Metal Ligand Aromatic Cation – π Interactions in Metalloproteins: Ligands Coordinated to Metal Interact with Aromatic Residues

Snežana D. Zarić,*[a] Dragan M. Popović,[b] and Ernst-Walter Knapp[b]

Abstract: Cation – π interactions between aromatic residues and cationic amino groups in side chains and have been recognized as noncovalent bonding interactions relevant for molecular recognition and for stabilization and definition of the native structure of proteins. We propose a novel type of cation – π interaction in metalloproteins; namely interaction between ligands coordinated to a metal cation—which gain positive charge from the metal—and aromatic groups in amino acid side chains. Investigation of crystal structures of metalloproteins in the Protein Data Bank (PDB) has revealed that there exist quite a number of metalloproteins in which aromatic rings of phenylalanine, tyrosine, and tryptophan are situated close to a metal center interacting with coordinated ligands. Among these ligands are amino acids such as asparagine, aspartate, glutamate, histidine, and threonine, but also water and substrates like ethanol. These interactions play a role in the stability and conformation of metalloproteins, and in some cases may also be directly involved in the mechanism of enzymatic reactions, which occur at the metal center. For the enzyme superoxide dismutase, we used quantum chemical computation to calculate that Trp163 has an interaction energy of 10.09 kcal mol^{-1} with the ligands coordinated to iron.

Keywords: amino acids · density functional calculations · metal complexes · metalloproteins · pi interactions

Introduction

It has recently become apparent that cation – π interactions are important for molecular recognition and stabilization in many biological systems. Experimental and theoretical studies provide an understanding of the nature of the cation – π interaction and show that this interaction is a strong, noncovalent one.[1] It is mainly electrostatic in character and is dependent upon the quadrupole moment of the aromatic moieties. A number of studies have established that cation – aromatic interactions play an important role in protein – ligand interactions.[2] Statistical analyses of crystal structures of proteins showed that the nitrogen atoms in the side chains of arginine, lysine, asparagine, glutamine, and histidine preferentially adopt positions close to the aromatic residues phenylalanine, tyrosine, and tryptophan.[3, 4]

Recent investigations into cation – π interactions in protein structures—between the cationic side chains of lysine and arginine and aromatic side chains—were based on energy criteria for selecting significant pairs of side chains. In this way, cation – π interactions of different molecular fragments were put on equal footing and it was possible to analyze their frequency of occurrence in proteins and to evaluate whether specific cation – π interaction pairs are preferred. These analyses showed that the tryptophan aromatic residue is the most likely to be involved in cation – π interactions in proteins, and that arginine is more likely than lysine to be capable of involvement in energetically stronger cation – π interactions. It was found that energetically significant cation – π interactions are common in proteins and that they probably contribute to protein stability. Some of the favorable cation – π interaction pairs contribute at least as much to protein stability and the structural profile of a native protein as do more conventional interactions. Hence, it was proposed that cation – π interactions should be considered in the same way as hydrogen bonds, salt bridges, and hydrophobic effects in analyses of the stability of native protein structure.[4]

There is experimental evidence for cation – π interaction in proteins in which a metal cation interacts directly with an aromatic group. In the crystal structure of hen egg-white lysozyme, an interaction between the sodium cation and the

[a] Prof. S. D. Zarić
Department of Chemistry
University of Belgrade
Studentski trg 16, P.O. Box
11001 Belgrade (Yugoslavia)
Fax: (+381) 11-638785
E-mail: zaric@helix.chem.bg.ac.yu

[b] D. M. Popović, Prof. E.-W. Knapp
Department of Biology, Chemistry, and Pharmacy
Institute of Chemistry
Free University of Berlin
Takustrasse 6, 14195 Berlin (Germany)
Fax: (+49) 30-83853464
E-mail: knapp@chemie.fu-berlin.de

  0947-6539/00/0621-3935 $ 17.50+.50/0

indole ring of tryptophan (residue Trp123) was observed.[5] The distance between the center of the indole ring of Trp123 and the Na^+ ion is 4.07 Å. However, an interaction between an aromatic π system and a metal complex as cation was neither observed nor investigated.

Recently, however, interactions of metal complexes as cation with π systems were investigated. In theoretical work, it has been shown that calculated interaction energies for cation–π systems are in agreement with experimental data,[6–18] and so it is possible to predict cation–π interactions by quantum chemical calculations. In our previous work, the interactions of π systems with the cationic metal complex $[Co(NH_3)_6]^{3+}$ were predicted by quantum chemical calculations. The bonding energies obtained using the B3LYP method for benzene, acetylene, and ethylene π complexes are 31.34 kcal mol^{-1},[19] 18.30 kcal mol^{-1}, and 17.02 kcal mol^{-1},[20] respectively. Since these calculations showed strong cation–π interactions, it was proposed[19, 20] that cation–π interaction may also play a role when $[Co(NH_3)_6]^{3+}$ interacts with nucleic acids.[21] Cation–π interactions with a cationic metal complex were observed in analyses of crystal structures of DNA and RNA. It was noticed that cation–π interactions between divalent cations and the π systems of bases are important for stabilization of unstacked conformations of DNA and RNA.[22, 23]

We propose a new type of cation–π interaction, with a metal complex as cation, in which ligands coordinated to the metal can form contacts with π systems. Such interactions occur in many biological molecular systems, including metalloproteins, where the metal center is often functionally relevant. In a basic investigation into metalloproteins, it was found that the first layer of a metal center involves hydrophilic atoms of ligands—such as carbonyl oxygen atoms, water, hydroxy groups, and sulfur—while the second layer of atoms involves hydrophobic groups.[24] These, however, belong mainly to the carbon-containing side chains of amino acids bearing the hydrophilic atoms ligated to the metal complex. In that study no attention was paid to aromatic groups in the second coordination shell. Aromatic groups found close to the zinc binding site in carbonic anhydrase[25, 26] are too distant to enter into cation–π interaction with a zinc ligand.

