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THE CH/π INTERACTION: SIGNIFICANCE IN MOLECULAR RECOGNITION*

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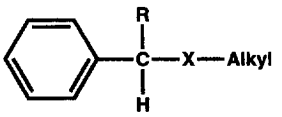
1. INTRODUCTION

Evidence has accumulated that attractive interactions are present between C–H groups and π-electron systems.[1,2] A suggestion for the presence of such an interaction, the CH/π interaction, has

* This paper is dedicated to Professor Sir D. H. R. Barton, the founder of concept of conformation, in this, his 77th year.

come from studies on conformational problems of a series of compounds bearing an aliphatic group on one side of the molecule and a phenyl group sited at the other terminus (**1**).

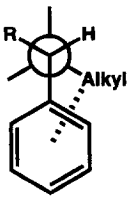


1

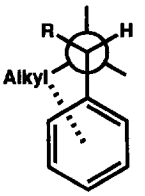
X = S
SO (threo)
SO (erythro)
SO_2
CH_2
CHOH (threo)
CHOH (erythro)
C=O

R = H
CH_3

Alkyl = Me, Et, iPr, tBu

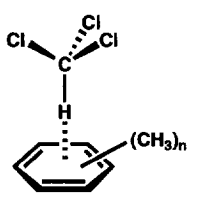


1a



1b

In almost every case studied,[3] the alkyl group has been shown to prefer synclinal orientations with respect to the phenyl group (**1a** and **1b**).[4] The conclusion regarding the conformational preferences did not vary significantly with changing conditions of measurement.[5] Experimental methods included X-ray,[6–8] NMR,[9–11] IR,[11,12] circular dichroism,[7] and dipole moment measurements.[13] Comparison of the experimental results (consideration on lanthanide-induced NMR chemical shifts) for alcohols (**1**, X = CHOH, R = H and CH_3) with those obtained from molecular mechanics calculations[14] indicated that an extra attractive interaction, different from the dispersion force, is present between alkyl and phenyl groups.[15] Suggestions have been made, based on these findings, that a variety of molecular phenomena, chemical as well as biochemical, are interpreted as consequences of this attractive force: the CH/π interaction hypothesis.[16]



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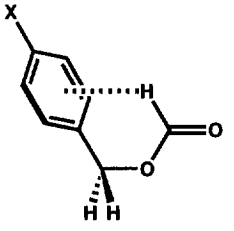
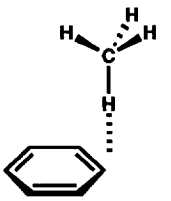


3

NMR studies on other systems such as triptycene derivatives,[17] **2**[18] and **3**[19] supported the above suggestion.[20] CH/π-Interacted species, in every case, have been found to become more favourable as the π-electron density of the aromatic ring increased, as was expected.

Theoretical support for the presence of such an attractive interaction has come from a semi-empirical molecular orbital calculation on methane/benzene binary system (**4**),[21] *ab initio* calculations on acetylene dimer (**5**),[22] benzene dimer (**6**),[23,24] and the methane/ethylene supramolecular system (**7**).[25]



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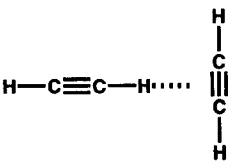

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6

T- or L-Shaped geometry has been demonstrated to be most stable in all of the above calculations. In such an arrangement, one of the C–H bonds is located above the π-orbital to give a maximum overlap between the interacting atoms. Table 1 summarizes computational results reported by Takagi *et al.* for interactions of CH_4 with ethane, ethylene and acetylene.[25]

Table 1

Energy components (in kcal/mole) for the CH_4 (A) plus C_2H_n (B: n = 2, 4, or 6) systems calculated by 4-31G basis set

B	ΔE	ΔE_{SCF}	ES	$CT_{B\rightarrow A}$	DISP
H-C≡C-H	-0.66	-0.47	-0.13	-0.52	-0.19
$H_2C{=}CH_2$	-0.88	-0.64	-0.14	-0.72	-0.24
CH_3-CH_3	-0.24	-0.05	-0.08	-0.08*	-0.19

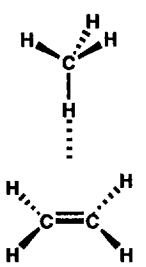
ΔE: stabilization energy with reference to isolated A and B.

ES: Electrostatic energy, CT: charge transfer energy due to electron migration from B to A, DISP: dispersion energy.

ΔE_{SCF} = ES + PL (polarization) + EX (exchange repulsion) + CT + MIX (higher order interactions)

*$CT_{B\rightarrow A}$ + $CTA_{\rightarrow B}$

As for the intramolecular system, positive overlap populations were found in conformers where CH/π interactions are stereochemically possible, by a 4-31G calculation of 1-phenyl-2-propanol (**8**).[26]



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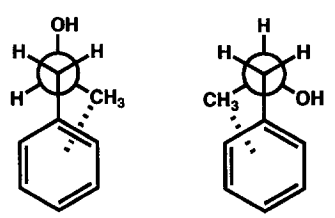

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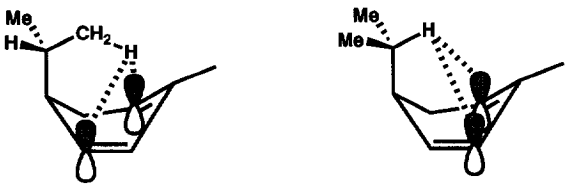
Unequivocal proof for the contribution of a charge-transfer interaction has come from considerations on optical rotatory strength of unsaturated compounds such as α-phellandrene (**9**), exomethylene steroids **10** and **11** as well as the methane/ethylene supramolecular model system.



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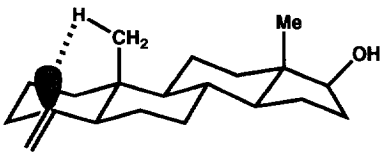

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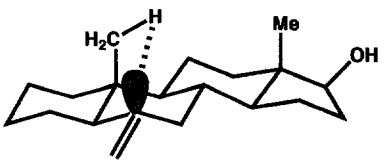

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Thus 4-31G calculations on iPr quasi axial conformers of **9** gave positive bond populations between H and sp^2 carbons which occupy positions geometrically advantageous for CH/π interaction; examination of the MO functions showed the relevant H and Csp^2 orbitals to be in phase both in the HOMO and LUMO in most cases.[27] Rotatory strengths were calculated on the basis of Rosenfeld theory, using AM1 molecular orbitals; an appreciable enhancement in CD intensity was demonstrated to occur for axial conformers where CH/π interactions are stereochemically possible. Further, rotational strengths calculated for 4-methylene-5α-androstane (**10**) and 6-methylene-5α-androstane (**11**) were found to be much greater than those for the respective molecules lacking the 10-axial methyl group,[28] in agreement with observation.[29,30]

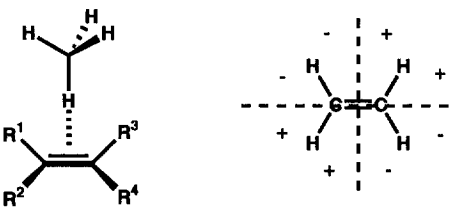


Figure 1. Optical rotatory strength of ethylene chromophore induced by a methane molecule placed at various position. Positive CD is induced when C–H is located in the region (+), and vice versa, thus giving rise to an octant centred at the midpoint of the C=C double bond.

In the case of the supramolecular system (Fig. 1),[28] the carbon atom of methane was fixed at points 3.6 or 3.8 Å above the plane of the ethylene ($R^1 = R^2 = R^3 = R^4 = H$) and one of the C–H bonds was kept perpendicular to the molecular plane. The optical rotatory power of the system was then calculated according to the above method.[31] Non-zero induced rotational strength has been obtained when the CH bond was located out of the symmetrical planes of the ethylene molecule. Since both components are achiral in the absence of interaction, the calculated rotational power of the π, π* transition should have originated from chiral distortion of the π-electron cloud of ethylene by a CH group of methane: an unequivocal demonstration for the presence of an orbital interaction between the molecules.[32]

2. CHARACTERISTICS OF CH/π INTERACTION

The properties of various types of hydrogen bonds are compared in Table 2.[1] Thus, the origin of this weak attractive force has been ascribed to a kind of hydrogen bond between a soft acid (CHs in an alkyl group) and a soft base (π system).[33]

Table 2

Type of H-bond	Energy of interaction			
Acid /Base	Delocalizative	Coulombic	Dispersive	Repulsive v.d.W.
CH(soft)/π(soft)	important	unimportant	important	similar
XH(hard)/π(soft)	important	weak	important	similar
CH(soft)/n(hard)	unimportant	important	unimportant	similar
XH(hard)/n(hard)	variable	strong	unimportant	similar

X = O or N n: lone pair

The CH/π interaction is characteristic of a relatively large contribution from delocalization (charge transfer from π to σ*) and dispersive interaction as compared to the normal H-bonding. A contribution from electrostatic interaction has been shown to be unimportant. A crucial point—unlike in the typical hydrogen bonding between a hard acid and a hard base—is that CH/π interaction can play its role in polar as well as in non-polar media; interactions of the above kind are hardly disturbed by the presence of water. This is important when considering molecular interactions in biological environments.

Enthalpy for a one unit CH/π interaction is small, around 1 kcal mol^{-1}. This estimate has been made from the following considerations: (i) slope regarding a ΔH versus σ plot for CH/π interaction in an IR experiment[12b] was found to be about one-half as compared to that of well-characterized OH/π interaction.[34–37] The enthalpy for the latter is known to be around 2 kcal mol^{-1}. (ii) Computation on a model compound, 1-phenyl-2-propanol (**8**), gave rise to a bond population for the CH/π interaction about half of that calculated for the OH/π interaction.[26] (iii) *Ab initio* calculation (4-31G) of a methane/ethylene supramolecular system (Table 1), gave ca 0.9 kcal mol^{-1} for a one unit CH/π interaction (total enthalpy; charge transfer from π to σ* is ca 0.7 kcal mol^{-1}).[25] One should keep in mind, however, that multiple CH groups can participate simultaneously in interactions with π groups (Fig. 2). The total enthalpy becomes sizeable, especially in interaction with compounds of higher molecular weight.

Groups which may be involved in CH/π interactions include methyl (Rln3 = 2.2 e.u.: TΔS = 0.65 kcal mol^{-1} at 300 K), isopropyl, long-chain alkyl groups, or CHs in an aromatic ring for the CH part. Unsaturated bonds (isolated or conjugated), aromatic groups such as those in amino acids (Phe, Tyr, Trp, His), nucleic acid bases, flavin, porphyrins, etc. make up the π part. This kind of interaction is entropically advantageous in that the chance for interaction is increased by organizing CHs and/or π groups into a discrete (often symmetric) chemical structure. This is important in understanding the behaviour of dynamically interacting molecular systems. The entropy effect, however, is not included generally in theoretical (MO or MM) calculations nor is it directly reflected in crystallographic results. The specificity of an interaction (in specific binding or selective reaction) is determined by the difference in free energy (ΔG^0 or $\Delta\Delta G^{\neq}$) of the two competing states (ground or transition state: Fig. 3). In view of this, it is pertinent to point out that a difference in free energy of ca 4 kcal mol^{-1} is sufficient for bringing about a 1000: 1 specificity. A contribution from the entropic term (or chance effect) would be appreciable.

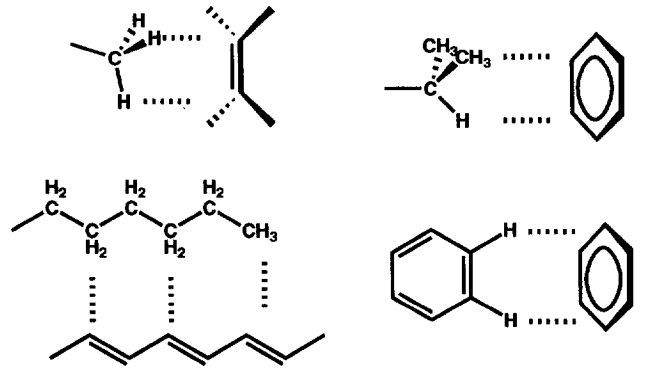


Figure 2. Schematic illustration showing characteristics of the CH/π interaction: multiple H atoms can interact simultaneously with multiple sp^2 atoms; the chance of participating in an interaction increases because of the symmetry of the groups.

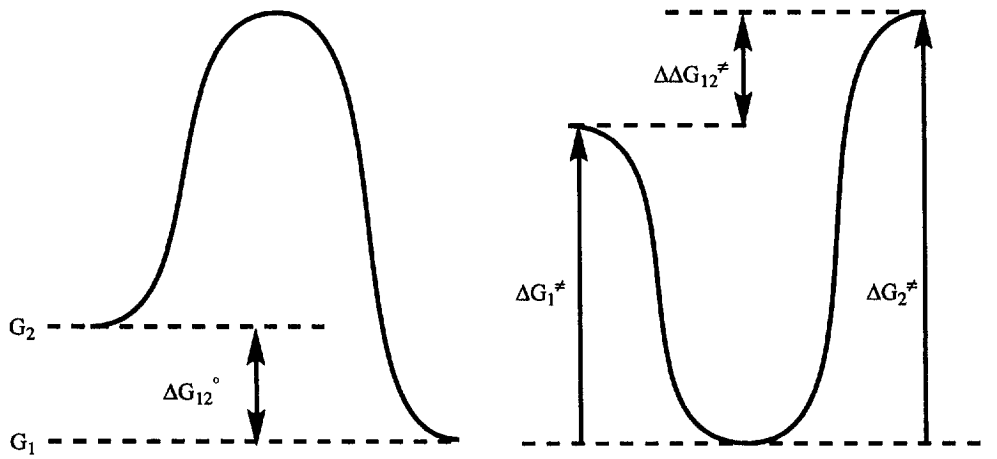


Figure 3. Energy profiles for a specific binding and a selective reaction. Difference of ca 4 kcal mol^{-1} in ΔG^0_{12} (G_2–G_1) or $\Delta\Delta G_{12}^{\neq}$ ($\Delta G_2^{\neq}$–$\Delta G_1^{\neq}$) is sufficient to bring about a 1000 to 1 selectivity.

