring of HisβD4. In molecule B/Q, on the other hand, Hγ1 and Hγ2 are in contact with HisβD4, while Hβ is free from the interaction. In both molecules, a Hβ of HisβD4 interacts with the tyrosine aromatic ring. A CH hydrogen in one of the geminal dimethyl groups in Val^{-2} is in contact with pTyr, intramolecularly. Here again, exchange of the partner occurs. Hγ2 of Val^{-2} is in contact with pTyr aromatic ring in molecule A/P, whereas in molecule B/Q it is Hγ1 which binds pTyr. The valine side-chain rotated accordingly ca. 120° around the Cα–Cβ axis.

Conservancy of the residues among 26 SH2 sequences was examined in order to see the relevance of the CH/π interactions. ArgβB5 is invariant throughout the family. This is reasonable since the bidentate ion-pair with phosphotyrosine plays a crucial role in the binding. ArgαA2 is conserved in 22 SH2 motifs. In two cases it is replaced by lysine and in two cases by glycine (SypN and SypC). LysβD6 is conserved in 10 proteins. In other cases this is replaced by arginine (5 motifs), valine (3), isoleucine (3), leucine (3), methionine, or proline. HisβD4 is conserved in 23 proteins but is replaced in other three cases by leucine, arginine or asparagine. Inspection of the molecular structures suggests that CHs in the side-chains can participate in interactions with the tyrosine aromatic ring. Residues are replaced but the necessary CH/π interactions are conserved.

Interactions involving lysine and arginine

In G proteins and SH2 complexes we saw a number of CH/π interactions around the aromatic rings of the ligands (CH/π network). The CH/π network certainly plays an important role, in cooperation with hydrogen

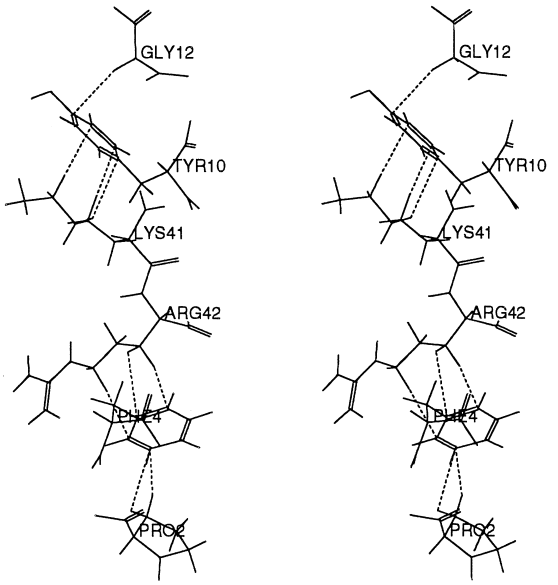

Figure 8. Lysine (arginine) CH/π interactions (stereo view) found in bovine pancreatic trypsin inhibitor BPTI (5PTI).

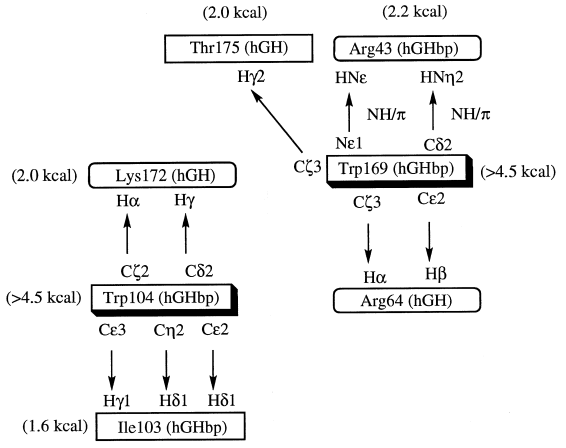

Figure 9. Lysine (arginine) CH/π interactions involved in the high-affinity binding site of human growth hormone-binding protein hGHbp complexed with human growth hormone hGH (3HHR). Numbers in parentheses correspond to the energetic contribution (in kcal mol^{-1}) of the side chain determined by point mutation. Aromatic residues are shaded.