Herein, we present the results of an analyis of metalloproteins geared to search for this novel type of metal ligand aromatic cation–π (MLACπ) interaction. For this purpose, we screened crystal structures of metalloproteins in the Protein Data Bank (PDB) and studied in more detail the structures of a selection of metalloproteins in which we found this specific cation–π interaction; between ligands coordinated to a metal cation and aromatic residues. As well as this, we used quantum-chemical calculations to compute the energy of this type of interaction for superoxide dismutase (SOD).[27]

Data Screening and Computational Methods

Crystal structures of proteins containing Ca, Co, Cr, Cu, Fe, Mg, Mn, Ni, and Zn as cations were obtained from the PDB. The structures of these proteins were screened for cation–π interactions between metal-coordinated ligands and aromatic residues, using geometric criteria. (These criteria were the same as those proposed by McFail-Isom et al.[22] for screening crystal structures of DNA for conventional cation–π interactions between a metal and a DNA aromatic base.) We searched for structures in which the distance between the metal and the center of the aromatic ring of an aromatic amino acid was less than $d_0 = 5.5$ Å and where the angle θ_0 of the normal of the aromatic ring plane with the distance vector between the center of the aromatic ring and the metal was less than 52° (for definitions of d_0 and θ_0 see Figure 1). In our application, the distance criterion was more conservative,

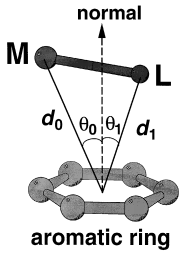

Figure 1. Distances (d_0, d_1), and angles (θ_0, θ_1) used to search for and to characterize MLACπ interactions. The distance d_0 is measured between the positions of metal cation (M) and the center of the aromatic ring participating in the MLACπ interaction. The angle θ_0 is defined as the angle of the normal of the aromatic ring plane with the distance vector $\boldsymbol{d}_0$. The distance d_1 and angle θ_1 with respect to the non-hydrogen ligand atom (L) closest to the ring center is defined analogously. The search criterion for MLACπ interaction in the PDB was $d_0 < 5.5$ Å, and $|\theta_0| < 52°$.

since it was applied to the metal atom, even though the cation–π interactions of interest here are those between the metal-coordinated ligand extending towards the aromatic ring and the aromatic group. The screening was performed for the aromatic groups in phenylalanine, tyrosine, and tryptophan. Although the side chain of histidine, with its aromatic ring, can also participate as an aromatic component in this type of cation–π interaction, we did not include it in this search. Since histidine is very often directly coordinated to a metal, we would have obtained a large number of structures that did not belong to the type of cation–π interaction we were looking for. On the other hand, if histidine were coordinated to a metal, it could be involved in an MLACπ interaction as a ligand, with partial positive charge transferred from the metal cation. Such histidines, however, would be found by our selection criteria. Screening the PDB using the described criteria provided a large number of structures in which an aromatic ring is close to a metal. We inspected these structures visually, and selected a number in which it was clear that an MLACπ interaction between a coordinated ligand and the aromatic ring of phenylalanine, tyrosine, or tryptophan existed. Data on these structures are compiled in Table 1.

The energy of the cation–π interaction in the protein SOD (PDB code 1avm, chain A) was calculated by using the density functional theory (DFT) with the Becke (B3) three-parameter exchange functional[28] and the Lee–Yang–Parr (LYP) correlation functional.[29] These B3LYP calculations were carried out using the Gaussian98 program.[30] For the carbon, nitrogen, and hydrogen atoms, STO-3G basis sets were

  0947-6539/00/0621-3936 $ 17.50+.50/0 *Chem. Eur. J.* **2000**, *6*, No. 21

Table 1. Metalloproteins showing metal ligand aromatic cation – π (MLACπ) interactions.[a]

Entry	Ref.	Name[a]	PDB code	Chain	Resolution[b]	Metal	CN	Charge[c]	Ligands[d]	Bond number[e]	Aromatic residues[f]	Distance d_1 [Å]	Angle θ_1 [°]
1	[54]	ADH	1adc	B	2.70	Zn^{2+}	4	0	(O) ethanol (C1)	2	Phe93	4.21	35.8
2	[55]	ADH	1ldy	B	2.50	Zn^{2+}	4	0	(NE2) His67 (CD2)	2	Phe93	4.28	35.2
									(O9) CXF378 (C7)	3	Phe93	3.46	19.6
3	[56]	ADH	1axe	B	2.20	Zn^{2+}	4	0	(NE2) His67 (CD2)	2	Trp93 (5)	3.77	21.5
									(O) ETF404 (C2)	2	Trp93 (6)	3.65	40.9
4	[56]	ADH	1axg	D	2.50	Zn^{2+}	4	0	(NE2) His67 (CD2)	2	Phe93	3.94	16.6
									(O) ETF404 (C2)	2	Phe93	3.94	44.6
5	[57]	ADH	2ohx	A	1.80	Zn^{2+}	4	0	(NE2) His67 (CD2)	2	Phe93	4.41	33.6
6	[70]	AO	1asp	A	2.59	Cu^{2+}	4	+1	(NE2) His62 (ND1)	3	Phe102	4.11	42.9
7	[63]	CBP	2cbp		1.80	Cu^{2+}	4	+1	(ND1) His39 (CE1)	2	Phe13	4.12	33.5
8	[62]	CheY	1chn		1.76	Mg^{2+}	6	0	H_2O300	1	Phe14	4.12	27.7
									H_2O301	1	Phe14	3.50	24.9
9	[71]	D9D	1afr	A	2.40	Fe^{2+}	4	0	(OE2) Glu143 (CG)	3	Trp139(5,6)	3.93	26.0
10	[72]	EndN	1ak0		1.80	Zn^{2+}	4	+1	(NE2) His15 (CD2)	2	Tyr16	3.69	14.1
11	[73]	FBC	1hfc		1.56	Zn^{2+}	4	+1	(NE2) His168 (CE1)	2	Phe174	4.00	38.0
12	[74]	GLI	2gli	A	2.60	Co^{2+}	4	0	(NE2) His129 (CD2)	2	Trp108 (5)	3.09	17.9
13	[75]	HeC	1lla		2.20	Cu^{+}	4	+1	(NE2) His364 (CD2)	2	Phe360	3.50	16.9
14	[76]	MMP-8	1mmb		2.10	Zn^{2+}	4	+1	(NE2) His147 (CE1)	2	Phe153	3.82	34.9
15	[77]	NPR	1npc		2.00	Ca^{2+}	6	+1	(OG1) Thr195 (CG2)	3	Tyr194	3.58	16.0
16	[78]	SBA	1sbd		2.52	Ca^{2+}	4	+1	(OD1) Asn130 (CB)	3	Trp132 (6)	3.37	27.7
17	[27]	SOD	1avm	A	1.55	Fe^{3+}	6	?	H_2O1	1	Trp163 (5)	3.35	16.3
									(OD2) Asp161 (CB)	3	Trp126 (6)	3.75	21.5
18	[59]	SOD	1mmm	A	2.20	Fe^{2+}	5	?	H_2O207	1	Trp169 (5)	3.12	14.4
									(OD2) Asp167 (CB)	3	Trp128 (6)	3.53	16.0
19	[60]	SOD	1ap5	A	2.20	$Mn^{2+(3+)}$	5	?	H_2O200	1	Trp161 (5)	3.52	25.4
									(OD2) Asp159 (CB)	3	Trp123 (6)	3.66	15.0
20	[61]	SOD	1ar4	A	1.90	$Mn^{2+(3+)}$	5	?	H_2O1	1	Trp163 (5)	3.29	19.7
									(OD2) Asp161 (CB)	3	Trp126 (6)	3.75	21.7
21	[58]	SOD	1mng(L)	B	1.80	Mn^{3+}	6	?	H_2O205	1	Trp168 (5)	3.57	19.7
									(OD2) Asp166 (CB)	3	Trp132 (6)	3.59	14.7
22	[58]	SOD	1mng(U)	B	1.80	Mn^{3+}	5	?	H_2O205	1	Trp168 (5)	3.52	26.9
									(OD2) Asp166 (CB)	3	Trp132 (6)	3.54	15.4
23	[79]	TNC	1tn4		1.90	Ca^{2+}	6	– 1	H_2O5	1	Tyr109	4.03	42.6