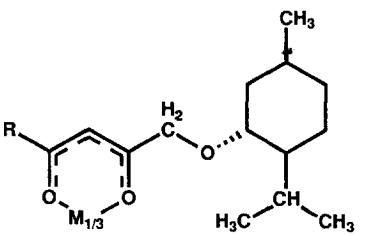
Discussion in the following sections will show the possibilities of the attractive force of such a nature in specific interactions in chemistry and biochemistry.

3. INTRAMOLECULAR INTERACTION

Intramolecular interactions where the CH/π interaction plays a role include problems of conformation.[38] In fact many X-ray crystallographic data indicate the occurrence of a CH/π interaction. The following are a few examples. Short atomic contacts were observed for (*SR*)/(*RS*) and (*SS*)/(*RR*)-1-(*p*-bromophenyl)ethyl *t*-butyl sulphoxides (distance about the relevant C/Csp^2: 3.32 and 3.33 Å, respectively), 2-(*p*-bromophenyl)-2,4,4,6-tetramethyl-1,3-dioxan (3.2 Å),[39] bis(2,4,6-tributylphenyl)phosphinic chloride (3.3 Å),[40] levopimaric acid (2.53 Å: $C^{17}H/C^8$, steroid numbering),[41] lumisterol (2.74 Å: $C^{18}H/C^8$),[42] pyrocalciferol (2.75 Å: $C^{18}H/C^8$),[43] and isopyrocalciferol (2.57 Å: $C^{18}H/C^8$).[44,45] The distances calculated by assuming van der Waals contact are ca 3.7 Å (2.0 Å for CH_3 and 1.7 Å for Csp^2) and 2.9 ~ 3.1 Å (1.2 ~ 1.4 Å for H and 1.7 Å for Csp^2), respectively, for C/Csp^2 and H/Csp^2. The conformational preference of fluorene derivatives reported by Nakamura *et al.*[46] is consistent with the presence of CH/π interaction. Methyl/phenyl short contacts were reported for a pair of conformational isomers of a 1-benzazocinone derivative and the results

were discussed in terms of the CH/π interaction.[47] Conformations of oligomeric flavanoids,[48] 1,6-disubstituted cyclooctatetraenes[49,50] and a layered superstructure of arrayed [2]pseudorotaxanes[51] were studied and the results were interpreted as supporting an attractive interaction between relevant groups.

Circular dichroism of certain compounds such as unsaturated terpenes[52–54] or steroidal ketones (for spectra at ca 200 nm)[55] show significant enhancement when an axial alkyl group is present close to the double bond. The "unusual" phenomena have been interpreted successfully on the basis of the CH/π interaction, or through-space hyperconjugation of the CH group with Csp^2.[56,57] Intramolecular interactions also include the problems of selectivity in diastereoface-differentiating reactions such as Prelog's system.[58] The extent of asymmetric synthesis has been shown to be greater for benzoyl formate (bearing a phenyl group) esters than for pyruvate (Me instead of Ph) esters. In remote functionalization reactions reported by Breslow *et al.*, sizeable selectivities were brought about in every case, when an aromatic group was incorporated with the reacting molecule.[59] Optical activation of aldehydes via the enamine with (*S*)-2-isopropyl-1-methylpiperazine was studied.[60] The result was interpreted as a CH/π (favourable isopropyl/phenyl) interaction in the enamine intermediate. Stereoselective formation of a variety of coordination complexes[61] was reported, where the contribution of the CH/π interaction has been demonstrated.



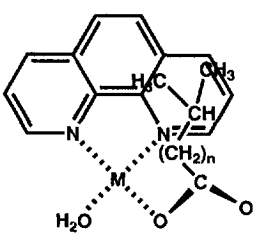
12

R = p-X-C_6H_4, C_8H_7, Alkyl

X = CH_3, H, Br

M = Co, Cr

Thus Okawa *et al.*[62] studied the selective formation of a series of coordination compounds such as **12** (R = phenyl or naphthyl) and found that stereoisomers (*fac* Δ) in which the menthyl group is face-to-face interacted to the aromatic ring were produced preferentially. The proportion of the predominant isomer increased if an electron-donating group was introduced in the aromatic ring; the reverse was true for an electron-withdrawing substituent. Such remarkable stereoselectivity was not observed with compounds bearing a non-aromatic group as R. Sigel *et al.*[63] observed favourable alkyl/aromatic interactions in conformations of a number of ternary coordination complexes such as **13**. The results were discussed in connection with the structure and function of metal enzymes.[64,65]



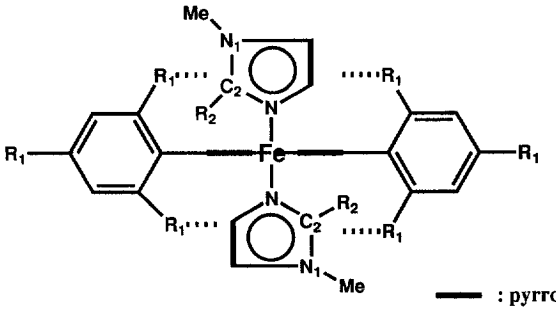
n = 0 to 4

M = Cu or Zn

13

Onaka *et al.*[66] reported short atomic contacts (H/Csp^2: 3.06 and 2.75 Å) in their crystallographic structures of [η^5-$CH_3C_5H_4$)Mn(CO)(1,1′-bis(diphenylphosphino)ferrocene)]. Jitsukawa *et al.*[67] studied the crystallographic structure of bis-(*N*-pyridoxy-L-phenylalaninato)cobalt complex and found that a methyl group is in proximity to the pyridoxy pyridine ring (C/Csp^2: 3.56 Å). This was argued

as evidence for a CH/π interaction and may be relevant for the geometry of the binding site of an enzyme, aspartate aminotransferase.[68] The finding recently reported by Nakamura and Nakamura[69] is interesting enough to merit special comment.



14

They studied the thermodynamics for the formation of low spin tetrakis(2,4,6-trialkylphenyl)porphyrinato-iron(III) (**14**). In contrast to the results obtained with lower alkyl analogues (R_1 = H, Me), triethylphenyl and triisopropylphenyl derivatives (R_1 = Et, iPr) showed a larger negative enthalpy of formation with 1,2-dimethylimidazole (R_2 = Me) than with 1-methylimidazole (R_2 = H) as the axially coordinated ligand. The attractive energy calculated for **14** (R_1 = iPr, R_2 = Me) was 7.8 kcal mol^{-1} greater than that of a lower analogue[70] (**14**, R_1 = H, R_2 = Me). The results demonstrate unequivocally the occurrence of an attractive interaction of the alkyl group with the imidazole moiety, most probably with its π-electron system.

Further, Dauben has recently commented upon the possibilities of involvement of the CH/π interaction in photochemistry.[71]

4. CH/π INTERACTIONS IN MOLECULAR RECOGNITION

Intermolecular interactions, or molecular recognition, where the CH/π interaction plays a role includes selectivity of organic reactions, specificities in solution[72] or surface phenomena such as chromatographic properties,[73,74] problems of electron transfer, properties of solid materials such as graphite and fullerene,[75] as well as substrate specificity of biologically important macromolecules.[76] This latter possibility was discussed briefly in our earlier papers[77] for several enzymes, immunoglobulins and haemoglobin. Also interesting in view of intermolecular interactions are problems of specificity in inclusion phenomena using cyclodextrins and synthetic macrocycles such as cyclophanes, calixarenes, etc. Here we explore the involvement of the CH/π interaction in the light of data available for several organic reactions, crystallographic structures of inclusion compounds and protein/specific ligand complexes.

4.1. *Selectivity in organic reactions*

4.1.1. *Enantioface-differentiating reactions.* Data listed in Tables 3-1 and 3-2 were extracted from the work of Mosher *et al.*[78] They studied the enantioface-differentiating reaction of ketones R_SCOR_L with a chiral Grignard reagent prepared from (+)-1-chloro-2-methylbutane. Table 3-2 gives the results obtained by reduction of alkyl phenyl ketones (**15**) with a Grignard reagent from (+)-1-chloro-2-phenylbutane (**16**). These chiral reductions gave rise to preferential formation of alcohols having the (*S*)-configuration, with a few exceptions for ketones bearing a *t*-butyl group.

Most remarkable of all is the difference in the optical yields listed in the two Tables. The extent of asymmetric synthesis is much greater in cases where a phenyl group is incorporated in both the

Table 3-1

Optical Yields [%ee] of the Reduction of Ketones with Grignard Reagent from (+)-1-chloro-2-methylbutane

R_S/R_L	tBu	cHex	Ph
Me	13	4	4
Et	11	9	6
iBu	6	16	10
iPr	0	2	24
tBu		2*	16

*(*R*)-enantiomer was obtained in excess

Table 3-2

Optical Yields [%ee] of the Reduction of Phenyl Alkyl Ketones (**15**) with Grignard Reagent from (+)-1-chloro-2-phenylbutane (**16**)

R/R'	Me	Et	iPr
Me	38	47	-
Et	38	52	66
iBu	-	53	-
iPr	59	82	80
tBu	22*	16	91

*(*R*)-enantiomer was obtained in excess

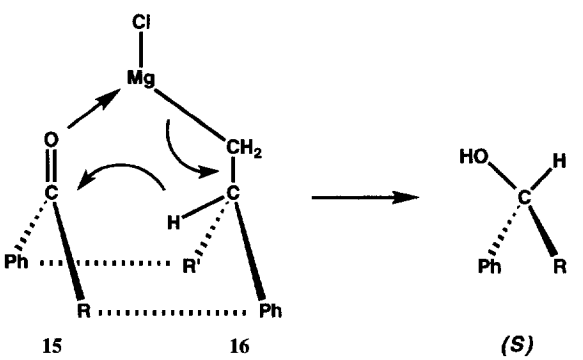


Figure 4.

Table 4

Optical Yields (%ee in *S*-enantiomer) of Reduction of Alkyl Phenyl Ketones with Chiral Grignard Reagents

R	X/Y	OCH_3	H	CF_3
C_2H_5	OCH_3	51	51	
C_2H_5	CH_3	54	52	10
C_2H_5	H	57	50	22
$CH(CH_3)_2$	H	84	81	58
$C(CH_3)_3$	H	16	16	-27*
C_2H_5	Cl	36	43	
C_2H_5	CF_3	22	22	10

*R-enantiomer was obtained in excess.

ketone **15** and the Grignard reagent **16**. This is understood if we assume an attractive interaction to play a role between the alkyl and the phenyl group at two points (Fig. 4). The attractive interaction is anticipated to increase with the number of CH groups suitably orientated with regard to the interaction versus Ph. This is what we see in Table 3-2. The isopropyl group was found to yield better results than isobutyl (Table 3-1, column 3; Table 3-2, column 2); this suggests that a face-to-face arrangement of the relevant groups (alkyl vs phenyl) is important for an effective CH/π interaction to take place.

Capillon and Guétté[79] studied the effect of substituents in an enantioface-differentiating reduction of phenyl alkyl ketones with chiral Grignard reagents. They found that the optical yield (giving rise to preferential formation of the *S*-enantiomer) decreased by introduction of an electron-withdrawing group on the aromatic ring of ketones or Grignard reagents (Table 4).

This is reasonable because the CH/π interaction will decrease on replacement of the substituent H by CF_3 or Cl (Fig. 5). The inverse will be true for compounds with an electron-donating

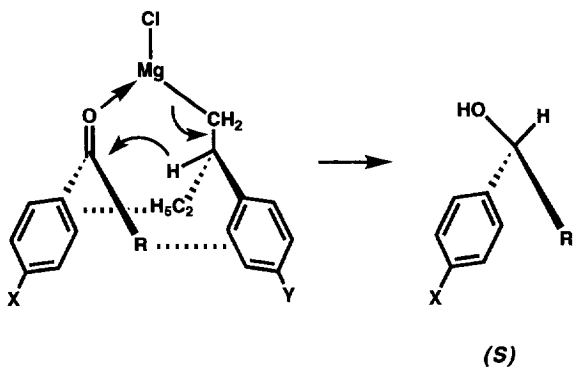
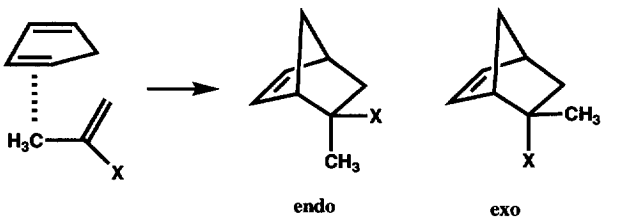


Figure 5.

substituent; this, however, is not very clear in Table 4. Cherest and Prudent studied the stereochemistry in hydride reduction of a series of ketones L-CHMe-CO-R (L = Ph and cyclohexyl; R = Me, Et, iPr and *t*Bu); the results were consistent with the presence of methyl/phenyl attractive interaction in the transition state which leads to the preferred product.[80]

4.1.2. *Coupling reactions.* Kobuke *et al.* studied the stereochemistry of Diels–Alder reactions of cyclopentadiene with a series of dienophiles, $CH_2 = C(CH_3)X$ (Fig. 6).[81]



X = CN, $COCH_3$, $COOCH_3$, CHO, COOH

Figure 6.

