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FOR THE RECORD

Protein recognition of ammonium cations using side-chain aromatics: A structural variation for secondary ammonium ligands

ANDREW R.C. RAINE,[1] CHIEN-CHIH YANG,[1] LEONARD C. PACKMAN,[1] SCOTT A. WHITE,[2] F. SCOTT MATHEWS,[2] AND NIGEL S. SCRUTTON[1,3]

[1] Centre for Molecular Recognition, Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW United Kingdom
[2] Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, Missouri 63110
[3] Department of Biochemistry, University of Leicester, Adrian Building, University Road, Leicester LE1 7RH United Kingdom

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Abstract: A model for the structure of dimethylamine dehydrogenase was generated using the crystal coordinates of trimethylamine dehydrogenase. Substrate is bound in trimethylamine dehydrogenase by cation-π bonding, but modeling of dimethylamine dehydrogenase suggests that secondary amines are bound by a mixture of cation-π and conventional hydrogen bonding. In dimethylamine dehydrogenase, binding is orientationally more specific and distinct from those proteins that bind tertiary and quaternary amine groups.

Keywords: ammonium ligands; flavoprotein; organic cation-π bonding; methylamine dehydrogenases

Because of their widespread occurrence in natural and synthetic bioactive molecules, the recognition of substituted ammonium ligands by proteins is of general interest. The three-dimensional structures of acetylcholinesterase (Sussman et al., 1991) and the McPC603 myeloma protein (Davis & Metzger, 1983), which bind acetylcholine and phosphorylcholine, respectively, illustrate that the quaternary ammonium moieties of the bound ligands are associated with aromatic side chains. This unconventional bonding between substituted ammonium ligands and π-donors is also seen in the clustering reactions of NH_4^+ and $MeNH_3^+$ with benzene derivatives (Deakyne & Meot-Ner, 1985) that show interaction energies ranging from 10 to 22 kcal mol^{-1}. Ab initio calculations and experimental data demonstrate that, for the purely chemical systems, the interaction is weakly polar, i.e., there is no π-donation into the bond (Deakyne & Meot-Ner, 1985). Amino-aromatic interactions also occur *within* protein molecules. A geometric analysis of 33 refined protein structures has demonstrated that positively charged or $\delta(+)$ side-chain amino groups show a statistical preference for making van der Waals' contact with the $\delta(-)$ π-electrons within 6 Å of the centroids of phenylalanine, tyrosine, and tryptophan residues (Burley & Petsko, 1986). The stabilization energies that amino-aromatic interactions contribute in the biological context are expected to be less than those seen for the purely chemical systems. The reason stems from the fact that stabilization in proteins is likely to be contributed from different and less aromatic residues than in the chemical clustering reactions, and is also affected by the physical properties of the protein interior and geometrical constraints. The wealth of information in the protein structure database has enabled an analysis of side-chain interactions with aromatics in protein structures to be undertaken. Unfortunately, the paucity of structural information for substituted ammonium ligand–protein interactions precludes a similar analysis for small molecule–protein recognition. Given the widespread occurrence and use of substituted bioactive amines, from a physiological and pharmacological viewpoint, this lack of information is disappointing.

Acetylcholinesterase and the McPC603 myeloma protein provide a structural framework for modeling and designing quaternary ammonium binding sites. Structural models are also required for tertiary and secondary ammonium ligands because many bioactive amines contain these functionalities. We have sought to find models for these ligands by studying the binding sites of proteins that bind the simplest ligands in this category, viz trimethylamine and dimethylamine, found, respectively, in trimethylamine dehydrogenase and dimethylamine dehydrogenase. Previous work performed by one of us on crystalline trimethylamine dehydrogenase soaked in the presence of the

Reprint requests to: N.S. Scrutton, Department of Biochemistry, University of Leicester, Adrian Building, University Road, Leicester LE1 7RH, United Kingdom; e-mail: NS101@mole.bio.cam.ac.uk.

substrate inhibitor tetramethylammonium chloride or the substrate trimethylamine demonstrated that these tertiary and quaternary ammonium ligands are accommodated in an aromatic "bowl" comprising three residues Tyr-60, Trp-264, and Trp-355 (Bellamy et al., 1989; Fig. 1), a finding that is in accord with those of acetylcholinesterase and the McPC603 myeloma protein. Dimethylamine dehydrogenase is highly related to trimethylamine dehydrogenase, but is specific for secondary amine substrates; trimethylamine dehydrogenase can be reduced by dimethylamine, but much less effectively than with the natural substrate trimethylamine. We conjectured that the binding site for dimethylamine in dimethylamine dehydrogenase would be altered in the substrate-binding aromatic bowl and that identification of the structural change would provide a model for the binding of secondary ammonium ligands in other natural and engineered proteins/peptides. To investigate this supposition, we undertook the primary structure determination of dimethylamine dehydrogenase and established its close relationship (63% identical) with trimethylamine dehydrogenase (Yang et al., 1995). On the basis of the sequence identity, we conclude that the structures of di- and trimethylamine dehydrogenase are homologous. Using the determined sequence, we have built a model for dimethylamine dehydrogenase using the refined crystallographic coordinates of trimethylamine dehydrogenase (Lim et al., 1986; unpubl. results) to identify the structural change that directs the binding of dimethylamine (Kinemage 1).