bonds, in binding their substrates in a specific manner. Of particular interest, among others, are the interactions involving the lysine side-chain. This type of interaction (lysine CH/π interaction) is found in a number of proteins and protein/substrate complexes. An examples is given in Figure 8 for interactions (Lys41/Tyr10) found in BPTI (PDB code 5PTI, resolution 1.0 Å). Figure 9 shows another interesting case found at the high-affinity binding site of human growth hormone-binding protein (hGHbp) with human growth hormone hGH (3HHR, resolution 2.6 Å).[34] Trp104 in hGHbp seems to be in CH/π contact at two points with Lys172 of hGH. Trp104 in turn is assumed, from the other side of the aromatic ring, with three CH/π interactions by Ile103 of hGHbp.

Arginine behaves like lysine. We see examples in BPTI (Figure 8: Arg42/Phe4) and at the high affinity binding site of the hGHbp/hGH complex (Figure 9). Trp169 of hGHbp, which is known to be crucial for the binding, is in CH/π contact with Arg64 and Thr175 of hGH. Trpl69 of hGHbp is assisted by Arg43 of the same protein with two NH/π interactions (Figure 9). Significant reductions in the binding affinity were reported to occur by point mutation of the above residues by alanine. Figure 10 shows an interesting example for a sequence of residues, Lys215/Tyr222/Arg213/Phe225/Arg211/Trp 186/Lys179, in hGHbp. The methylene hydrogens of the lysines and arginines are involved in a stacked manner with aromatic rings of the aromatic residues, like a Dogwood sandwich. Four and three methylenes, respectively, are present in the lysine and arginine side-chain. It is certain that these residues are effective, with use of the linearly arranged CH and the terminal NH[35] groups, in stabilizing the three-dimensional structure of proteins and protein/ligand complexes.