[a] Abbreviations: EndN, endonuclease; FBC, fibroplast collagenase; GLI, zinc finger protein GLI; HeC, hemocyanin; MMP-8, metalloproteinase-8; NPR, neutral protease; SBA, soybean agglutinin; others are given in the text. [b] The resolution of the crystal structure. [c] Total charge of the first coordination sphere. In some cases the exact value of this charge is uncertain, since the redox state of the metal is unclear, or it is not clear whether a water ligand is neutral (HOH) or negatively charged (OH^-). [d] Ligands (ETF, trifluoroethanol) taking part in metal ligand-aromatic cation – π interaction (MLACπ). The atoms, denoted in brackets are the ligand atom coordinated to metal (to the left of ligand name) and the non-hydrogen ligand atom closest to the center of the aromatic ring (to the right of ligand name). [e] The number of bonds in the ligand (including the bond to the hydrogen atom extending towards the aromatic ring) which are involved in the MLACπ interaction. In some structures, there are two ligands interacting with the same or with two different aromatic residues. [f] The tryptophan indole group consists of two aromatic rings: a five-membered and a six-membered ring. Both can take part in MLACπ interactions. The digit 5 or 6 given in brackets after the name of the tryptophan indicates which ring of the indole ring system is involved in the MLACπ interaction.

chosen, while LANL2DZ was used for the iron atom. In previous theoretical work on cation – π interactions, Hartree – Fock and MP2 methods were mainly used.[6–18] The MP2 method was used very successfully for some transition metal complexes,[31–34] but no good results were obtained for the first row transition metal complexes.[35–37] In contrast, DFT methods give good results for all transition metal complexes, including those of the first row.[38–50] Accordingly, the DFT method was used in this work, because of the presence of first-row transition metal complexes. It was shown that the B3LYP method is suitable for characterizing cation – π interactions by calculating the geometry and bonding energy of the NH_4^+–C_6H_6 complex with LANL2DZ basis sets.[19] The obtained bonding energy of 16.43 kcal mol^{-1} and geometry are in good agreement with previous results, where bonding energies are in the range from 16.3 to 22.2 kcal mol^{-1}.[6, 12–16] Recently, reliable results were also obtained for other cation – π systems using DFT methods.[51–53]

Results and Discussion

We have found that these specific cation – π interactions—between a coordinated ligand and the aromatic ring of phenylalanine, tyrosine, and tryptophan—can be observed in a number of crystal structures of metalloproteins. Data relating to MLACπ interactions in different crystal structures are compiled in Table 1. With less conservative search criteria, many more metalloproteins containing MLACπ interactions can be found. These data show that this type of interaction exists in numerous metalloproteins, and that different metals and ligands can be involved. Table 1 lists the abbreviated name of the protein and the resolution of the crystal structure, metal, ligand, and aromatic residue involved in the MLACπ interaction, as well as the PDB code.

In all of the observed cases in which the MLACπ interaction was identified, the ligand participating in the interaction possesses a hydrogen atom extending towards the

aromatic ring of the molecular group involved in the cation – π interaction. A partial positive charge on the ligand, induced by the charge on the metal, is situated on the hydrogen atom interacting with the aromatic ring. With the exception of the basic blue copper protein from cucumber (CBP), the positions of hydrogen atoms are not available from the crystal structure in any of the protein structures listed in Table 1. Therefore, it was not possible to ascertain the distance between aromatic ring and interacting hydrogen atom directly. Hence, the geometry of the special cation – π interaction is characterized by the distance d_1 (between the center of the aromatic ring and the non-hydrogen ligand atom closest to the ring center) and the angle θ_1 (between the corresponding distance vector $\boldsymbol{d}_1$ and the normal of the aromatic ring plane), as explained in Figure 1. For the protein structures with MLACπ interaction that we found, the distance d_1 varies between 3.09 and 4.41 Å. The angle θ_1 is often smaller than 30°, but can become as large as 45°. By visualizing the corresponding protein structures, we often observed that the ligand atom closest to the aromatic ring was oriented so that the attached hydrogen atom would extend towards a bond rather than towards the center of the aromatic ring. This is particularly the case when the angle θ_1 is large. Similar geometries for cation – π interactions were found by quantum chemical calculations on the $[Co(NH_3)_6]^{3+}$–benzene model system.[19]

For some metalloproteins, a number of crystal structures that may involve more than one chain are available. For the same protein, all crystal structures possess the same MLACπ interaction, though the redox state and the coordination number may vary (see column 8, lower part of Table 1). In some metalloproteins, more than one ligand coordinated to the same metal cation interacts with the same aromatic group; as, for instance, in alcohol dehydrogenase (ADH).[54, 55, 56, 57] In other proteins, such as superoxide dismutase (SOD),[58, 27, 59, 60, 61] different ligands (water and asparagine) can interact with different aromatics (in SOD, two different tryptophans). As well as the name of the ligand involved in the MLACπ interaction, the ligand atom directly bound to the metal (to the left of the ligand name in Table 1) and the non-hydrogen atom closest to the aromatic ring (to the right of the ligand name in Table 1) are also given, in brackets (column 10 in Table 1). (For water as ligand, that information is obvious and so not given, since the oxygen atom of water is closest to the metal and also to the aromatic ring involved in the MLACπ interaction.)

The indole group of tryptophan consists of two aromatic rings: one five-membered and one six-membered. Both can take part in MLACπ interactions and they can even do this simultaneously. See, for instance, the structure of Δ9-stearoyl-acyl carrier protein desaturase (D9D), entry 9 in Table 1. The aromatic ring actively participating in MLACπ interaction is denoted with the digit 5 or 6 in brackets after the name of the aromatic residue (see Table 1). In SOD, the water always interacts with the five-membered ring, whereas the asparagine interacts with the six-membered ring of another tryptophan. In the mutant Phe93Trp of ADH, both ligands interact with the same tryptophan residue. The histidine interacts with the five-membered, the trifluoroethanol (ETF) with the six-membered ring.