The sequences of tri- and dimethylamine dehydrogenases are 63% identical but, within the active site region, the identity is almost perfect. In contrast, sequence conservation is lowest in those residues that comprise the subunit interface of the enzyme dimer (43% identical). In the cell, this no doubt reflects the need for the subunits of dimethylamine dehydrogenase to recognize "self" rather than subunits of trimethylamine dehydrogenase. Residues in the core of the eightfold β/α barrel (Raine et al., 1994) are also highly conserved as are those forming the putative electron tunneling pathways from flavin to 4Fe-4S (Wilson et al., 1995). Within the active site, those residues involved in the chemistry of demethylation are totally conserved (Fig. 2). These include the cysteine residue that forms a covalent link to the C6 position of the flavin, the tentatively assigned active site tyrosine base, a histidine residue required for the decay of a covalent intermediate (Rohlfs & Hille, 1994), and an arginine residue thought to stabilize developing negative charge on the N1 and C2 carbonyl of FMN during catalysis (Kinemage 1). The only difference between the two active sites is the exchange of Tyr-60 in trimethylamine dehydrogenase for a glutamine residue in dimethylamine dehydrogenase (Kinemage 1). Assuming the dimethylamine to be bound as the ammonium cation, we have been able to position dimethylamine in the active site of our model using the coordinates for tetramethylammonium chloride taken from the crystallographic analysis of trimethylamine dehydrogenase soaked in the presence of this quaternary ammonium cation inhibitor. In our model, Gln-60 is ideally positioned to make a conventional hydrogen bond from the side-chain amide carbonyl to the N-H hydrogen of dimethylamine (Kinemage 1). The remaining methyl groups are positioned to make cation-π ionic interactions with the two tryptophan residues in much the same way as they do in trimethylamine dehydrogenase. The model indicates that a single amino acid substitution is responsible for the switch in substrate specificity in these two amine dehydrogenases. In dimethylamine dehydrogenase, the hydrogen bond between Gln-60 and substrate holds the substrate in a specific orientation to enable the catalytic machinery to gain access to one of the substrate methyls—the site of oxidation (Kinemage 1). In trimethylamine dehydrogenase, there is no requirement for orienting specifically the substrate in the aromatic bowl—the substrate possesses C3 rotational symmetry and, in principle, any of the methyl substituents can be oxidized. Substrate presentation in dimethylamine dehydrogenase is therefore more orientationally specific, and this specificity is acquired through the provision of a conventional hydrogen bond to substrate.

We predict that the structural variation seen for the recognition of secondary ammonium ligands in dimethylamine dehydrogenase will also be a feature of other protein molecules that associate with secondary ammonium groups. Following a simple mutagenic change, the possibility arises for proteins that associate with secondary ammonium ligands that they can be persuaded to accept tertiary or quaternary ligands and vice versa. This would be an exciting prospect given the widespread occurrence of natural and synthetic bio-active organic ammonium ligands.

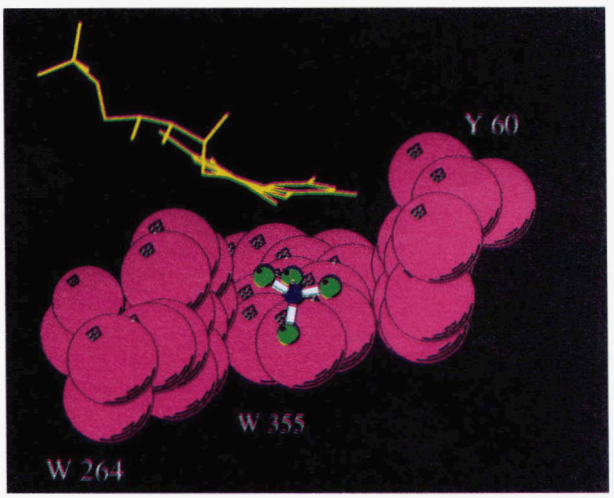

Fig. 1. The substrate-binding "aromatic bowl" in trimethylamine dehydrogenase. The coordinates used are those of the 2.4 Å structure of trimethylamine dehydrogenase (Lim et al., 1986; S.A. White & F.S. Mathews, unpubl. Brookhaven code 1TMD) and inhibitor coordinates (tetramethylammonium) were also taken from a 2.4 Å study (S.A. White & F.S. Mathews, unpubl. results). Flavin mononucleotide is depicted in yellow, tetramethylammonium in green and blue, and the three aromatic residues comprising the substrate-binding bowl in purple. The graphics representation was generated using MOLSCRIPT software (Kraulis, 1991).