They found an appreciable *endo*-orientating tendency for the methyl group as opposed to polar unsaturated groups, X. The result was attributed to the presence of the attractive force of the methyl group which orientates itself to stabilize the transition state leading to the *endo* product.

Closs and Moss[82] studied the effect of alkyl substitution on the stereochemical outcome in an addition reaction of aryl carbenes to a series of olefins, $CH_2 = CHR$ (Fig. 7). The *syn*/*anti* preference in the cyclopropane formation was shown to decrease as R became larger; from methyl (R = Me, 3.1), to ethyl (2.1), isopropyl (1.4), and to *t*-butyl (0.45).

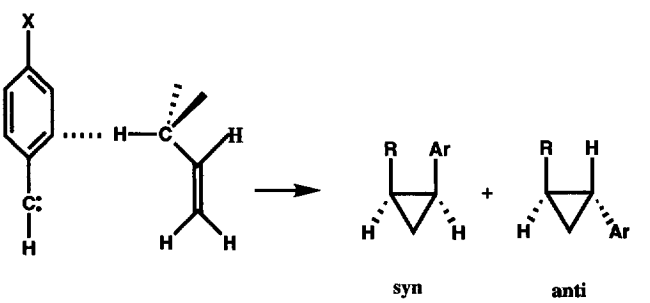
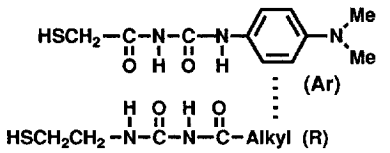


Figure 7.

Our interpretation is that the number of CH bonds involved effectively in the CH/π interaction (in stabilizing the transition state leading to the formation of *syn*-isomer) decreases upon successive methylation of R ($\underline{CH_3}$ to $\underline{CH_2}Me$, to $\underline{CH}Me_2$, and to CMe_3). In support of this hypothesis, introduction of a second methyl group on the olefin (isobutene) resulted in a significant increase in the reaction rate.[83] An increase in the *syn*/*anti* product ratio was also noted by substituting X from H to Me and then to MeO. This is reasonable in view of the presence of the CH/π interaction.

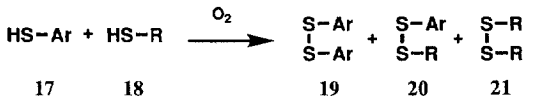
Endo *et al.*[84] studied an oxidative coupling reaction of a pair of thiols **17** and **18** to disulphides **19–21**. The ratio ($r = 2 \times$ **[20]**/**[19]**) of the resulting disulphides, unsymmetrical disulphide **20** over a

symmetrical one **19** or **21**, represents the extent of molecular recognition of the interacting species.



17

18



17 18 19 20 21

The recognition has been found to be most specific when an isopentyl group was introduced as the alkyl moiety (R) in **18**. Such a remarkable dependence of the selectivity on the structure of R was not observed when a series of straight-chain alkyl groups was used. In the case of isopentyl, *r* was reported to be 21.2, but it decreased on replacing R from iC_5H_{11} by a smaller alkyl group (iC_3H_7 or iC_4H_9), or a longer one (iC_6H_{13} or iC_7H_{15}).

Inspection of CPK molecular models suggests that the complex (associated with the use of two NH/O hydrogen bonds) of the reacting species is stabilized by a CH/π interaction in the case of **17**/**18** (heterogeneous complex) as compared to the cases of **17**/**17** or **18**/**18** (homogeneous complex). Interaction **17**/**18** would be most effective if **18** bears a branched alkyl group with an appropriate length; a face-to-face arrangement of the relevant groups seems to be essential for an effective CH/π interaction to take place.

4.1.3. *Enzyme mimetic catalytic reactions.* Guthrie *et al.* studied a base catalysed β-elimination reaction[85] and an ester hydrolysis[86] with an enzyme model (Fig. 8).

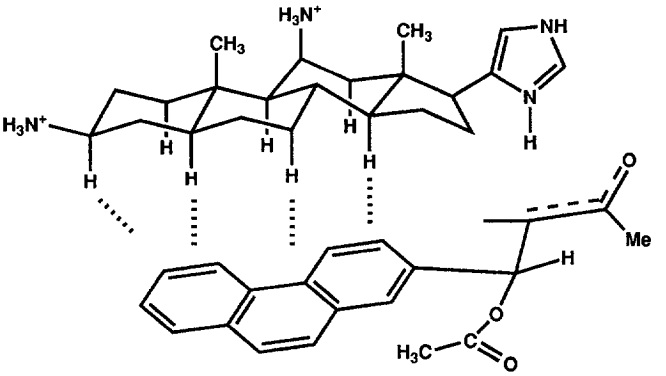


Figure 8.

The rates of the catalytic reactions were found to be enhanced regularly on replacement of the phenyl in the substrate by the naphthyl and then by the phenanthryl group. The surface area of the π-plane becomes larger in this order. The results were interpreted as a consequence of the "hydrophobic" effect.[87] We think it appropriate to reconsider this in the light of the CH/π interaction hypothesis.

4.2. *Inclusion complexes*[88]

4.2.1. *Cyclodextrin complexes.* The inner surface of the cavity of cyclodextrins is lined with many hydrogens (C^3H, C^5H and C^6H of glucose) and is therefore capable of forming a CH/π interacted complex with aromatic guests. Harata studied the thermodynamics of complex formation of cyclodextrins and their derivatives with substituted benzenes in aqueous solution.[89] Negative values were obtained for ΔH and ΔS. The results were interpreted to indicate that complex formation is due to tight binding of the guests within the cavity of the host molecule, in other words, the driving force of the complex formation is enthalpic in origin.

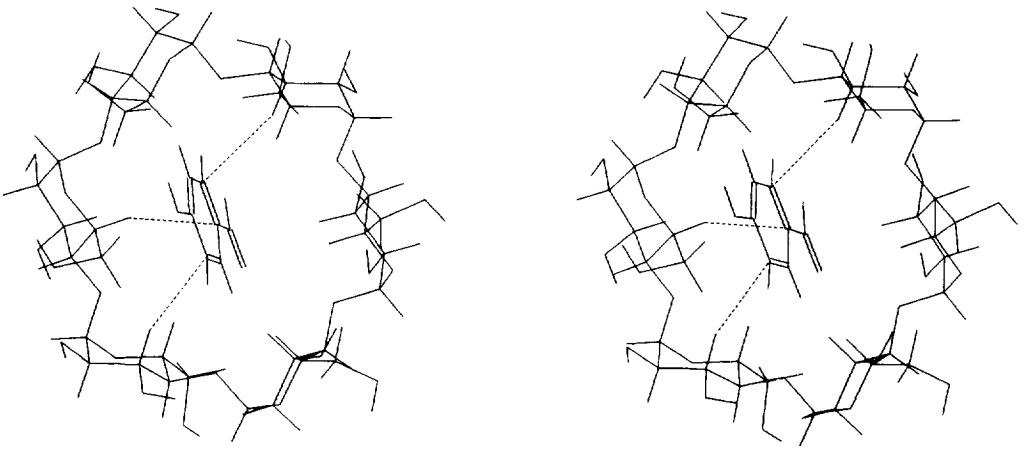
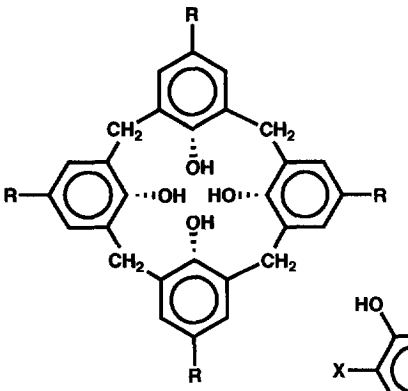


Figure 9. Stereo view showing short atomic contacts in α-cyclodextrin/*p*-nitrophenol complex (hydrogen atoms were generated). Dotted lines indicate CH/C(aromatic) contacts shorter than 3.0 Å.

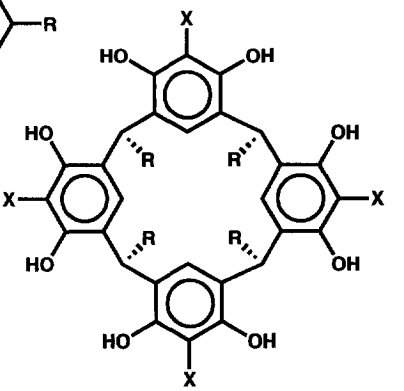
Short CH(cyclodextrin)/C(aromatic guests) distances were in fact found in X-ray crystallographic data of cyclodextrin complexes reported by Harata *et al.*[90] In Figure 9 an example is given for the α-cyclodextrin/*p*-nitrophenol complex.[91] CH/Csp^2 distances, 2.96, 3.00 and 2.85 Å, are shown between CHs in the glucose moiety and aromatic carbons.[92] Favourable CH/C(phenyl) interactions were found also in *m*-nitroaniline (2.90 and 2.92 Å)[93] and 1-phenylethanol complexes of α-cyclodextrin,[94] a complex of trimethyl-β-cyclodextrin with *p*-iodophenol,[95] complexes with aromatic guests (*p*-iodoaniline, benzaldehyde, *p*-nitrophenol and flurbiprofen) of α- and/or β-cyclodextrin and their permethylated derivatives,[96] as well as for complexes of β-cyclodextrin with *m*-iodophenol, 4-biphenylacetic acid[97] and 2-naphthoic acid.[98] Favourable methyl/phenyl interactions in the crystal structure of a β-cyclodextrin complex with fenoprofen [2-(3-phenoxyphenyl) propionic acid] have been reported.[99] The above results are in line with the findings of Armstrong *et al.*[100] that separation of drug enantiomers, by HPLC with immobilized β-cyclodextrin, are most effectively accomplished in cases where at least one aromatic moiety is present in the solute molecule.

4.2.2. *Calix*[4]*arene complexes.*[101] Andreetti *et al.* were the first to report the crystallographic structure of an inclusion complex of calixarene. Thus a methyl group in the guest toluene was shown to be surrounded by four phenyl rings of *p-t*-butyl-calix[4]arene (**22**: R = *t*Bu), whereas the toluene aromatic ring is sandwiched by two *t*-butyl groups of the host.[102] The same type of inclusion complex was also reported for the tetramethoxy derivative of **22** (R = *t*Bu): distances between relevant carbon atoms and the aromatic ring are 3.54 and 3.77 Å.[103] The problem was studied for a pyridine complex in view of involvement of the CH/π interaction.[104] Thus the experimental result has been

shown to be best reproduced in molecular mechanics calculations by introducing parameters for CH/π interaction in the force fields.



22



23 : R = $CH_2CH_2SO_3Na$

Of particular interest, in this respect, are X-ray and thermochemical results reported by Perrin *et al.* for *p*-xylene complexes of *p*-isopropyl-calix[4]arene (**22**: R = iPr).[105] The most stable 1:1 complex loses a xylene molecule on heating to give a 1:2 complex, and then rearranges finally to form an empty macrocycle. The perpendicular distances between a xylene methyl group and the nearest aromatic ring of the host are 3.65 Å for the 1:1 complex and 3.7 Å for the 1:2 complex. For the xylene-free compound, the methyl group in an isopropyl substituent lies inside the cavity towards the benzene ring (methyl/phenyl distances 3.51 and 3.54 Å). The substrate specificity for similar types of compounds (**23**) has been studied by Kobayashi *et al.*[106]

Table 5

Binding constants (K/mole) for the complexation of **23** [R = $(CH_2)_2SO_3Na$] with various guests in D_2O at 25°

	N^+H_4	MeN^+H_3	$Me_2N^+H_2$	Me_3N^+H	Me_4N^+	Me_3COH
X = H	1	1	3	30	160	4
$X = CH_3$					1500	19
X = OH					1800	24

Stability of the inclusion complex was found to increase with progressive methylation of the guest ammonium chloride (Table 5). That this is not merely a consequence of the bulk or electrostatic effect was shown, since replacement of X in the host from H by a more electron-donating group such as CH_3 or OH resulted in remarkable increases in the stability of the complexes. This is

consistent with our expectation that the extent of the CH/π interaction becomes greater if the π-electron density of the aromatic ring increases. Trimethyl ammonium chloride was shown to be more specific than *t*-butyl alcohol as a substrate. This is reasonable since the complexing ability of the guest will increase if the hydrogens in CH_3 become more acidic. The above result, together with the findings reported by Andreetti *et al.* and Perrin *et al.*, provide an unequivocal demonstration of the importance of CH/π interactions in determining specificities of molecular complexes.

Specific inclusions of a similar type were reported for **22** (R = *t*Bu) with aromatic guests such as phenol,[107] anisole[108] and benzene.[109] Acetonitrile (CH_3/phenyl 3.80 Å),[110] ethanol,[111] acetone,[112] methyl sulphate,[113] methylene chloride (CH_2/phenyl 3.54 Å)[114] and 4-(dimethylamino)benzonitrile[115] also form inclusion compounds with calix[4]arenes. Methyl or methylene groups of the guests point to the cavity of the host molecule, the inner surface of which is lined with many π-electrons, thus demonstrating the importance of the CH/π interaction.