In SOD, the distances d_1 between the tryptophan—the aromatic group involved in the MLACπ interaction—and the oxygen atom of coordinated water range from 3.12 to 3.57 Å. This is generally shorter than the corresponding distances—between the two water oxygen atoms and Phe14—in the bacterial chemotaxis protein (CheY)[62], which are 3.50 and 4.12 Å. Interestingly, in the mutant Phe93Trp of ADH, the distances between tryptophan and the histidine and ETF ligands are 3.77 and 3.65 Å, respectively; shorter than in the native protein, where Phe93 is the aromatic group involved in cation – π interactions, with distances of 3.94 Å. The shortest distance ($d_1 = 3.09$ Å) occurring in Table 1 also relates to an interaction with tryptophan. Hence, it seems that tryptophan enters into the strongest interactions with coordinated ligands. This is in agreement with calculated electrostatic potential surfaces around aromatics—which are a qualitative guide to cation – π interactions—and with analyses of protein structures that showed that tryptophan is most likely to be involved in cation – π interactions.[1, 2, 4, 8, 9] Calculated interaction energies of benzene and indole ring with Na^+ also indicated that the indole ring interacts more strongly than benzene.[5] This agreement with previous results demonstrates that the nature of the new type of cation – π interaction (MLACπ), in which ligands coordinated to a metal interact with aromatic molecular groups, is related to other already observed and investigated cation – π interactions.

The number of bonds in the ligand (including the bond to the hydrogen atom extending towards the aromatic ring) that are involved in the cation – π interaction is given in column 11 of Table 1. The MLACπ interaction will generally be stronger, the fewer the ligand bonds lying between the atom coordinated to metal and the interacting hydrogen atom. Water is the ligand with the smallest number of bonds between the atom coordinated to a metal cation and the interacting hydrogen atom, since it involves just a single O–H bond. The largest number of bonds that we found in a ligand is three; see column 11 of Table 1. From the above reasoning, we expect that MLACπ interactions with water as ligand are the strongest. As a matter of fact, almost the smallest distance d_1, at 3.12 Å, was found between water and tryptophan in an Fe^{2+} SOD.

Four selected structures involving the new type of cation – π interaction—namely blue copper protein phytocyanin (CBP, PDB code 2cbp),[63] bacterial chemotaxis protein (CheY, PDB code 1chn),[62] superoxide dismutase (SOD, PDB code 1avm/A),[58] and alcohol dehydrogenase (ADH, PDB code 1adc/B)[54]—will now be discussed in more detail. The geometry of the MLACπ interaction in these structures, with different metals, ligands, and aromatic groups, is depicted in Figure 2.

Phytocyanin: The exact biological function of phytocyanin, the basic blue copper protein from cucumber (CBP),[63] is unknown; however, it is considered that phytocyanin is an electron transport protein[63] or is involved in redox reactions.[64] In the crystal structure of phytocyanin, there are two histidines (His39 and His84) coordinated to copper(II), as well as one cysteine (Cys79) and one methionine (Met98) (see Figure 2 A). As is typical for blue copper proteins, the bond to one ligand, Met98, is rather long (2.61 Å).[63] The Phe13

  0947-6539/00/0621-3938 $ 17.50+.50/0 *Chem. Eur. J.* **2000**, *6*, No. 21

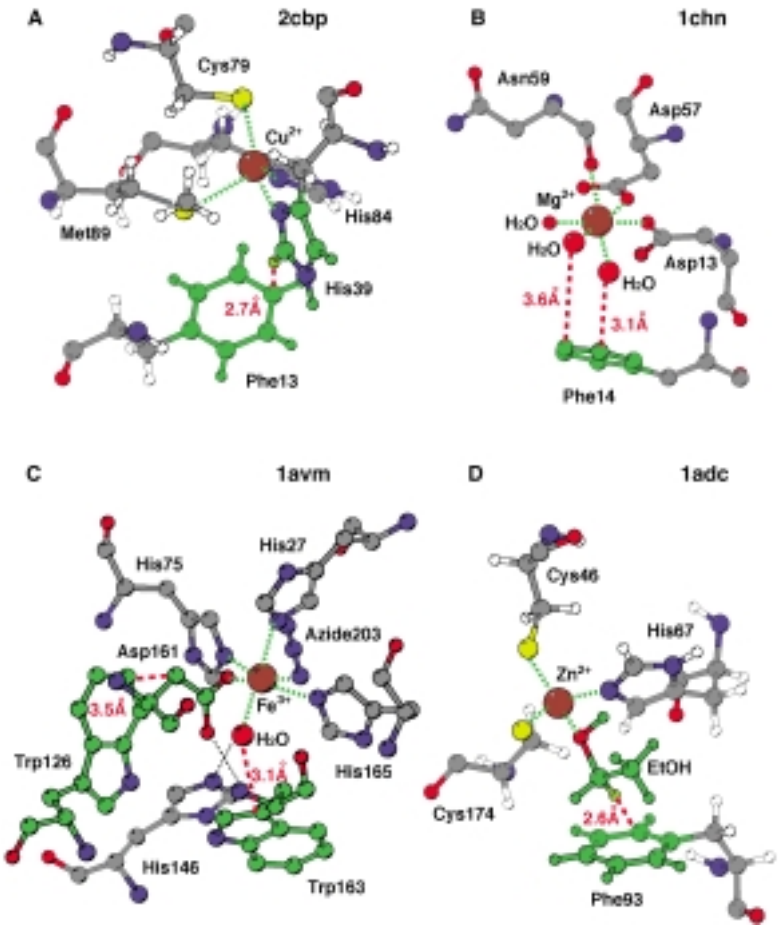

Figure 2. Four examples of the new type of cation – π interaction (MLACπ). Ball-and-stick presentation of a selection of four metal complexes in proteins with cation – π interactions. A: blue copper protein phytocyanin (CBP, PDB code 2cbp), B: bacterial chemotaxis protein (CheY, PDB code 1chn), C: superoxide dismutase (SOD, PDB code 1avm/A), and D: alcohol dehydrogenase (ADH, PDB code 1adc/B). All ligands (amino acids, water, substrates, and inhibitors) coordinated to the metal center and all aromatic residues relevant for MLACπ interaction are displayed. The color is as follows: side chains of aromatic residues and ligands involved in MLACπ interaction in dark green, water oxygen atoms and ligand atoms in red, the metal in brown, the hydrogen atom extending towards the aromatic ring which participates in MLACπ interaction in light green, while for all other atoms a standard color is used. Red dashed lines represent distances between the aromatic group and ligand atoms closest to it. Green dashed lines represent the coordination bonds of ligands with the metal involved in MLACπ interaction. Black dashed lines represent hydrogen bonds.

residue is close to the metal complex, the center of the phenyl ring is 5.31 Å away from the copper. In this high-resolution (1.8 Å) crystal structure, the positions of hydrogen atoms were determined, allowing us to observe the distance between the interacting hydrogen atom and the aromatic ring directly. The cation – π interaction occurs between the metal ligand His39 and the aromatic group Phe13. The distance from the hydrogen atom of His39 to the center of the aromatic ring of Phe13 is 3.16 Å, whereas the distance to the closest carbon atom of the aromatic ring is 2.71 Å. The distance between the carbon atom of His39, the hydrogen atom of which is involved in the interaction, and the center of the Phe13 aromatic ring is 4.12 Å (Table 1).