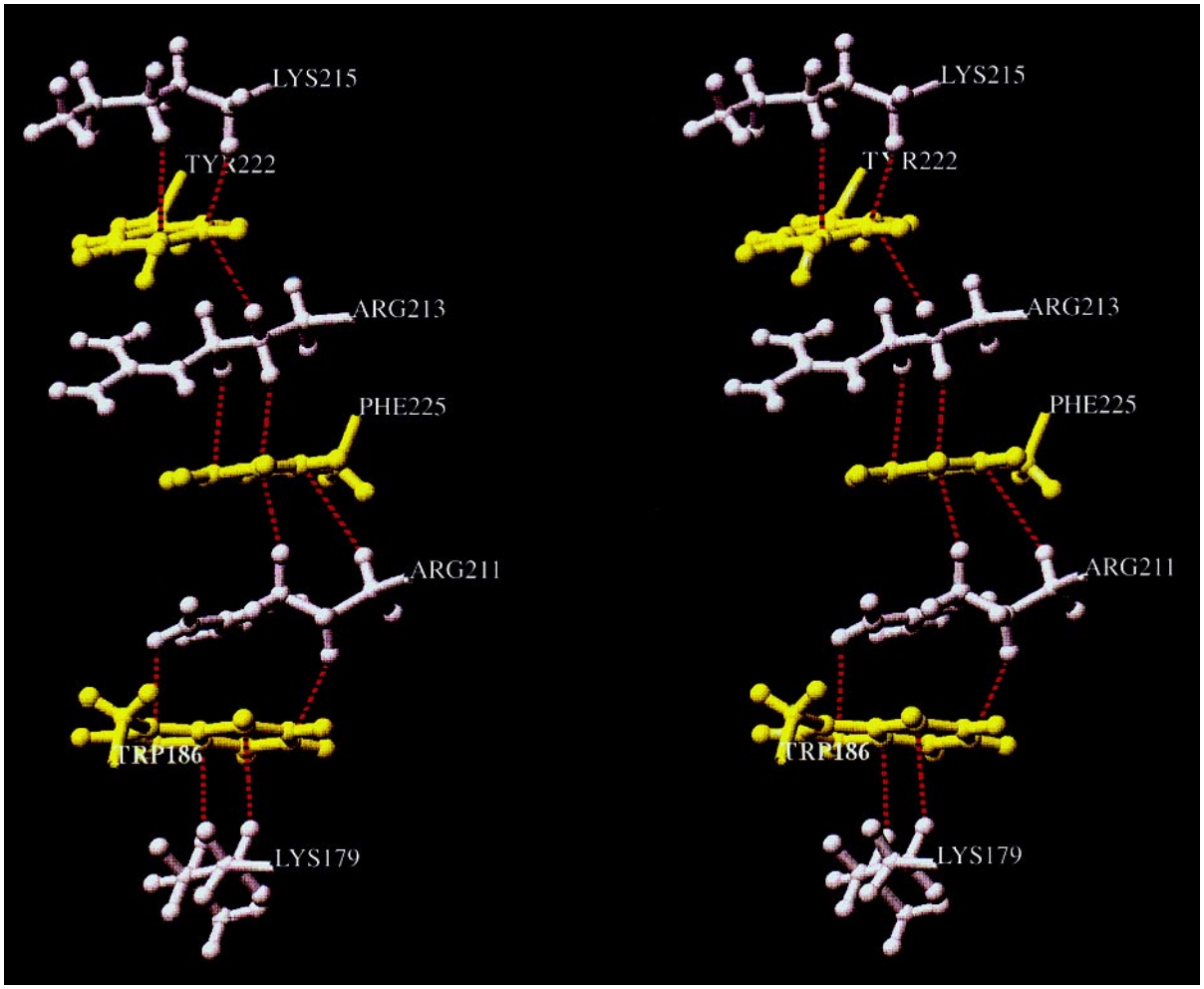

Figure 10. A stack of lysine (arginine) CH/π interactions found in human growth hormone (stereo view). Yellow: aromatic residues. White: lysine and arginine.

Conclusion

The higher-order structure of proteins relevant to cell biology are rapidly being elucidated at the atomic level by means of modern analytical methods such as X-ray crystallography and NMR spectroscopy. The molecular forces involved there should thus be analyzed with the same level of accuracy. In view of the discussions raised as above it seems reasonable to suggest that a considerable part of the nonpolar interactions, hitherto broadly ascribed to the van der Waals force or the so-called hydrophobic interaction[36] should be attributed to a more specific attractive force. Available data in the literature, such as substituent effects on spectral data,[37] conformational equilibria[38] and thermochemical results,[39] demonstrate that the CH/π interaction is not merely a conventional dispersion force. According to theory[2c,40] and experimental evidence, we believe that the CH/π interaction is largely originated by a charge transfer process from the π system to the antibonding orbital of the C–H bond (π→σ*). Superimposed on this effect is the dispersion force. The electrostatic force, though not very important, also contributes.

In other words, the CH/π interaction is the weakest extreme of hydrogen bond, which occurs between hydrogens attached to carbon (C–H) and π electron systems. The CH/π interaction can therefore play its role in polar as well as non-polar media, unlike conventional hydrogen bonds and the so-called hydrophobic interaction. Though weak (around 1 kcal mol^{-1} for a one unit interaction),[2] a unique feature of the CH/π interaction is that the relevant groups (CH as well as π) are ordinarily arranged in an organized fashion and thus gives rise to a considerable stabilization for the specific binding of interacting molecules; this is especially true for interactions involving chemical species of high-molecular weight such as proteins. The concept of the CH/π interaction is useful in the engineering of macromolecules including proteins and in designing biologically active substances such as drugs and agrochemicals.

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