4.2.3. *Other types of inclusion complexes.*[116] Odashima *et al.*[117] studied the crystallographic structure of 1,6,20,25-tetraaza[6.1.6.1]paracyclophane with durene and found short atomic contacts between sp^2 carbons in the aromatic part of the host and a methyl group in the guest molecule. Kyuno *et al.*[118] studied the complexation of a zinc porphyrin compound. They found that secondary amines such as azetidine, pyrrolidine and diethylamine, which fit the cavity effectively, bind well to the porphyrin host. The results were interpreted in terms of attractive interligand interactions; an important contribution from the CH/π interaction has been suggested. Favourable methyl or methylene/aromatic ring interactions were also noted for a methyl ammonium complex with a speleand,[119] a xylene complex of 1′,1″-dimethyl-dispiro[1,6,20,25-tetraoxa[6.1.6.1]paracyclophane-13,4′:32,4″-bispiperidine],[120] and methanol, ethanol and acetonitrile complexes of cavitands.[121] Methanol, ethanol and propanol were reported to form clathrates with 2-[*o*-(triphenylphosphoranylidenamino)benzyliden]amino-1-*H*-2,3-dihydroindazol-3-one.[122] Formation of a methanol clathrate with α,α,α′,α′-tetraphenyl-1,3-dioxolane-4,5-dimethanol has been reported.[123]

4.3. *Protein/ligand complexes*

We will now explore the involvement of the CH/π interaction in biological systems in the light of data presently available for proteins and protein/ligand complexes. A computer programme (CHPI) was written to examine interactions between X–H groups (X = C, N, O or S) and π systems. The method is shown in Fig. 10.

The π-system may be an aromatic group (five- or six-membered, or fused) or a double bond (C=C, C=N or C=O). The hydrogen may be a part of an alkyl group (CH_3, CH_2 or CH), CH in an aromatic ring, N^+H_3, NH_2, NH, OH or SH group. To participate in a XH/π interaction, a hydrogen is positioned above the π plane though not necessarily directly above the carbon atom (region 1 in Fig. 10).

In order to cover other possibilities (regions 2 and 3 in Fig. 10), several kinds of H/Xsp^2 distance and angle parameters were defined. Hydrogens were generated on non-hydrogen atoms and the positions optimized when possible.[124] For a histidine side-chain, a hydrogen atom was laid on Nδ1. The programme was thus run with crystallographic coordinates of proteins and protein/ligand complexes from the Brookhaven Protein Data Bank (PDB). Interactions of H versus the closest sp^2 atoms with correct angle parameters were collected.

There is a large body of literature reporting the structures and functions of proteins. The following are a few examples which were selected rather arbitrarily to obtain insight into our thesis: the CH/π interaction is playing a role generally in biopolymer interactions. The proteins reported here include the classic ones as well as those of current interest.

4.3.1. *Haemoglobin* (*carp parvalbumin*). The structure of haemoglobin was extensively studied by Perutz *et al.*[125,126] Table 6 is a sample output from the above programme (CHPI) for α subunit of horse deoxyhaemoglobin[127] (αβ dimer, 2DHB, resolution 2.8 Å; PDB code and the resolutions are shown hereafter in the References). Figure 11 presents a global view of the α subunit.

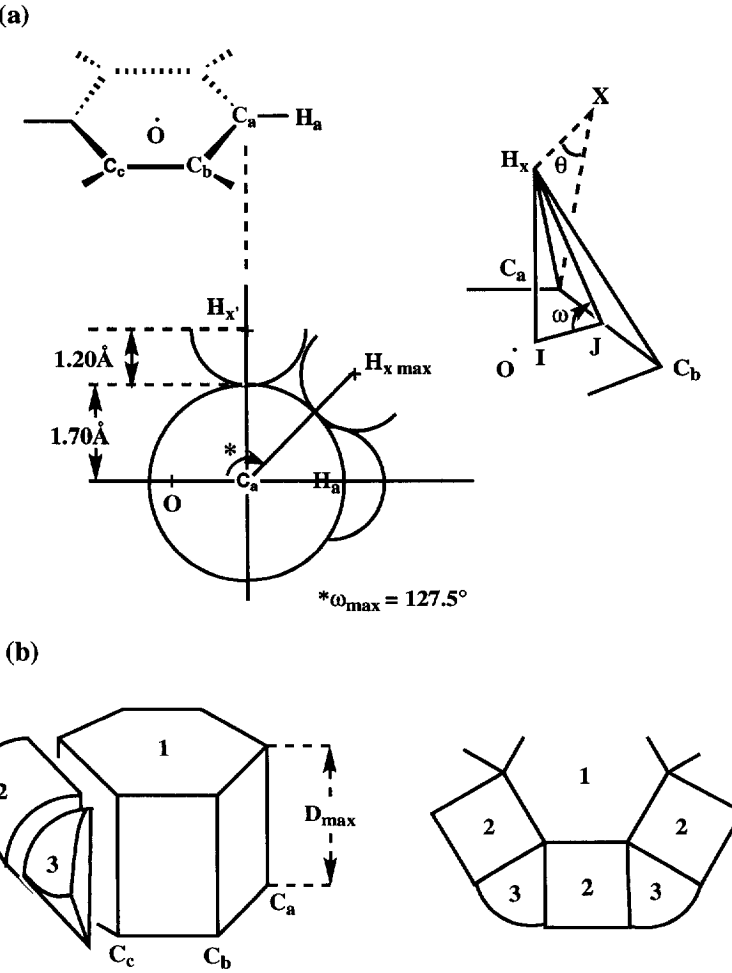


Figure 10. Method for finding XH/π contacts (X = C, N, O or S). An example is given for a six-membered π-system. (a) O: Centre of the plane. C_a and C_b: nearest and second nearest sp^2-carbons, respectively, to the H_x hydrogen. ω: Dihedral angle defined by C_aOC_b and $H_xC_aC_b$ planes. θ: H_x–X–C_a angle. D_{PLN}: perpendicular distance between H_x and nearest π-plane (H_xI). D_{ATM}: H_x/C_a interatomic distance. D_{LIN}: distance between H_x and line C_a–C_b (H_xJ). (b) Regions to be searched. Region 1: zone where H_x is just above the ring. Regions 2 and 3: zones where H_x is out of Region 1 but may interact with π-orbitals. Programme was run to search for short XH_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); $\omega_{max} = 127.5°$, $-\omega_{max} < \omega < \omega_{max}$; $\theta < 70°$.

Chart 1 shows H/Csp^2 distances (D_{ATM}) demonstrated for (a) the α-subunit, (b) the α/β-interface, and (c) the β-subunit, respectively. Labels in parentheses correspond to stereochemical notation of globins. Greek letters following H and C (or N) indicate atoms relevant to the contact. In the above charts we see many CH/π contacts shorter than van der Waals distances. We do not discuss the interatomic distances in detail, however, in view of limitations to the precision in the crystallographic determination for proteins. Histidine behaves as an aromatic residue. This was expected, but more impressive is that the interactions of haem with aromatic residues are assumed by other interactions, thus forming CH/π networks. The haem group represents a large π-system and has been known to be surrounded by a number of non-polar residues. They are invariant and have important biological significance; substitution of one residue by another results in serious hereditary diseases due to decreased ability of the globin molecule to hold haem.[128]

Table 6

```
     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 FIV    CG   CD1  NE1  CE2  CD2
PRTN TRP 2 SIX    CE2  CD2  CE3  CZ3  CH2  CZ2

RANGE  -127.50   < OMEGA < 127.50
RANGE     0.00   < THETA <  70.00
RANGE     0.00   < Dmax  <   3.05
```

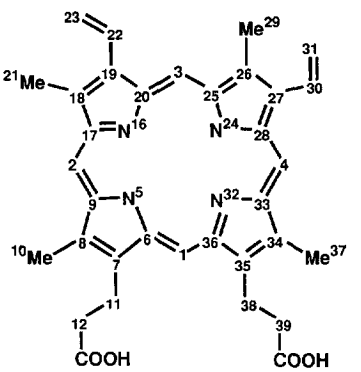
pi ID	RES	VPI	I	N	VATM	HX ID	RES	VATM	N	geometry DATM	DPLN	DLIN	OMEGA	THETA	RG
A 14	TRP	FIV	1	1	CG	A 70	VAL	HCG2	15	2.48	2.26	2.46	113.44	13.81	2
A 14	TRP	SIX	2	5	CH2	A 17	VAL	HCG1	11	3.06	3.01	3.01	87.31	17.12	1
A 14	TRP	SIX	2	6	CZ2	A 66	LEU	HCB	9	3.02	2.98	3.00	96.83	0.99	2
A 24	TYR	SIX	1	2	CD1	A 17	VAL	HCG1	10	2.57	2.06	2.56	126.47	31.39	2
A 24	TYR	SIX	1	4	CZ	A 20	HIS	HCB	9	2.93	2.93	2.93	90.47	35.78	2
A 24	TYR	SIX	1	2	CD1	A109	LEU	HCD2	19	2.77	2.17	2.73	127.33	52.48	2
A 24	TYR	SIX	1	4	CZ	A112	HIS	HCB	9	2.92	2.83	2.88	100.74	52.46	2
A 33	PHE	SIX	1	3	CE1	A 32	MET	HCE	17	3.06	3.04	3.04	93.97	29.93	2
A 33	PHE	SIX	1	1	CG	A 33	PHE	HN	2	2.93	2.62	****	116.49	68.41	3
A 33	PHE	SIX	1	6	CD2	A 48	LEU	HCD2	19	2.66	2.56	2.59	98.66	9.11	2
A 36	PHE	SIX	1	3	CE1	A100	LEU	HCB	8	2.62	2.42	2.55	107.98	15.59	2
A 36	PHE	SIX	1	5	CE2	A100	LEU	HCD2	19	2.90	2.78	2.85	77.13	13.27	1
A 42	TYR	SIX	1	4	CZ	A 93	VAL	HCA	4	2.63	2.41	2.55	109.09	22.55	2
A 42	TYR	SIX	1	5	CE2	A 93	VAL	HCG1	10	2.65	2.64	2.64	91.97	10.57	2
A 42	TYR	SIX	1	1	CG	A 93	VAL	HCG2	16	2.84	2.77	2.77	89.00	6.05	1
A 43	PHE	SIX	1	2	CD1	A 33	PHE	HCZ	20	2.54	2.40	2.44	78.73	42.63	1
A 43	PHE	SIX	1	4	CZ	X#LG1	LIG	HC37	66	3.04	2.97	2.98	84.18	32.77	1
A 45	HIS	FIV	1	5	CD2	A 46	PHE	HCD2	14	2.87	2.82	2.86	99.17	39.79	2
A 46	PHE	SIX	1	1	CG	A 43	PHE	HCD2	14	2.69	2.59	2.66	77.21	26.21	1
A 46	PHE	SIX	1	3	CE1	A 43	PHE	HCE2	18	2.79	2.64	2.72	103.85	44.84	2
A 58	HIS	FIV	1	4	NE2	A 43	PHE	HCZ	20	2.86	2.42	****	122.39	31.94	3
A 58	HIS	FIV	1	1	CG	A 46	PHE	HCE1	16	2.75	2.20	****	126.80	37.50	3
A 58	HIS	FIV	1	2	ND1	A 46	PHE	HCZ	20	2.97	2.49	2.93	121.83	29.46	2
A112	HIS	FIV	1	1	CG	A 24	TYR	HCE2	18	2.99	2.95	****	99.52	59.08	3
A117	PHE	SIX	1	6	CD2	A110	ALA	HCA	4	2.96	2.84	2.92	103.58	31.62	2
A117	PHE	SIX	1	5	CE2	A122	HIS	HCB	9	2.56	2.51	2.52	95.47	24.85	2
A122	HIS	FIV	1	5	CD2	A106	LEU	HCD1	13	2.60	2.20	2.59	121.91	56.60	2
A128	PHE	SIX	1	4	CZ	A 70	VAL	HCG2	14	3.05	2.85	****	110.50	23.96	3
A128	PHE	SIX	1	4	CZ	A132	VAL	HCG2	15	3.12	3.03	3.06	83.08	50.86	1
A140	TYR	SIX	1	5	CE2	A 88	ALA	HCA	4	2.85	2.79	2.82	97.34	42.32	2
X#LG1	LIG	OL2	1	2	C1	A 58	HIS	HCE1	16	2.53	2.52	2.52	91.74	8.30	2
X#LG1	LIG	FIV	2	2	C6	A 58	HIS	HCE1	16	3.00	2.56	****	121.31	22.16	3
X#LG1	LIG	FIV	2	4	C8	A 83	LEU	HCD1	14	2.35	1.97	****	123.06	8.40	3
X#LG1	LIG	FIV	2	5	C9	A 87	HIS	HCE1	16	2.72	2.52	2.65	108.31	32.88	2
X#LG1	LIG	OL2	3	2	C2	A 62	VAL	HCG2	16	3.01	2.94	2.95	94.60	29.08	2
X#LG1	LIG	FIV	4	5	C20	A 98	PHE	HCE1	16	2.95	2.42	2.95	124.71	28.73	2
X#LG1	LIG	FIV	4	3	C18	A136	LEU	HCD1	14	2.87	2.66	2.87	112.12	52.36	2
X#LG1	LIG	OL2	5	2	C3	A 98	PHE	HCE1	16	2.75	2.37	2.73	119.82	49.85	2
X#LG1	LIG	FIV	6	4	C27	A 93	VAL	HCG1	11	2.58	2.53	****	101.24	10.79	3
X#LG1	LIG	OL2	7	2	C4	A 93	VAL	HCG2	15	2.67	2.44	****	114.05	14.97	3
X#LG1	LIG	FIV	8	5	C36	A 58	HIS	HCE1	16	2.81	2.58	****	113.33	29.07	3
X#LG1	LIG	FIV	8	5	C36	A 91	LEU	HCD1	13	2.74	2.66	2.70	79.89	42.75	1
X#LG1	LIG	FIV	8	3	C34	A 91	LEU	HCD2	19	2.83	2.39	2.80	121.35	31.35	2
X#LG1	LIG	OLE	10	1	C31	A 93	VAL	HCG1	10	2.95	2.93	****	96.17	61.59	3
X#LG1	LIG	OLE	10	2	C30	A 93	VAL	HCG1	11	2.53	2.35	2.51	111.03	33.44	2

no.of H/pi interactions : 45

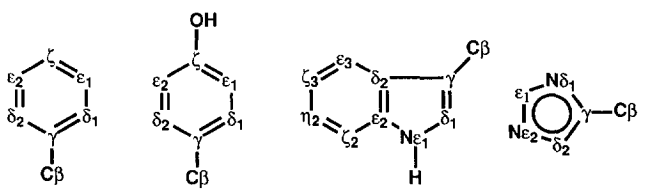
Figure 11. Stereo view of haemoglobin α-subunit. Dotted lines indicate short CH/π contacts.