Bacterial chemotaxis protein: Large conformational changes in the bacterial chemotaxis protein (CheY)[62] are caused by the bonding of a magnesium cation, which is known to be essential for the function of this protein. This structure contains a hexacoordinate magnesium(II) cation (see Figure 2B); these coordinated ligands are characteristic for magnesium.[65] There are three amino acids acting as ligands: namely Asp57, Asp13, and Asn59, together with three water molecules. The distance d_0 from the magnesium atom to the center of the aromatic ring of Phe14 is 4.89 Å. The aromatic amino acid Phe14 is involved in two cation – π interactions with two of the coordinated water molecules, H_2O301 and H_2O300 (see Table 1). The distances between the oxygen atoms of the coordinated water molecules and the center of the phenyl ring are 3.50 and 4.12 Å, respectively, and the angles θ_1 are 24.9 and 27.7°. In the crystal structure, the interaction of H_2O301 with the aromatic ring of Phe14 was originally classified as a hydrogen bond with the phenyl ring.[62] Although at greater distance from Phe14, the other water molecule H_2O300 may also be considered to interact with Phe14. According to our analysis, the interactions of the water molecules with the phenylalanine are examples of MLACπ interaction rather than hydrogen bonding.

Superoxide dismutase: In Figure 2C, we show structural details of the metal complex of SOD from *Propionibacterium shermanii* (PDB code 1avm/A), containing a hexacoordinate iron(III) center, with one ligand a negatively charged azide. In the corresponding iron(II) SOD structure (PDB 1*mmm*/A), the azide is lacking and the iron center is pentacoordinate. There are six structures of FeSOD and MnSOD listed in Table 1, with data for the cation – π interaction of coordinated water with tryptophan. Two of these have a pentacoordinate metal center (Fe^{2+}SOD, Mn^{3+}SOD); the other four possess a hexacoordinate metal center. Since the charge state of the water ligand, and in some cases also the redox state of the metal center, are uncertain, the total charge of the first coordination sphere cannot be given for the SODs.

In the iron(III) SOD structure, with a resolution of 1.55 Å, there are two tryptophans close to the metal center.[58] Both are involved in cation – π interactions. There are four amino acids coordinated to the iron: three histidines (His27, His75, His165), and one aspartate (Asp161). The other two ligands are water and azide. The MLACπ interaction with the shortest distance is the interaction of a coordinated water

with the five-membered ring of Trp163. The distance from the center of the five-membered ring to the iron is $d_0 = 5.24$ Å and the angle is $\theta_0 = 27.3^\circ$. The distance from the oxygen atom of the coordinated water to the center of the five-membered ring is $d_1 = 3.35$ Å with the angle $\theta_1 = 16.3^\circ$. The MLACπ interaction in SOD may be important for the mechanism of the enzymatic reaction, since it has been proposed that the coordinated water is involved in a functionally relevant proton transfer process.[27]

The second interaction occurs with the six-membered ring of Trp126. The distance of the center of the six-membered ring to the metal ion is $d_0 = 5.42$ Å and the angle is $\theta_0 = 51.4^\circ$. Trp126 interacts with the CB atom of the coordinated ligand Asp161 (see Figure 2C). The distance between the center of the six-membered ring to the CB atom is 3.75 Å with an angle of 21.5°. This cation–π interaction is probably weaker than the first one, since the CB atom is two bonds away from the oxygen atom OD2 coordinating with the iron atom.

Importantly, the two tryptophans involved in cation–π interactions in SOD are among the twelve residues of FeSOD and MnSOD which are strictly conserved.[66] These tryptophans probably play an important role in the enzymatic reaction mechanism of SOD, and the cation–π interactions may be important for their function. Interestingly, the tryptophan that interacts with water is destroyed by the reaction of hydrogen peroxide with FeSOD.[67]

Density functional and electrostatic calculations on the MnSOD active site complex showed that, for accurate energetic calculations, it is essential to involve the second-shell ligands not directly bound to the central metal.[68] Since the indole ring of Trp168 was not included in these computations, it would be interesting to find out how the involvement of tryptophan in DFT calculations would influence the results.

Calculated MLACπ interaction for superoxide dismutase: For the FeSOD structure (PDB code 1avm) the energy of the cation–π interaction was computed by using DFT calculations. This protein was chosen for the computation as its X-ray structure is the one of highest resolution (1.55 Å) for which we found this new type of cation–π interaction. We expected a strong MLACπ interaction in this case, since water H_2O1 as ligand interacts with the π system of tryptophan Trp163. The oxygen–metal distance between the coordinated water and iron is, at 2.15 Å, relatively large, and so it is assumed that H_2O rather than OH^- is present. To ascertain the cation–π interaction, we considered a molecular model of the FeSOD containing the relevant molecular components coordinated to iron(III) and Trp163 (see Figure 3). Of the iron ligands (His27, His75, His165, azide, Asp161, and H_2O), the histidines were modeled by imidazole rings, and the aspartate as acetate. The tryptophan was modeled as indole ring system and an ammonia was used for the backbone of Trp163 to model the hydrogen bond, with the oxygen atom of the aspartate extending away from the iron. The imidazole ring of His146, which might form a hydrogen bond with one hydrogen atom of the water ligand, was also included. Hydrogen atoms were added with the aid of CHARMM22.[69] Subsequently, the positions of the hydrogen atoms were optimized by energy minimization, while the coordinates of all non-hydrogen atoms were fixed. To avoid artifacts during the optimization procedure, the atomic partial charge of the iron was reduced to the value +1.20 and the charge on the aspartate oxygen atom directly bound to the iron was increased to the value −0.36, as compared to its value in the CHARMM forcefield, which is −0.76. The atomic charges on azide were chosen as −0.5 for the nitrogen closest to the iron and −0.25 for the other two. As a result of this optimization, one hydrogen atom of the water ligand makes a hydrogen bond with the nitrogen atom of His146 (H–N distance of 2.17 Å), while the other hydrogen atom extends towards the edge of the five-membered ring of the indole system. The distances from this hydrogen atom to the two nearest carbon atoms of Trp163 are 2.31 and 2.50 Å. The quantum-chemical computation was performed after removal of the His146. The energy of the MLACπ interaction, calculated as the bonding energy of the metal complex (iron with its coordinated ligands) with the indole ring system of tryptophan, amounted to 10.09 kcal mol^{-1}. This interaction energy is comparable to a strong hydrogen bond.

