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Identification of a potential metal cation–π binding site in the structure of a thermophilic *Bacillus stearothermophilus* triosephosphate isomerase mutant

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A potential metal cation–π interaction between a sodium cation (Na^+) and the indole ring of a tryptophan residue was detected in the crystallographic structure (2 Å) of the thermophilic *Bacillus stearothermophilus* triosephosphate isomerase H12N/K13G mutant (bTIMmut). The cation–π binding site is located near the surface of the protein, the alkali metal ion facing the benzo ring of Trp9. The presence of Phe21 and Glu17 close to Trp9 could indicate an additional role for those residues in the stability of the sodium–indole interaction. The sodium cation lies in a position that is occupied by CE and NZ of Lys13 in the wild-type structure.

1. Introduction

Evidence has accumulated during the past two decades that cation–π interactions play an important role in biological molecular recognition and enzyme catalysis (Gallivan & Dougherty, 1999; Choi *et al.*, 1998; Ma & Dougherty, 1997; Dougherty, 1996; Flanagan *et al.*, 1995; Luhmer *et al.*, 1994). The cation–π interaction is thought to be responsible for the binding of the quaternary ammonium group of acetylcholine (ACh) to a tryptophan residue in the nicotinic receptor (Zhong *et al.*, 1998; Axelsen, 1994). Cation–π interactions have been considered in diverse other systems such as voltage-gated ion (K^+) channels, the cyclase enzymes of steroid biosynthesis and enzymes that catalyze methylation reactions involving *S*-adenosylmethionine (Ma & Dougherty, 1997). The interaction between a sodium cation and a tryptophan residue has also recently been identified in a 2 Å crystallographic structure of hen egg-white lysozyme (Wouters, 1998). A more conclusive example is from rhodanese, where Trp287 is in close contact with a Cs^+ ion (Kooystra *et al.*, 1988).

The present work suggests the presence of similar alkali-metal cation–π binding site involving the lateral chain of a Trp residue in the structure of a thermophilic *B. stearothermophilus* triosephosphate isomerase mutant (bTIMmut).

2. Material and methods

Details of the crystallization and data collection have been presented elsewhere (Alvarez *et al.*, 1999). The protein (7 mg ml^{-1}, 5 m*M* MES pH 6.5, 1 m*M* NaN_3, 2 m*M* 2-phosphoglycolate) crystallizes against a 25%(*w*/*v*) PEG 4000 solution in 100 m*M* sodium acetate buffer pH 5.0 as a homodimer. Both subunits define the asymetric unit of a primitive orthorhombic crystal lattice belonging to space group $P2_12_12$, with unit-cell parameters a = 78.13, b = 107.91, c = 70.98 Å. The structure of bTIMmut complexed with the competitive inhibitor 2-phosphoglycolate was refined to a model with good geometry and crystallographic quality (PDB code 2btm; Table 1).

The electron density [8.5σ in an unbiased ($F_o - F_c$) electron-density map] corresponding to the proposed cation–π binding site was initially assigned to a water molecule (water 50 and 75 in 2btm). In the present work, the structure was refined with an Na^+ ion replacing the water molecule [*X-PLOR* parameters for the Na^+: mass = 22.9898, non-bonded 0.01 2.067 0.01 2.067 (Lennard–Jones parameters E in kcal mol^{-1}, and σ)]. In the final model, the temperature structure factor for the cation is 39.9 and 44.0 Å^2 for molecules *A* and *B*, respectively.

3. Results and discussion

In the course of our study on the thermostability of triosephosphate isomerases (TIM), the effect of a double mutation in the wild-type TIM from the thermophilic bacterium *B. stearothermophilus* was analysed and the crystal structure of this TIM mutant was refined at 2.4 Å resolution. The double mutation, H12N and K13G (His12 is mutated to an aspargine and a glycine residue replaces Lys13) affects residues present in loop 1, located at the dimer interface of the protein and seems to play a role in the thermostability of the protein (Alvarez *et al.*, 1999).

The protein crystallizes as a dimer. In both monomers, a residual density peak [8.5σ in an unbiased ($F_o - F_c$) electron-density map] appears in the difference Fourier close to Trp9

Table 1
Data collection and refinement statistics.

Crystallographic refinement	
Resolution (Å)	30.0–2.40
Final R	0.176
R_{free}	0.221
Theoretical total No. of reflections in resolution range	24110 (100.0%)
No. of unobserved reflections (no entry or $\|F\| = 0$)	1378 (5.7%)
Total No. of reflections used	22732 (94.3%)
No. of reflections in working set	21623 (89.7%)
No. of reflections in test set	1109 (4.6%)
R.m.s.d. for ideal value	
Bonded main-chain atoms (Å)	1.342
Bonded side-chain atoms (Å)	2.430
Angle main-chain atoms (°)	2.080
Angle side-chain atoms (°)	3.417

(Fig. 1). It was originally assigned as a water molecule but finally, based on geometric criteria, was assigned as a sodium cation (see §2).