An interesting feature is that the geminal dimethyl groups in valine and leucine [Cγ1 and Cγ2 in Val93α (Val93 in α-subunit) and Val67β (Val67 in β-subunit), Cδ1 and Cδ2 in Leu91α] are all involved in interaction with the porphyrin moiety. Within the globin molecules, we note the same type of interaction for Val93α/Tyr42α, Val137β/Phe71β, and Leu141β/Phe103β. At the boundary

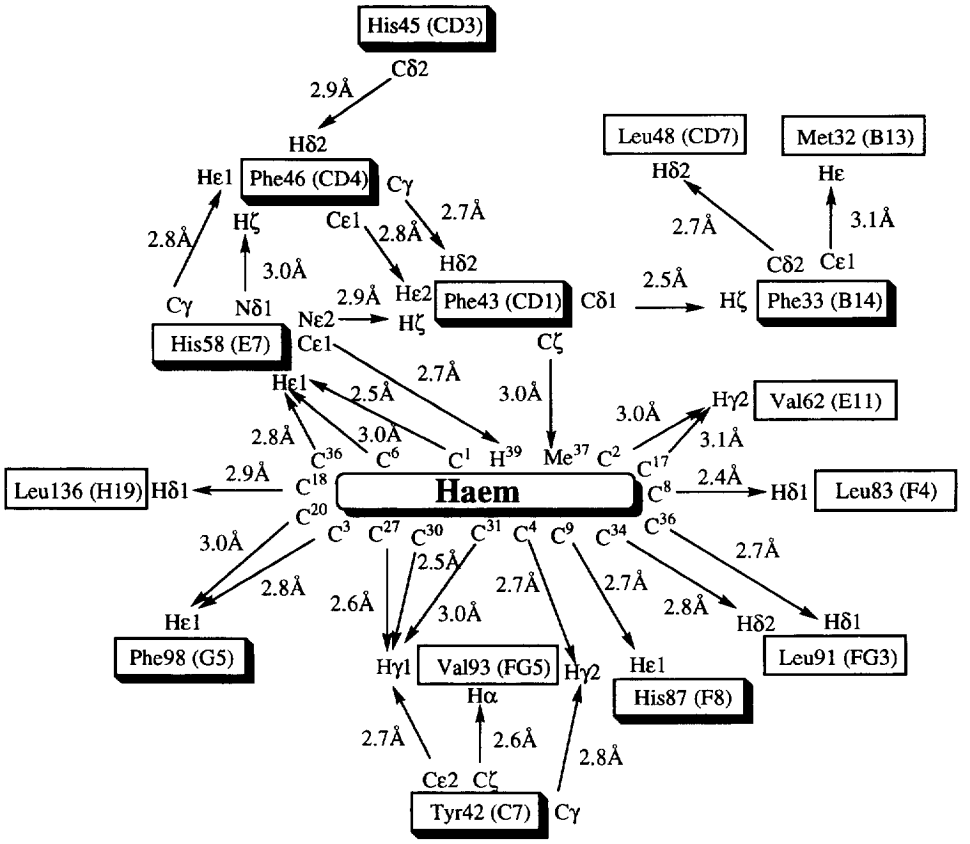
Numbering of atoms in haem and aromatic rings



Haem



Phe Tyr Trp His



π (donor) ⟶ σ* (H: acceptor)

Chart 1(a).

Chart 1. CH/π networks in (a) haemoglobin α-subunit, (b) boundary of the subunits, and (c) β-subunit (PDB file 2DHB). Numbers (in Å) refer to CH/$C_{sp}2$ distances (D_{ATM}). Labels in parentheses are stereochemical notations for globins by Kendrew *et al.* Greek letters indicate atoms relevant to the CH/π contact. Interatomic distances are shown for the closest ones among atoms in the respective aromatic ring.

of monomers α and β, there is an interaction of this type for Val34β/His122α. This kind of interaction (Fig. 12) is found also in other proteins (but not very often).

We think the above interactions involving geminal dimethyl side-chains versus aromatic rings

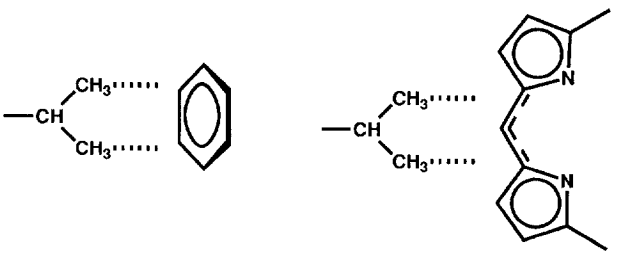


Figure 12. Geminal dimethyl CH/π interaction.

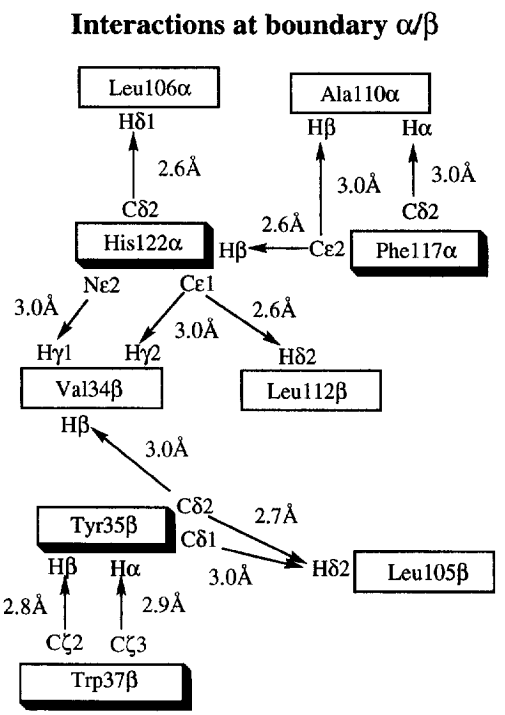


Chart 1(b).

illustrate the importance of multiple CH/π interactions.[129] This may explain why only residues bearing a branched alkyl group (valine, leucine and isoleucine) are found in nature whereas amino acids with a straight aliphatic side-chain are absent. Neither homoalanine **24**, norvaline **25**, norleucine **26**, nor homonorleucine **27** are found in proteins (Fig. 13). A speculation is that amino acids with straight-chain aliphatic groups are less favourable in interactions of various sorts (e.g. CH/π, CH/n or van der Waals), as compared to branched ones, and thus dropped out in the process of natural selection.

The CH/π contacts of haem with aromatic residues (including His) are shown for Phe43, His58, His87 and Phe98 in α-subunit, and for Phe42, His63, His92 and Phe103 in β-subunit. Phe43α interacts with other aromatic residues (Phe33, Phe46, His58 and indirectly with His45), thus locating itself at a key position in the network. The invariance[130] and biological importance[131,132] of residues Phe43α and Phe42β is well known. In addition to these, we see a number of CH/π contacts between aromatic rings. They are Tyr24α/His112α, Tyr24α/His20α, Tyr24α/His112α, Phe117α/His122α, Phe42β/His63β, His63β/Phe45β, Trp15β/Phe71β, Trp37β/Tyr35β and Phe122β/Tyr130β.

A specific attractive interaction between aromatic groups has been known to be quite general for smaller molecules in crystals[133] and in solution for a variety of synthetic compounds,[134] somatostatin derivatives,[135] flavinyl peptides,[136] nicotineamide-adenine dinucleotide and its analogues.[137] In particular, Sigel[138] and associates have studied extensively the conformational equilibria of a number of ternary complexes bearing biologically important moieties, such as ATP or amino acids, as ligands.[139] In every case, they found that the conformer in which aromatic moieties on both side of the molecules are close to each other is preferred. An example is given in Figure 14 for [M(phen)(ATP)] complex. Okawa *et al.* found short interatomic distances between aromatic carbons (3.44, 3.34, 3.29 Å) in a tetrahedral Zn complex of *N*-(*R*)-1-phenylethylsalicylideneimine.[140]

As to the interactions in proteins, Edmundson *et al.* pointed out the abundance of aromatic residues in the antigen binding pocket of immunoglobulins.[141] A calcium-binding myogen was found

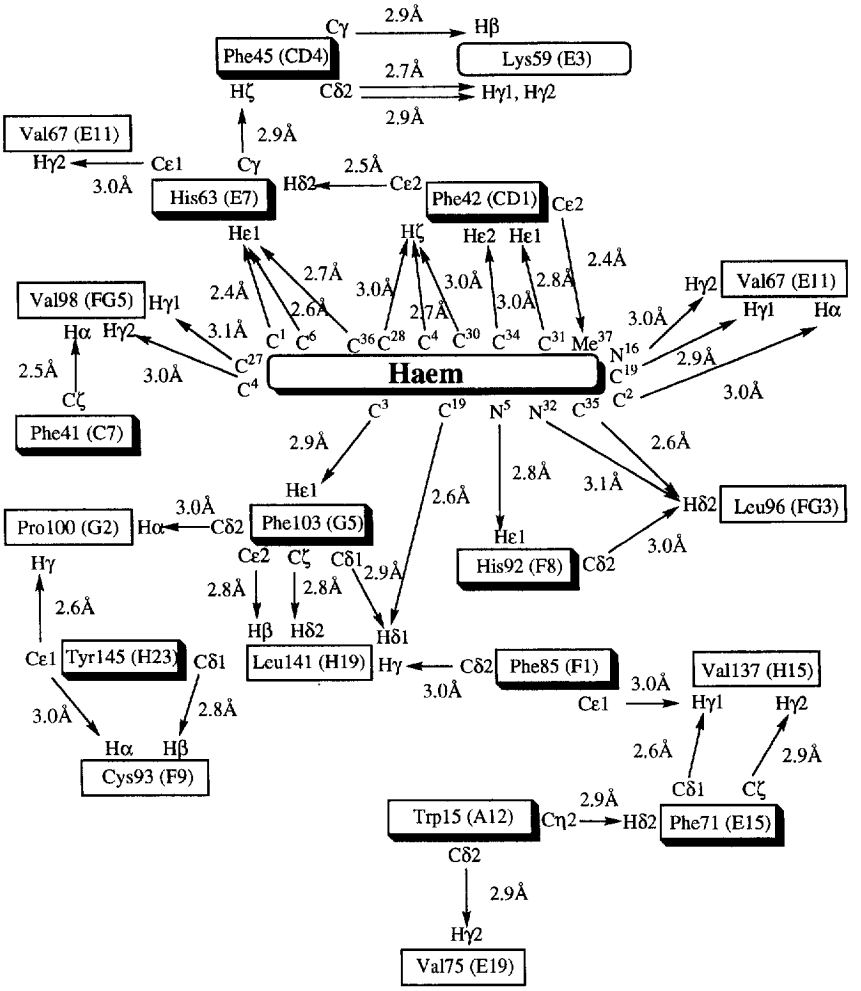


Chart 1(c).

to form a cluster consisting of many phenylalanines at the "hydrophobic" core of the protein.[142] This problem was studied later by examining statistically a large number of protein structures and it has been known that approximately T-shape (edge-to-face) or L-shape (cogwheel) arrangements of aromatic rings are dominant.[143,144] This type of interaction has been known to be stable in the crystallographic structure of benzene and in MO calculations. We therefore examined interactions in carp parvalbumin, which is known to have a non-polar core consisting of eight phenylalanines.[145] Chart 2 gives the results.

Eight phenylalanines and a histidine have in fact been shown to participate in a CH/π network. There we see a number of contacts shorter than the van der Waals distance. In the present survey only interactions with approximately T- or L-shape arrangements of the aromatic rings have been collected since the CH/π interaction was expected to occur only in such an arrangement (see Fig. 10). There is much debate as to the origin of the arene/arene attractive interaction.[146–148] We think it most probable that aromatic/aromatic interactions found in proteins are CH/π in type (hereafter referred to as aromatic CH/π interactions).

4.3.2. *Lysozyme*. Chart 3 presents CH/π interactions which have been demonstrated for a hen egg-white lysozyme/substrate complex.[149] A CH/π network is shown around the ligand, tri-*N*-acetylchitotriose $(GlcNAc)_3$. Trp111 is found at the centre of a smaller network.