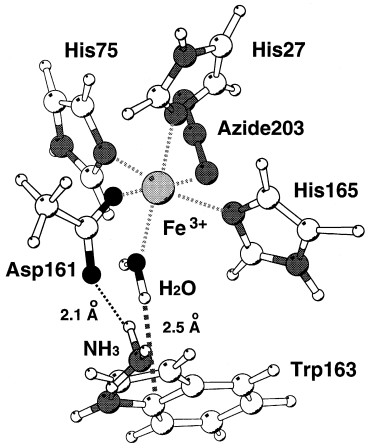

Figure 3. Model of active site of FeSOD (PDB 1avm/A) used in DFT calculation. The iron ligands were modeled from the side chains of the amino acids His27, His75, His165, azide, Asp161, and H_2O1 of the first coordination shell, using PBD coordinates as explained in text. The histidines were modeled by imidazole rings, the Asp161 was replaced by acetate. The aromatic side chain of Trp163 was modeled as an indole ring system, the backbone NH group of Trp163 was replaced by ammonia. The energy of the MLACπ interaction calculated as the bonding energy of the metal complex (iron with its coordinated ligands) and the indole ring of Trp163 was 10.09 kcal mol^{-1}.

Alcohol dehydrogenase: The structure of horse liver alcohol dehydrogenase (LADH) with PDB code 1adc/B contains CPAD (5-β-D-ribofuranosylpicolinamide adenine dinucleotide), an isosteric analogue of nicotinamide adenine dinucleotide (NAD), the cofactor in native LADH.[54] CPAD exhibits competitive inhibition of LADH with respect to NAD and binds to LADH in a very similar way to NAD. Alcohol that binds to the LADH–CPAD system is not oxidized, and the LAHD–CPAD–alcohol complex is a potentially useful model system for studying the process of alcohol binding and dehydrogenation. In the crystal structure of the LAHD–CPAD–alcohol complex,[54] solved with a resolution of 2.7 Å,

0947-6539/00/0621-3940 $ 17.50+.50/0 Chem. Eur. J. 2000, 6, No. 21

zinc(II) is tetracoordinate. Three of the ligands are amino acids: two cysteines (Cys46 and Cys174) and one histidine (His67). The fourth ligand is the bound ethanol (Figure 2D). Close to the metal is the aromatic ring of Phe93. The distance d_0 from the zinc atom to the center of the phenyl ring is 5.45 Å, with an angle θ_0 of 20.5°. The Phe93 is in close contact with the coordinated ethanol. The hydrogen atoms of ethanol were modeled into this structure; the positions of the hydrogen atoms of the ethanol -CH_2- group are available in the PDB structure (the hydrogen atoms of the ethanol methyl group are also added in the structure shown in Figure 2D). The distance from the ethanol -CH_2- group hydrogen atom closest to the center of the phenyl ring is 3.19 Å, and to the closest carbon atom of the phenyl ring 2.64 Å.

In other structures of alcohol dehydrogenases that we found while searching for the MLACπ interaction, there are interactions between different ligands coordinated to the zinc atom and Phe93 (Table 1). In the 2ohx/A structure,[57] there is an interaction of the coordinated His67 with Phe93. In the ADH structures 1axg/D[56] and 1ldy/B,[55] there are interactions between Phe93 and two coordinated ligands: His67 and trifluoroethanol (ETF) or cyclohexylformamide (CXF), respectively. In the ADH structure 1axe/B,[56] there is the mutation Phe93Trp. Trp93 is involved in cation – π interactions with the coordinated ligands His67 and ETF. The distances associated with cation – π interactions with Trp93 are shorter, and probably stronger than the interactions with Phe93. It was found experimentally that the mutation from Phe93 to Trp93 increases the tunneling of hydrogen in the hydride transfer reaction from alcohol to NAD.[56] The question is whether there is a connection between a stronger cation – π interaction and an increase in hydrogen tunneling. In the ADH crystal structure (1adc/B), ethanol engages in an MLACπ interaction with Phe93, which may be needed to place the second hydrogen atom at the carbon C1 of ethanol in a position suitable for hydrogen transfer to NAD^+, such that a replacement of Phe93 by Trp93 would increase the hydrogen tunneling. As a consequence, the cation – π interaction of the alcohol with Phe93 may play a role in the reaction mechanism of the enzyme ADH.

Conclusion

A novel type of interaction is proposed. In this metal ligand aromatic cation – π interaction (MLACπ), a ligand coordinated to a metal cation interacts with an aromatic component. The metal lends positive charge to the ligand, which in turn gains cationic character to interact strongly with aromatic moieties. We searched systematically in the PDB, using conservative geometric criteria, to find such interactions between ligands coordinated to a metal and aromatic entities. It turned out that this type of interaction is ubiquitous in metalloproteins. Examples were found for each of the three aromatic residues searched for: phenylalanine, tryptophan, and tyrosine. Practically all metals commonly occurring in enzymes can participate in such interactions. The ligands involved in the MLACπ interaction can be amino acids and water, as well as different substrates and inhibitors. The indole ring system of tryptophan possesses two aromatic entities: a six- and a five-membered ring. Both were observed to participate in the MLACπ interaction, and they can even do this simultaneously. From the atomic separations, we can conclude that, of the aromatic amino acids, tryptophan produces the strongest MLACπ interactions, and that, among the ligands, water produces the strongest interactions.

We found a number of typical examples of this interaction in the PDB. Four structures in which we found the new type of cation – π interaction were selected in order to study it in more detail. For the enzyme superoxide dismutase, we used quantum-chemical computations to ascertain that Trp163 has an interaction energy with the ligands coordinated to the iron center of 10.09 kcal mol^{-1}. In some cases we have indications that this specific interaction may be relevant for enzyme function.

Acknowledgement

We would like to thank Dr. Matthias G. Ullmann for useful discussions. This work was supported by the Serbian Ministry of Science and Technology (grant No. 02E09) and by the Deutsche Forschungsgemeinschaft SFB 498 Project A5, SFB 312 Project D7, the GRK 80/2 and the GRK 268. S.Z. was supported by a DAAD fellowship.