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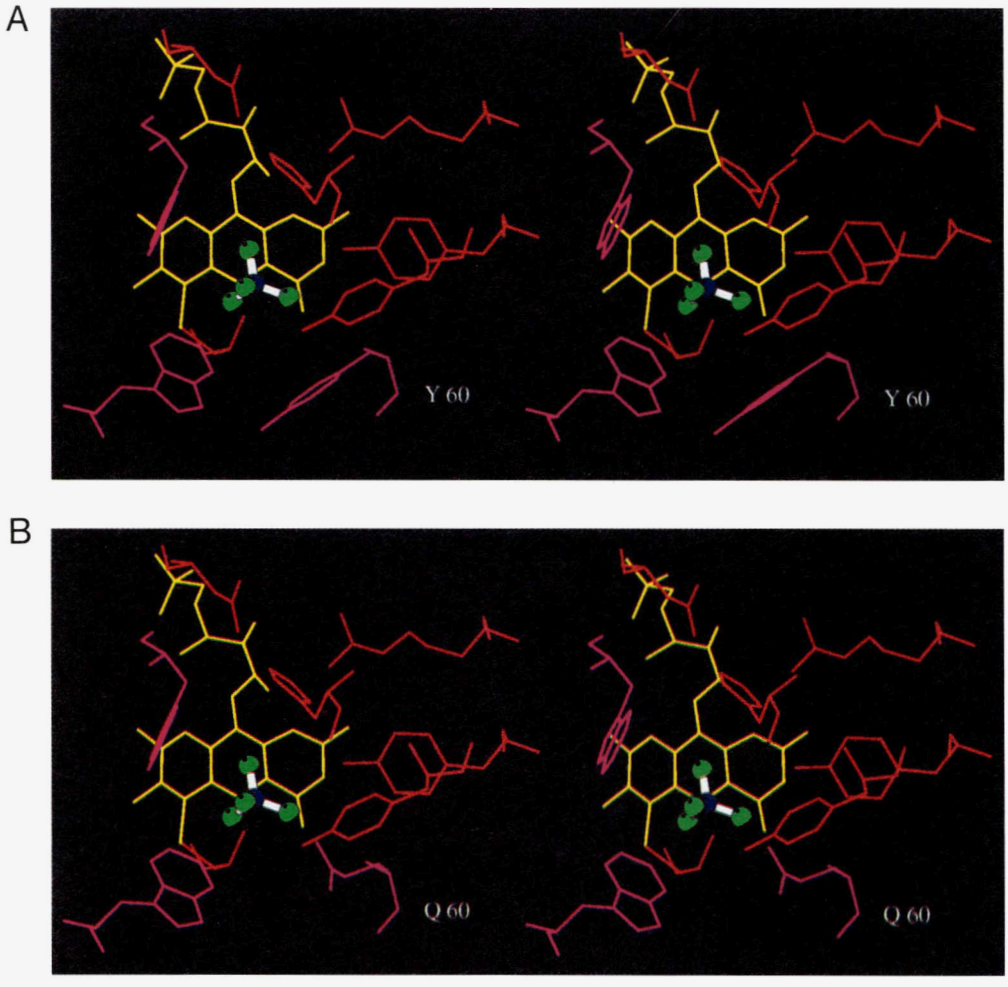

Fig. 2. Stereo views of the active sites of (**A**) trimethylamine dehydrogenase and (**B**) the modeled dimethylamine dehydrogenase. The dimethylamine dehydrogenase model was constructed using the automatic point mutation tool in the software package QUANTA (Molecular Simulations, Inc.). QUANTA preserves the positions of all atoms that are common to both original and mutated side chains and new atoms are placed in an extended conformation. Because the side chain of residue-60 is the only difference in the active sites of trimethylamine and dimethylamine dehydrogenases, the dimethylamine dehydrogenase model was minimized with only the N-atom of tetramethylammonium included and allowing only the side chain of Gln-60 to move. The minimization was performed using XPLOR (Brünger, 1992) and the PARAM 19 force field. In the minimized model, the χ_1, χ_2, and χ_3 of Gln-60 are equal to 56°, 179°, and 89°, consistent with a relaxed side-chain conformation. Gln-60 does not make unfavorable van der Waals contacts with neighboring residues. The coordinates used are those detailed in the legend of Figure 1. Flavin mononucleotide is depicted in yellow, the substrate-binding residues in purple, and the remaining active site residues in red. The inhibitor is shown in green and blue and for reference it is also shown in the dimethylamine dehydrogenase model at the equivalent location to that determined crystallographically for trimethylamine dehydrogenase. Modeling suggests that residue Gln-60 in dimethylamine dehydrogenase is suitably positioned to make a hydrogen bond to the N-H of dimethylamine. The distance between the $O_{\epsilon 1}$ of Gln-60 and the N of tetramethylammonium in the minimized model of dimethylamine dehydrogenase is 3.1 Å. This would correspond to a hydrogen bond length of approximately 2.1 Å, which agrees well with a statistical analysis of hydrogen bond lengths taken from high-resolution protein structural data (Baker & Hubbard, 1984). In our model, the angles defined by the atoms C_δ, $O\epsilon_1$ of Gln-60 and the nitrogen of tetramethylammonium is 154°. This would correspond to a C-O···H hydrogen bond angle between Gln-60 and dimethylamine of approximately 150°, which also agrees well with statistical data taken from high-resolution protein structures (Baker & Hubbard, 1984). The graphics representation was generated using MOLSCRIPT software (Kraulis, 1991).

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