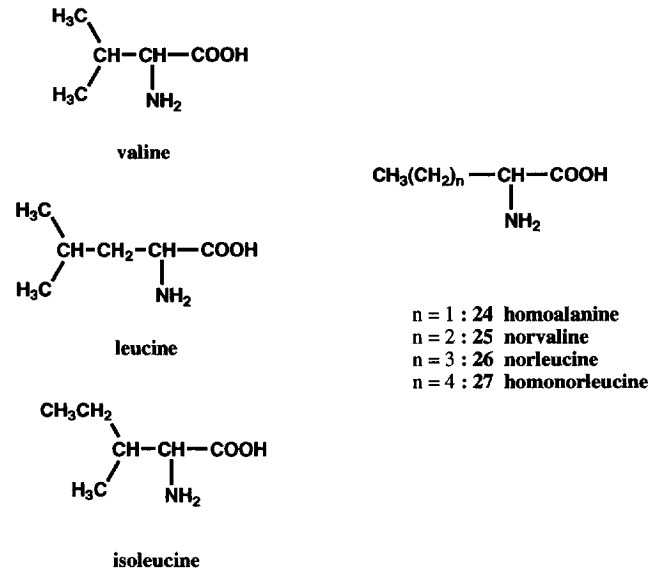


Figure 13. Naturally occurring amino acids, and those absent in nature (**24**–**27**).

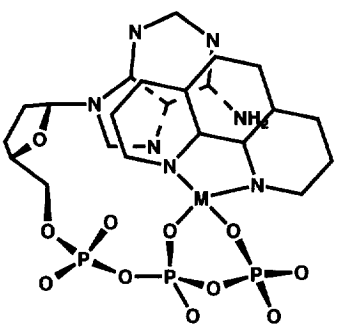


Figure 14. Preferred conformation for ternary complex $[M(phen)(ATP)]^{2+}$, M = Ca, Mg, Mn, Zn or Cu. ATP: adenosine triphosphate; phen: phenanthroline.

The pyranose rings (A, B and C) of the substrate were reported by Phillips *et al.*[150] to be proximate to Trp62 and Trp63. The methyl group in the terminal acetamide in ring C has also been known to be close to Trp108; this is compatible with observations that N-acetylation is essential for sugar oligomers to be a good substrate.[151] Here, Nε1 in Trp108 has been shown to be in contact with a hydrogen in the acetyl group in ring (pyranose) C of the ligand. Cδ1 and Cδ2 in Trp62 are close to C^4H (ring C) and C^5H (ring B), respectively. Cζ2 of Trp63 is close to $C\underline{H}_2OH$ (ring B). Interactions of the ligand with Trp108, Trp62 and Trp63 are shown to be assisted by a CH/π network involving Leu56, Trp28, Leu17, Arg61, Leu75 and Ile98. Of particular interest, in this respect, are recent results reported by Muraki *et al.* They showed that the residue in position 63 (Trp in avian and Tyr in human lysozyme) must be an aromatic one, from a study with enzymes prepared by site-directed mutagenesis.[152] Enzymatic activities and crystallographic structures were compared for Y63F (Tyr63 was converted to Phe. Y: tyrosine, P: phenylalanine), Y63W (W: tryptophane), Y63L (L: leucine), Y63A (A: alanine) with native human lysozyme. Properties of Y63F and Y63W were found to be comparable to those of the wild enzyme whereas those of Y63L and Y63A are not.

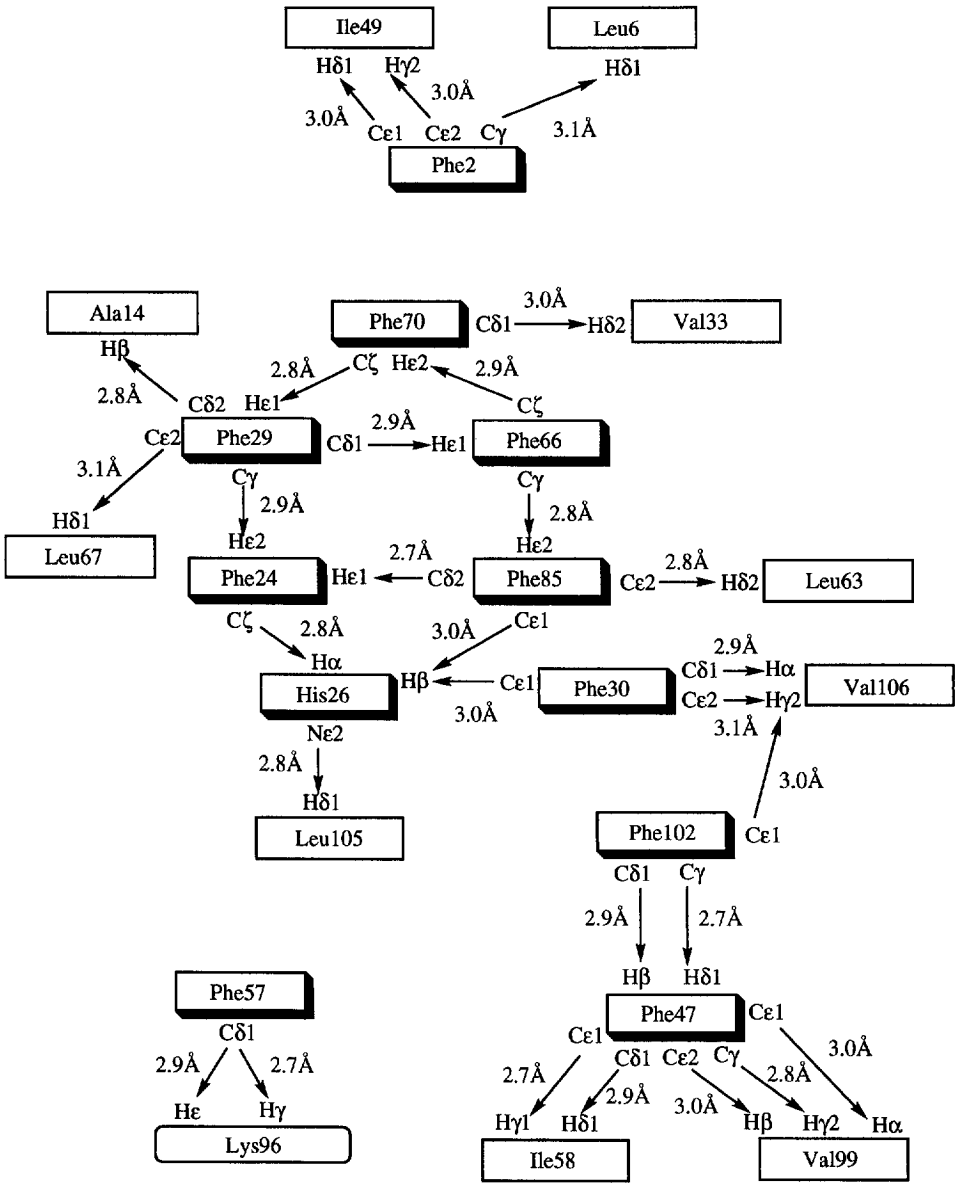


Chart 2. CH/π networks in carp parvalbumin (5CPV).

Somewhat unexpected but very interesting are the interactions observed between lysines and the aromatic residues: Lys1/Phe3, Lys33/Phe38, Lys96/Tyr20 and Lys116/Trp111. In every case multiple pairs of atoms are involved in the interaction. Lysine is classified generally as a basic amino acid and thus considered to be responsible for the formation of salt-bridges or hydrogen-bonds. We suggest that lysine plays a role by CH/π interaction with aromatic residues to stabilize the structure of proteins, in cooperation with stronger bonding forces.

Four methylenes are present in the lysine side-chain between Cα and the terminal amino group. As shown here and elsewhere, XHs in CH_2 and NH_2 are involved simultaneously in multiple XH/π bonds, thus providing an environment for dynamic interactions with specific substrates. This type of interaction (Fig. 15, hereafter referred to as the lysine CH/π interaction)[153] is found often in other

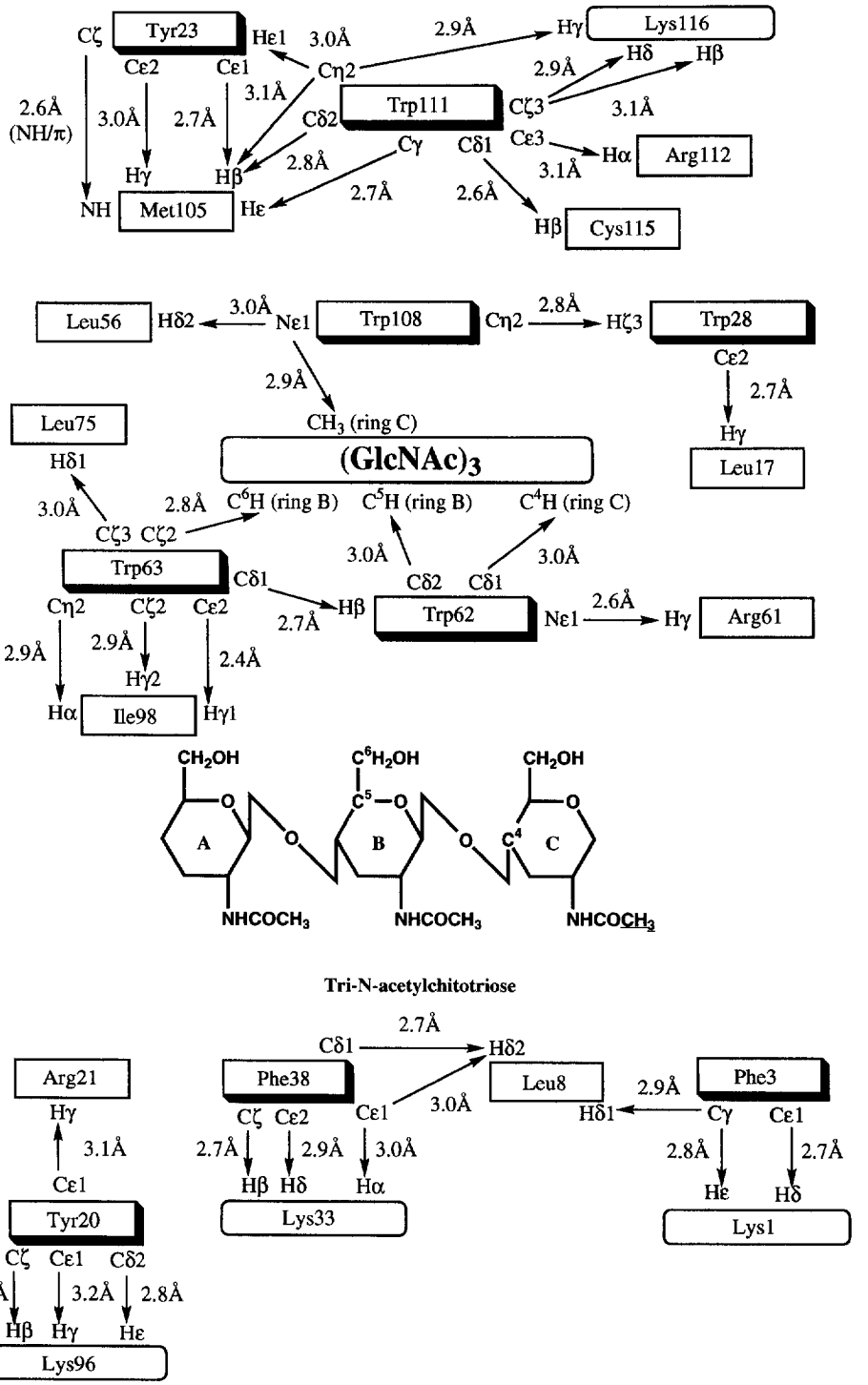


Chart 3. CH/π interactions in the lysozyme/tri-*N*-acetylchitotriose complex (1HEW).

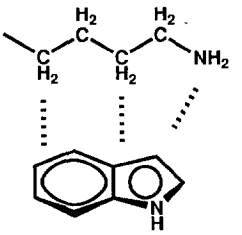


Figure 15. Lysine CH/π interaction.

proteins. The CH/π network, incorporating geminal dimethyl, aromatic CH/π, as well as lysine CH/π interaction support our thesis that multi-point (as well as multi-chance) interaction is essential in building a stable and flexible structure of a macromolecule.

4.3.3. D-*Xylose isomerase* (*c*-H-*ras p*21 *protein*, *v-src SH2 domain*). Xylose isomerase catalyses the isomerization of D-xylose and the conversion of D-glucose to fructose. D-Sorbitol is a specific inhibitor, since the molecule closely resembles an open-chain configuration involved in the transition state of the reaction. Crystallographic structures of complexes of this protein with specific substrates and inhibitors were studied.[154] Chart 4 summarizes some of the CH/π networks revealed for a protein/sorbitol complex.

It was reported that the sorbitol molecule takes up a linear arrangement of atoms C^1-C^2-C^3-C^4-C^5 and the indolyl ring of Trp136 is lined with a consecutive arrangement of CH groups of the inhibitor. The temperature factors for the inhibitor are low, suggesting that the interaction involved is strong. Short CH/π contacts have in fact been shown for Trp136 with the ligand (C^4H/Cγ, C^2H/Cδ2 and C^1H/Cη2). These interactions are arranged in a CH/π network. However, only one CH/π interaction has been found between Trp136 of the protein complexed with a cyclic sugar substrate.[155] An important contribution from the CH/π interaction in stabilizing the transition state is evident.