[1] D. A. Dougherty, *Science* **1996**, *271*, 163 – 168.
[2] J. C. Ma, D. A. Dougherty, *Chem. Rev.* **1997**, *97*, 1303 – 1324.
[3] S. K. Burley, G. A. Petsko, *FEBS Lett.* **1986**, *203*, 139 – 143.
[4] J. P. Gallivan, D. A. Dougherty, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 9459 – 9464.
[5] J. Wouters, *Protein Sci.* **1998**, *7*, 2472 – 2475.
[6] J. W. Caldwell, P. A. Kollman, *J. Am. Chem. Soc.* **1995**, *117*, 4177 – 4178.
[7] R. A. Krumpf, D. A. Dougherty, *Science* **1993**, *261*, 1708 – 1710.
[8] S. Mecozzi, A. P. West Jr., D. A. Dougherty, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 10566 – 10571.
[9] S. Mecozzi, A. P. West Jr., D. A. Dougherty, *J. Am. Chem. Soc*. **1996**, *118*, 2307 – 2308.
[10] P. C. Kearney, L. S. Mizoue, R. A. Kumpf, J. E. Forman, A. McCurdy, D. A. Dougherty, *J. Am. Chem. Soc.* **1993**, *115*, 9907 – 9919.
[11] R. C. Dunbar, S. J. Klippenstein, J. Hrusak, D. Stocking, H. Schwarz, *J. Am. Chem. Soc.* **1996**, *118*, 5277 – 5283.
[12] K. S. Kim, J. Y. Lee, S. J. Lee, T.-K. Ha, D. H. Kim, *J. Am. Chem. Soc*. **1994**, *116*, 7399 – 7400.
[13] J. Y. Lee, S. J. Lee, H. S. Choi, S. J. Cho, K. S. Kim, T.-K. Ha, *Chem. Phys. Lett*. **1995**, *232*, 67 – 71.
[14] C. A. Deakyne, M. Meot-Ner (Mautner), *J. Am. Chem. Soc*. **1985**, *107*, 469 – 474.
[15] J. Mavri, J. Koller, D. Hadzi, *J. Mol. Struct. (Theochem)* **1993**, *283*, 305 – 312.
[16] H. Basch, W. J. Stevens, *J. Mol. Struct. (Theochem)* **1995**, *338*, 303 – 315.
[17] E. Cubero, F. J. Luque, M. Orozco, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 5976 – 5980.
[18] W. Zhong, J. P. Gallivan, Y. Zhang, L. Li, H. A. Lester, D. A. Dougherty, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 12088 – 12093.
[19] S. D. Zarić, *Chem. Phys. Lett.* **1999**, *311*, 77 – 80.
[20] S. D. Zarić, *Chem. Phys.*, in press.
[21] Y.-G. Gao, H. Robinson, J. H. van Boom, A. H.-J. Wang, *Biophys. J.* **1995**, *69*, 559 – 568.
[22] L. McFail-Isom, X. Shui, L. D. Williams, *Biochemistry* **1998**, *37*, 17105 – 17111.
[23] X. Shui, L. McFail-Isom, G. G. Hu, L. D. Williams, *Biochemistry* **1998**, *37*, 8341 – 8355.