Protein/carbohydrate interactions were studied extensively by Quiocho *et al.*[156] They reported crystallographic structures of periplasmic proteins of bacterial origin such as L-arabinose-binding protein,[157] D-galactose-binding protein (GBP),[158] D-maltose-binding protein,[159] and complexes with their specific substrates. For instance, D-glucose in a GBP/glucose complex was found to be sandwiched by aromatic residues; stacking of "hydrophobic" patches with C^3H, C^5H and C^6H of glucose by Trp183 and C^2H, C^4H by Phe16 was reported.[160] A programme search by CHPI with GBP/glucose complex[161] has shown Cε3, Cε2 of Trp183 and Cδ2 of Phe16 to be in CH/π contact with C^3H, C^5H and C^2H of the ligand, respectively. It is clear that axially orientated methine CH bonds in saccharides can participate simultaneously in interactions with sp^2 carbons of the aromatic rings.

The lysine CH/π interaction has been shown in xylose isomerase for Lys41/Trp305, Lys182/His219 and Lys294/Trp19. Note that the aromatic rings have contacts with several methylene hydrogens in the lysine side-chain (Chart 4). Lys182, Lys294 and His219 are invariant within five species examined.[162] Trp19 (WWWWA: W = tryptophane, A = alanine), Trp305 (WWWYY: Y = tyrosine) and Lys41 (KKRAA: K = lysine, R = arginine), are fairly well conserved.

Crystallographic structures of c-Harvey-*ras* p21 protein complexes were reported recently.[163-165] There are two lysine CH/π interactions around its specific ligand, GppNp, a GTP analogue. It is evident that the side-chains in Lys117 and Lys147 play a role, by the lysine CH/π interaction, in stabilizing the bonding with the guanine moiety (Fig. 16 and Chart 5).

These residues are conserved within many guanine nucleotide-binding proteins of various sources.[166,167] The guanine aromatic ring is in CH/π contact with Phe28 (aromatic CH/π interaction) and this is assisted by interactions with Lys147 and Asp30.

Of special interest, in this connection, is the crystal structure of the phosphotyrosine recognition domain SH2 of v-src complexed with ligands.[168] The carbon atoms of the Lys203 chain were reported

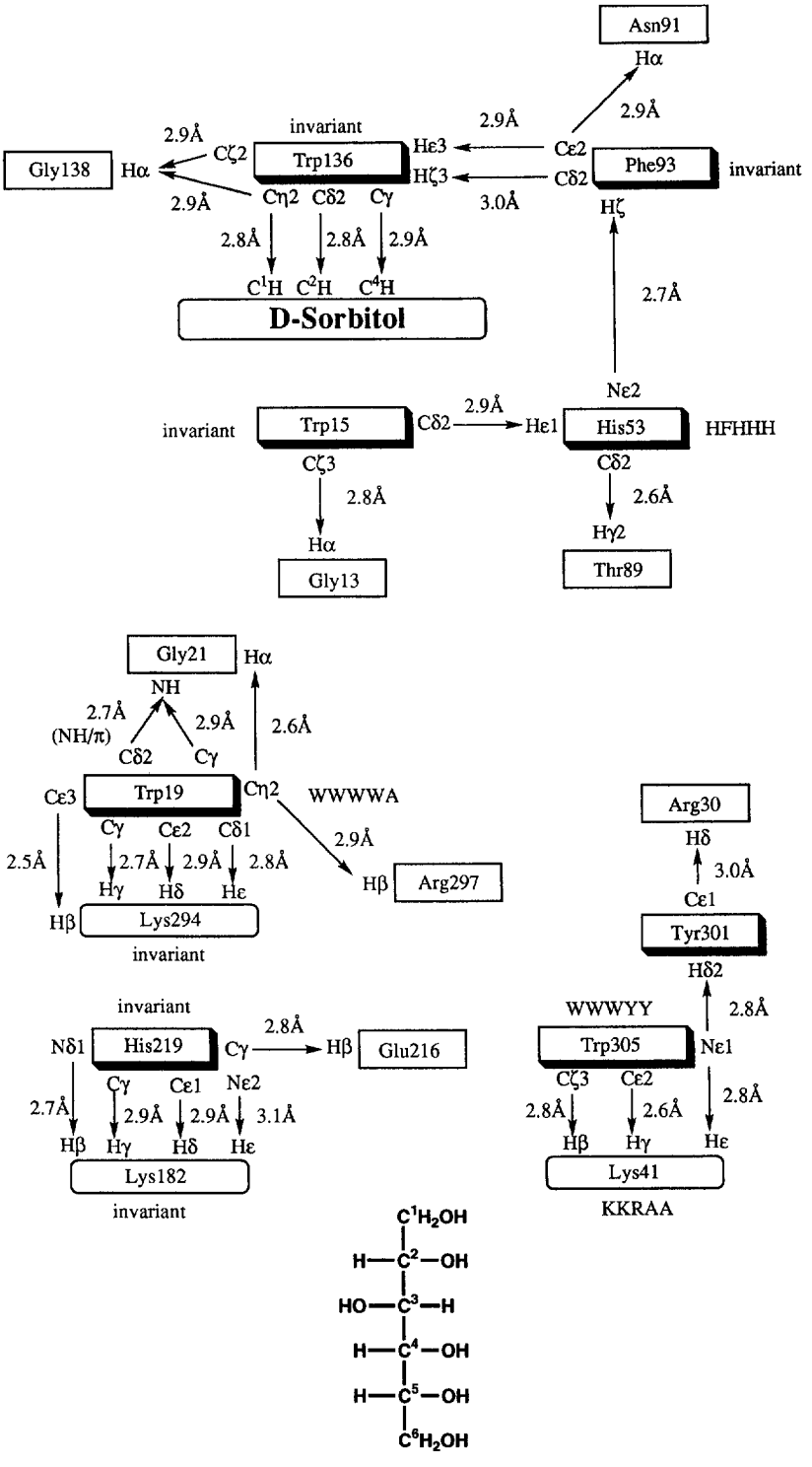


Chart 4. Parts of CH/π interactions in xylose isomerase/D-sorbitol complex (4XIA). F: phenylalanine, Y: tyrosine, H: histidine, A: alanine, W: tryptophane, K: lysine, R: arginine.

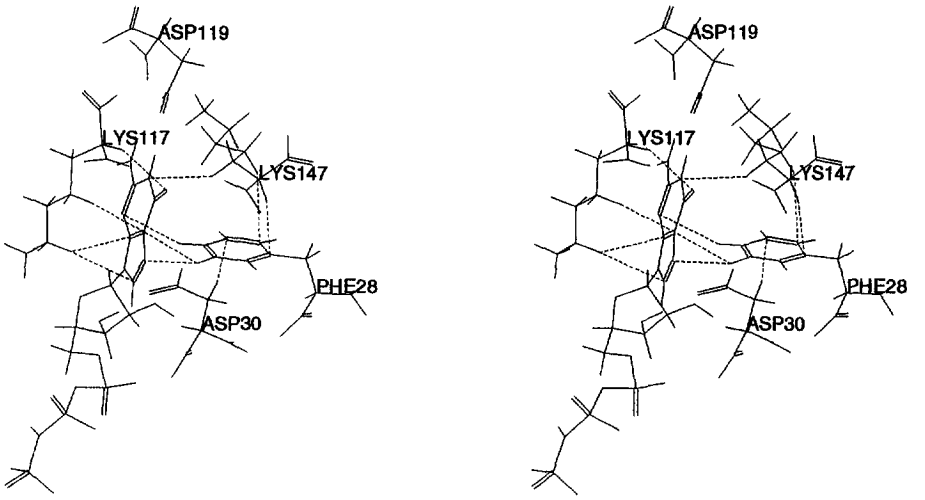
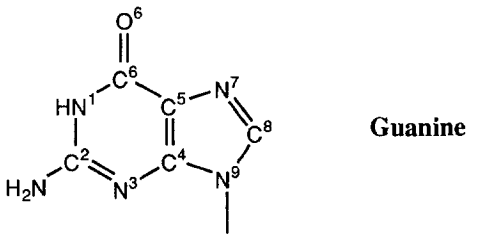


Figure 16. Stereo view of the guanine-binding region of ras p21 protein/GppNp complex. Side-chains in Phe28, Lys117 and Lys147 play a cooperative role in stabilizing the binding.



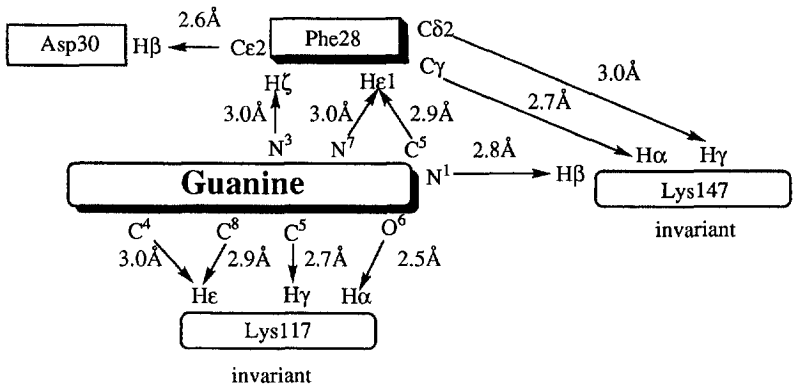


Chart 5. CH/π network around the ligand in c-H-ras protein p21/GppNp complex (5P21). GppNp: a GTP analogue.

to form a "hydrophobic" platform for the aromatic ring of the substrate phosphorylated tyrosine peptide (Tyr-Val-Pro-Met-Leu) and the amino group in the lysine residue is involved in a so-called amino/aromatic ring interaction. One of the amino terminal nitrogens in Arg155 was reported to be 3.1 Å above the centre of the aromatic ring. We examined these points by the programme CHPI.

The results are shown in Figure 17 and Chart 6. It is clear that the CHs in Lys203 participate in CH/π interactions with the aromatic carbons of the tyrosine, whereas the amino NH was found to be somewhat more remote.[169] An amino group in the Arg155 side-chain interacts with the aromatic

ring (NH/π interaction) of the phosphorylated tyrosine. The valine side-chain in the ligand is involved in CH/π interactions with Tyr202 (Fig. 17).

4.3.4. *FK506-Binding protein.*[170] Schreiber *et al.* reported the crystallographic structure of a complex of FK506-binding protein (FKBP: an immunophilin) with FK506 (**28**, an immuosuppressant). The ligand was found to interact with a number of aromatic residues (Tyr26, Phe36, Phe46, Phe48, Trp59, His87, Tyr82 and Phe99).[171]

A CH/π network including the above residues (except Tyr82 which may have an OH/π contact with the ligand) has been shown around **28** (Chart 7, Fig. 18). Trp59 interacts with three methylene hydrogens of the pipecolinyl ring. His87 is in contact with a methylene hydrogen of the pyranose ring. Short C/O contacts were pointed out to be present between FKBP (Phe99, Phe36) and carbonyl oxygens of the α-diketone in **28**. Hε2 of Phe36 (to O = C^9, ω 105°, θ 18°; see Fig 10 and Chart 7) and Hζ of Phe99 (to O = C^8, ω 106°, θ 21°) have indeed been found to be proximate to the carbonyl oxygens. These aromatic hydrogens position themselves somewhere above the respective C=O plane. Thus at present it is not clear whether the interactions are CH/π or CH/n (n: lone pair) in type.[172]

4.3.5. *Immunoglobulin McPC603 (acetylcholine esterase).* A mouse myeloma immunoglobulin fragment, Fab McPC603, has long been known to have a specific affinity with phosphorylcholine. For apparently obvious reason, the specificity of this protein was attributed to electrostatic interaction between positive versus negative charges in the ligand and globulin molecules.[173] Dougherty and Stauffer[174] recently discussed the problem on the basis of an attractive interaction between an ammonium cation and a π-electron system. According to their argument, a polar interaction of Me_3N^+ in the ligand with the negatively charged surface of the aromatic rings in the protein plays a central role.

Coulombic or polar interactions of similar nature are important.[175] However, we think the above phenomenon can more adequately be accommodated in the context of the CH/π interaction. To be effective, a CH hydrogen needs not necessarily be polarized (see discussions in section 4.2.2). The hydrogens in Me_3N^+ are positively charged as compared to those in normal aliphatic groups and therefore are more prone to CH/π interaction. Approximately 10-fold increase in the complexing ability of Me_3N^+H from that of Me_3COH (Table 5) may reflect this.