  0947-6539/00/0621-3941 $ 17.50+.50/0

[24] M. M. Yamashita, L. Wesson, G. G. Eisenman, D. Eisenberg, *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 5648 – 5652.
[25] J. A. Hunt, C. A. Fierke, *J. Biol. Chem.* **1997**, *33*, 20364 – 20372.
[26] J. A. Hunt, M. Ahmed, C. A. Fierke, *Biochemistry* **1999**, *38*, 9054 – 9062.
[27] M. Schmidt, C. Scherk, O. Iakovleva, H. F. Nolting, B. Meier, F. Parak, *Inorg. Chim. Acta* **1998**, *275 – 276*, 65 – 72.
[28] A. D. Becke, *J. Chem. Phys.* **1993**, *98*, 5648 – 5652.
[29] C. Lee, W. Yang, R. G. Parr, *Phys. Rev. B* **1988**, *37*, 785 – 789.
[30] Gaussian 98, Revision A.6, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery, Jr., R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. Cammi, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Cui, Q., K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. Cioslowski, J. V. Ortiz, B. B. Stefanov, G. Liu, A.Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, C. Gonzalez, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, J. L. Andres, C. Gonzalez, M. Head-Gordon, E. S. Replogle, and J. A. Pople, Gaussian, Inc., Pittsburgh PA **1998**.
[31] A. W. Ehlers, G. Frenking, *J. Am. Chem. Soc*. **1994**, *116*, 1514 – 1520.
[32] S. Zarić, M. Couty, M. B. Hall, *J. Am. Chem. Soc.* **1997**, *119*, 2885 – 2888.
[33] S. Zarić, M. B. Hall, *J. Phys. Chem.* **1997**, *101*, 4646 – 4652.
[34] Z. Lin, M. B. Hall, *Coord. Chem. Rev.* **1994**, *135/136*, 845 – 879.
[35] P. E. M. Siegbahn, *J. Am. Chem. Soc*. **1996**, *118*, 1487 – 1496.
[36] A. Ghosh, D. F. Bocian, *J. Phys. Chem.* **1996**, *100*, 6363 – 6367.
[37] S. Niu, M. B. Hall, *J. Phys. Chem. A* **1997**, *101*, 1360 – 1365.
[38] S. Niu, M. B. Hall, *J. Am. Chem. Soc.* **1997**, *119*, 3077 – 3086.
[39] J. L. C. Thomas, M. B. Hall, *Organometallics* **1997**, *16*, 2318 – 2324.
[40] S. Zarić, M. B. Hall, *J. Phys. Chem.* **1998**, *102*, 1963 – 1964.
[41] J. L. C. Thomas, C. W. Bauschlicher Jr., M. B. Hall, *J. Phys. Chem.* **1997**, *101*, 8530 – 8539.
[42] D. L. Strout, S. Zarić, S. Niu, M. B. Hall, *J. Am. Chem. Soc.* **1996**, *118*, 6068 – 6069.
[43] S. Niu, M. B. Hall, *J. Am. Chem. Soc*. **1998**, *120*, 6169 – 6170.
[44] S. Niu, S. Zarić, D. L. Strout, C. A. Bayse, M. B. Hall, *Organometallics* **1998**, *17*, 5139 – 5147.
[45] P. E. M. Siegbahn, R. H. Crabtree, *J. Am. Chem.Soc*. **1997**, *119*, 3103 – 3113.
[46] D. H. Jones, A. S. Hinman, T. Zigler, *Inorg. Chem*. **1993**, *32*, 2092 – 2095.
[47] C. Rovira, P. Ballone, M. Parrinelo, *Chem. Phys. Lett.* **1997**, *271*, 247 – 250.
[48] C. Rovira, K. Kunc, J. Hutter, P. Ballone, M. Parrinelo, *Int. J. Quant. Chem.* **1998**, *69*, 31 – 35.
[49] T. Zigler, *Can. J. Chem*. **1995**, *73*, 743 – 761.
[50] C. Rovira, K. Kunc, J. Hutter, P. Ballone, M. Parrinelo, *J. Phys. Chem. A* **1997**, *101*, 8914 – 8925.
[51] X. J. Tan, H. L. Jiang, W. L. Zhu, K. X. Chen, R. Y. Ji, *J. Chem. Soc. Perkin Trans.* **1999**, 107 – 111.
[52] P. C. Miklis, R. Ditchield, T. A. Spencer, *J. Am. Chem. Soc*. **1998**, *120*, 10482—10489.
[53] J. B. Nickolas, B. P. Hay, D. A. Dixon, *J. Phys. Chem. A* **1999**, *103*, 1394 – 1400.
[54] H. Li, W. H. Hallows, J. S. Punzi, W. Pankiewicz, K. A. Watanabe, B. M. Goldstein, *Biochemistry* **1994**, *33*, 11734 – 11744.
[55] H. Cho, S. Ramaswamy, V. Plapp, *Biochemistry* **1997**, *36*, 382 – 389.
[56] B. J. Banson, T. D. Colby, J. K. Chin, B. M. Goldstein, J. P. Klinman, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 12797 – 12802.
[57] F. Colonna-Cesari, D. Perahia, N. Karplus, H. Eklund, C. I. Branden, O. Tapia, *J. Biol. Chem.* **1986**, *261*, 15273 – 15280.
[58] M. S. Lah, M. M. Dixon, K. A. Pattridge, W. C. Stallings, J. A. Fee, M. L. Ludwig, *Biochemistry* **1995**, *34*, 1646 – 1660.
[59] R. A. Edwards, M. M. Whitteker, J. W. Whitteker, G. B. Jameson, E. N. Baker, *J. Am. Chem. Soc.* **1998**, *120*, 9684 – 9685.
[60] Y. Guan, M. J. Hickey, G. E. O. Borgstahl, R. A. Hallewell, J. R. Lepock, D. O'Connor, Y. Hsien, H. S. Nick, D. N. Silverman, J. A. Tainer, *Biochemistry* **1998**, *37*, 4722 – 4730.
[61] M. Schmidt, B. Meier, F. Parak, *J. Biol. Inorg. Chem.* **1996**, *1*, 532 – 541.
[62] L. Bellsolell, J. Prieto, L. Serrano, M. Coll, *J. Mol. Biol.* **1994**, *238*, 489 – 495.
[63] J. M. Guss, E. A. Merritt, R. P. Phizackerley, H. C. Freeman, *J. Mol. Biol.* **1996**, *262*, 686 – 705.
[64] A. M. Nersissian, C. Immoos, M. G. Hill, P. J. Hart, G. Williams, R. G. Herrmann, J. S. Valentine, *Protein Sci.* **1998**, *7*, 1915 – 1929.
[65] T. Dudev, J. A. Cowan, C. Lim, *J. Am. Chem. Soc.* **1999**, *121*, 7665 – 7673.
[66] T. Hunter, K. Ikebukuro, W. H. Bannister, J. V. Bannister, G. J. Hunter, *Biochemistry* **1997**, *36*, 4925 – 4933.
[67] F. Yamakura, R. L. Rardin, G. A. Petsko, D. Ringe, B. Y. Hiraoka, K. Nakayama, T. Fujimura, H. Taka, K. Murayama, *Eur. J. Biochem.* **1998**, *235*, 49 – 56.
[68] J. Li, C. L. Fisher, R. Konecny, D. Bashford, L. Noodleman, *Inorg. Chem.* **1999**, *38*, 929 – 939.
[69] A. D. MacKerell, Jr., D. Bashford, M. Bellot, R. L. Dunbrack, Jr., J. D. Evanseck, M. J. Field, S. Fischer, J. Gao, H. Guo, S. Ha, D. Joseph-McCarthy, L. Kuchnir, K. Kuczera, F. T. K. Lau, C. Mattos, S. Michnick, T. Ngo, D. T. Nguyen, B. Prodholm, W. E. Reiher III, B. Roux, M. Schlenkrich, J. C. Smith, R. Stote, J. Straub, M. Watanabe, J. Wiórkiewicz-Kuczera, D. Yin, M. Karplus, *J. Phys. Chem.* **1998**, *102*, 3586 – 3616.
[70] A. Messerschmidt, R. Ladenstein, R. Huber, M. Bolognesi, L. Avigliano, R. Petruzzelli, A. Rossi, A. Finazzi-Agro, *J. Mol. Biol.* **1992**, *224*, 179 – 205.
[71] Y. Lindqvist, W. Huang, G. Schneider, J. Shanklin, *EMBO J.* **1996**, *15*, 4081 – 4092.
[72] A. Volbeda, A. Lahm, F. Sakiyama, D. Suck, *EMBO J.* **1991**, *10*, 1607 – 1618.
[73] J. C. Spurlino, A. M. Smallwood, D. D. Carlton, T. M. Banks, K. J. Vavra, J. S. Johnson, R. S. Cook, J. Falvo, R. C. Wahl, T. A. Pulvino, J. J. Wendloski, D. L. Smith, *Proteins Struct. Funct. Genet.* **1994**, *19*, 98 – 109.
[74] N. P. Pavletich, C. O. Pabo, *Science* **1993**, *261*, 1701 – 1707.
[75] B. Hazes, K. A. Magnus, C. Bonaventura, J. Bonaventura, Z. Dauter, K. H. Kalk, W. G. J. Hol, *Protein Sci.* **1993**, *2*, 597 – 619.
[76] F. Grams, M. Crimmin, L. Hinnes, P. Huxley, M. Pieper, H. Tschesche, W. Bode, *Biochemistry* **1995**, *34*, 14012 – 14020.
[77] W. Stark, R. A. Pauptit, K. S. Wilson, J. N. Jansonius, *Eur. J. Biochem.* **1992**, *207*, 781 – 791.
[78] R. L. Olsen, A. Dessen, D. Gupta, S. Sabesan, J. C. Sacchettini, C. F. Brewer, *Biochemistry* **1997**, *36*, 15073 – 15080.
[79] A. Houdusse, M. L. Love, M. N. Dominguez, Z. Grabarek, C. Cohen, *Structure* **1997**, *5*, 1695 – 1711.

Received: February 18, 2000 [F2304]

  0947-6539/00/0621-3942 $ 17.50+.50/0