Hasan *et al.* studied the kinetics of binding of acetylcholine (ACh) analogues such as 3,3-dimethylbutyl acetate, 4-*t*-butylthio-2-butanone, or 3,3-dimethylbutanol. They reported that the above neutral compounds bind as effectively as ACh, to the same subsite of the enzyme, acetylcholine esterase (AChE).[176] This demonstrated that positive charge makes little contribution if any to

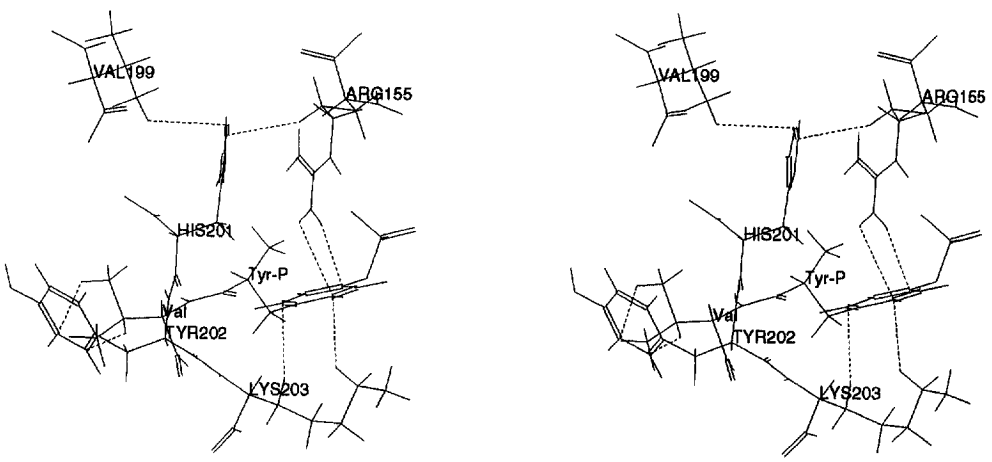


Figure 17. Stereo view of the tyrosine-binding region of a SH2 domain/phosphotyrosyl peptide complex.

the binding. Comparisons of quaternary compounds (Me_3N^+-R and Me_3C-R) as ligands with corresponding lower analogues (Me_2N^+H-R and Me_2CH-R) showed the former to be more effective than the latter with regard to the binding capabilities to AChE. From this they argued that the stereochemical structures of the ligand were important, on the grounds of the complementarity to the binding site geometry of the enzyme. We think that the binding force involved here can be understood in terms of the CH/π interaction; the number and probability for CHs to be participated in the interaction will have an appreciable effect in stabilizing the structure of the complexes.

Chart 8 shows CH/π interactions around phosphorylcholine in immunoglobulin McPC603.[177] Cγ, Nε1, Cε3 and Cζ2 in Trp107H (H; heavy chain), and Cε1 and Cζ in Tyr100L (L: light chain) are in fact found to be in contact with the hydrogens of CH_3N^+. Cζ2 in Trp107H and Cε1 in Tyr100L are close to a methylene hydrogen in the ligand.

Sussman *et al.* studied the crystallographic structure of AChE.[178] In a docking study, the Me_3N^+ group of substrate ACh was put on to the aromatic side-chain of Trp84, which has been known to be important for binding of the substrate. There we see (by CHPI; data not shown) the interaction of ACh with Trp84 to be supported, from the rear side of the indole ring, by Met83 side-chain with the aid of the following CH/π interactions (Hγ/Cδ2, Hγ/Cδ1 and Hε/Cζ2). An aromatic CH in Trp84 (Hζ2) has been found to be CH/π-interacted with Tyr442 (Cζ) which, in turn, is assumed by interactions with Leu430 (Hδ2/Cδ2) and Ile439 (Hβ/Cδ2). Thus a CH/π network plays a role in stabilizing the enzyme/substrate complex in a flexible manner, certainly in collaboration with other bonding forces.

The active site of AChE was found to lie at the bottom of a deep gorge and a substantial portion of the gorge is lined with many aromatic residues. The antigen-binding region of several immunoglobulin fragments was also reported to have a close-packed cluster of aromatic side-chains.[179,180] It is tempting to speculate that specific antigens of the antibodies are piloted to the combining site by the CH/π interaction. The mechanism of "aromatic guidance" proposed by Sussman *et al.* may represent an illustration of the CH/π interaction and might thus more adequately be termed as "CH/π-piloted pathway."

To summarize, the concept of CH/π interaction will provide a useful and essential means of analysing protein structures. It is probable that interactions of this kind play a role in interactions involving nucleic acids.

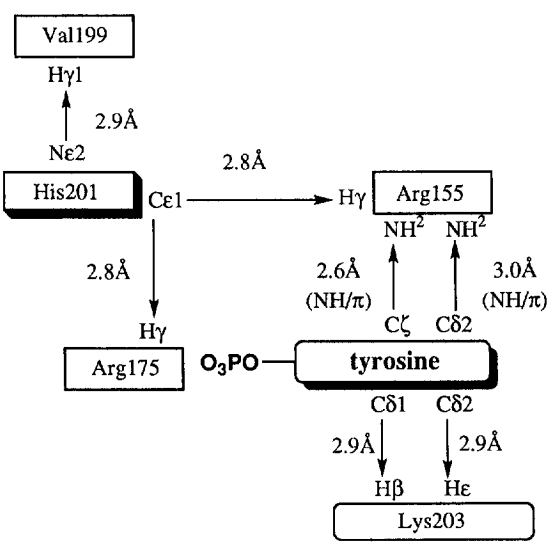


Chart 6. CH/π network around the ligand in a SH2 domain/phosphotyrosyl peptide complex (1SHA).

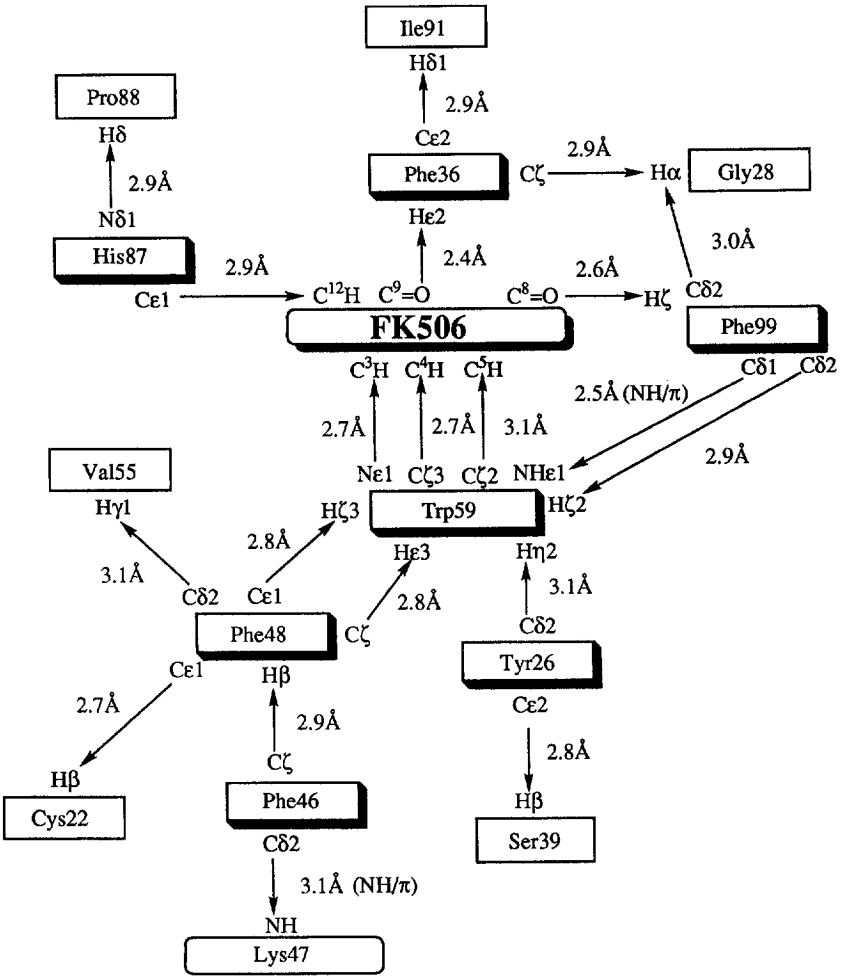
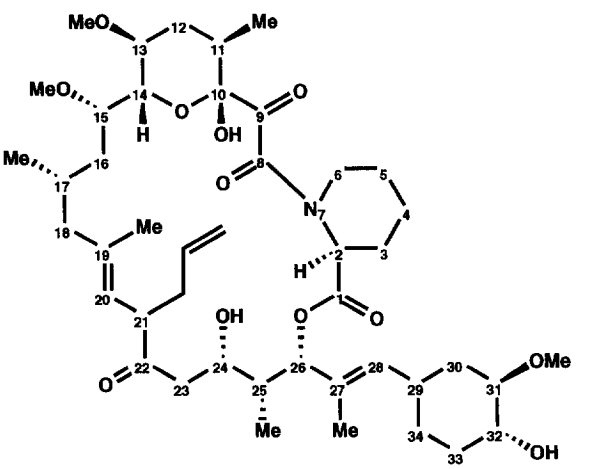


Chart 7. CH/π network around the ligand in FK506-binding protein/FK506 complex (1FKF).



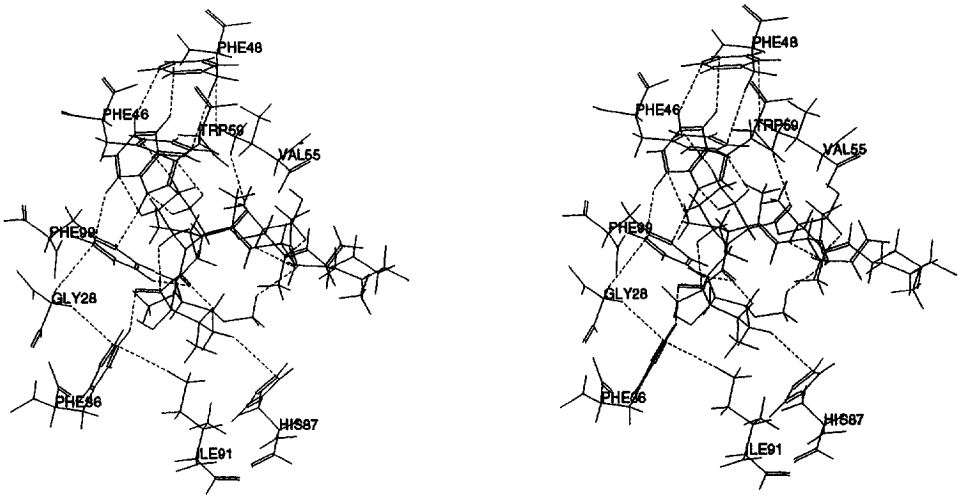
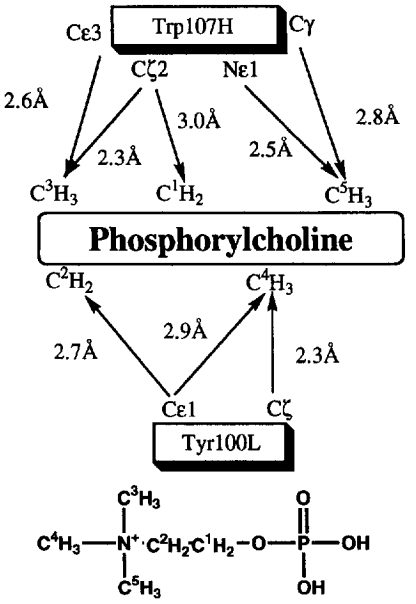


Figure 18. Stereo view of the ligand-binding site of FKBP/FK506 (**28**) complex. A CH/π network is disclosed around FK506.

5. CONCLUSION

On the basis of the foregoing discussions, we conclude that the CH/π interaction plays an important role in molecular recognition, in determining specificities for organic reactions and inclusion complexes, as well as controlling specific functions of biopolymers. Higher order structures of proteins should be considered against this background. Stronger forces such as hydrogen bonding are important, but it is certain that interactions other than the H-bond, occurring between non-polar groups, are very important in maintaining the low-entropy structure of macromolecules.



Phosphorylcholine

Chart 8. CH/π interactions involving the ligand in McPC603/phosphorylcholine complex (2MCP).

Discrimination of the CH/π interaction from the attractive part of the van der Waals force is difficult. A considerable fraction of the CH/π interaction undoubtedly originates from the dispersion force. The latter, on the other hand, constitutes a major part of the van der Waals interaction. However, the van der Waals force is quite an ambiguous concept representing a blend of a variety of non-specific interactions, attractive as well as repulsive. Besides the London dispersion force, the attractive part of the van der Waals force consists of a number of terms from polar interactions, such as charge/dipole, dipole/multipole, multipole/multipole interactions.

As for the CH/π interaction, this represents an extreme case (but abundant in nature) of hydrogen-bonding,[1] which occurs between a soft acid and a soft base. The CH/π interaction obviously includes the dispersion force, however, contribution from charge transfer (or hyperconjugation through the space) is appreciable. Contribution from the Coulombic force, on the other hand, is unimportant. The CH/π interaction, therefore, can play its role in polar media as well as in a non-polar atmosphere, unlike normal H-bonding.

The enthalpy for a one unit CH/π interaction is small. However, groups involved in the CH/π interaction are often engaged simultaneously in interactions with multiple atoms. Furthermore, according to symmetry (e.g. three-fold axial symmetry for CH_3), CH groups have a larger chance to be involved in an interaction, as compared to, e.g. OH/π or OH/O hydrogen-bonding, thus giving rise to appreciable effects on the free energy of competitive states in a dynamically interacting molecular system. This point is crucial in understanding the role of weak secondary forces. Examples were given in previous sections by, e.g. CH/π network, aromatic CH/π and lysine CH/π interaction.

The secondary forces involved in specific biopolymer interactions should never be too strong.[181] Instead, in order to assume a rapid recombination of interacting molecules and to be compatible with the living cell, they should be moderately weak and must have a proper orientation dependence. In view of this, the CH/π interaction, among others, represents a most general and effective one. Interactions such as OH/π (see Refs 34–37), NH/π,[182–184] CH/n (n = lone pair: CH/O, CH/N etc.; see Ref. 10b)[185–194] and those involving multipoles[195] are important as well in understanding the behaviour of biomolecules. Our knowledge about weak chemical interactions is still far from complete.

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