The potential cation–π binding site in bTIMmut is near the surface of the protein, a fact also noticed for the lysozyme sodium–Trp complex (Wouters, 1998). This could be related to the great desolvation energy of the cation, the lateral chain of the tryptophan competing with aqueous solvation. The environment (Glu17, Phe21, Leu237) close to Trp9 in the cation-binding site (Fig. 1, Table 2) plays an additional role in the stability of the sodium–indole interaction.

Interestingly, the cation–π site is located in the region where mutations were introduced. In fact, the cation lies in a position that is occupied by the lateral chain (CE/NZ) of Lys13 in the wild-type structure (Delboni *et al.*, 1995; PDB code 1btm). Extensive general statistical analysis of side-chain interactions in well resolved protein structures showed that Tyr and Trp are over-represented as nearest neighbours of both Lys and Arg (Gallivan & Dougherty, 1999). In particular, cation–π interactions are found to be common among structures in the Protein Data Bank. It is clearly demonstrated that, when a cationic side chain (Lys or Arg) is near an aromatic side chain (Phe, Tyr or Trp), the geometry is biased toward one that would experience a favorable cation–π interaction. Among the aromatics, a strong bias towards Trp is clear. The lysine–tryptophan interaction often involves a CH_2 group (CE) of Lys, rather than its terminal (NZ) NH_3^+. This is also the case in the structure of the wild-type bTIM: CE carries a substantial positive charge (like a methyl of a NMe_4^+ group) and its contact (Table 2) with the face of an aromatic will be a cation–π interaction. Thus, in the mutated (H12N/K13G) bTIM structure, the sodium cation occupies a cation-stabilizing position filled by a Lys side chain in the wild type.

Table 2
Experimental geometry of the cation (M^+)–π interaction (in Å).

B_{eq} values of the atom of the protein involved in the interaction are given in italic (in Å^2). Centr defines the centroid of a benzo ring.

	bTIMmut	Na	Wild type	NZ_13	CE_13
Chain *A*					
CD2_9	*20.9*	4.36	*13.4*	5.68	4.52
CE2_9	*22.9*	4.40	*16.0*	5.44	4.57
CZ2_9	*21.5*	4.60	*16.8*	5.43	4.83
CH2_9	*23.9*	4.71	*18.6*	5.65	5.01
CZ3_9	*23.0*	4.67	*13.1*	5.88	4.96
CE3_9	*21.1*	4.51	*9.02*	5.91	4.75
Centr_9		4.36		5.68	4.52
OE1_17	*45.9*	5.36	*38.3*	4.53	4.94
O_17	*24.0*	5.06	*23.1*	4.98	5.47
O_237	*27.3*	3.58	*16.4*	2.68	3.26
O_12	*20.8*	5.03	*39.3*	5.93	4.93
CD2_21	*19.1*	4.29	*8.6*	4.06	4.57
Centr_21		5.38		5.29	5.57
Chain *B*					
CD2_9	*19.5*	4.250	*18.6*	5.33	3.90
CE2_9	*20.2*	4.32	*18.9*	4.93	3.67
CZ2_9	*19.9*	4.46	*19.3*	5.43	3.90
CH2_9	*20.5*	4.48	*15.3*	5.00	4.28
CZ3_9	*17.7*	4.40	*14.8*	5.80	4.48
CE3_9	*16.3*	4.29	I18.5	5.76	4.31
Centr_9		4.14		5.20	3.86
OE1_17	*43.1*	5.26	*38.1*	4.91	5.70
O_17	*31.6*	4.84	*17.6*	5.15	5.30
O_237	*27.6*	3.97	*10.3*	3.64	4.46
O_12	*27.1*	4.75	*34.8*	5.96	5.64
CD2_21	*22.2*	5.56	*13.3*	3.58	3.69
Centr_21		5.61		4.83	4.70

The geometry (Table 2) of the cation–π interaction observed in the bTIMmut structure (mean aromatic C—Na^+ distance = 4.54 and 4.36 Å for chains *A* and *B*, respectively; distance between the aromatic plane and the cation = 4.36 and 4.14 Å for chains *A* and *B*, respectively) is consistent with the geometry observed in the crystal structure of small molecules (Wouters, 1998). In particular, for 44 cation–π interactions involving a sodium ion deposited in the Cambridge Structural Database (Allen & Kennard, 1993), the distances between the aromatic C atom and Na^+ range from 2.83 to 4.95 Å. Confirmation of cation–π interaction between alkali-metal ions and the indole ring were recently discussed for synthetic compounds (De Wall *et al.*, 1999). Their experimental results show that in the case of their synthetic receptors, the pyrrolo rather than the benzo ring is the π donor.

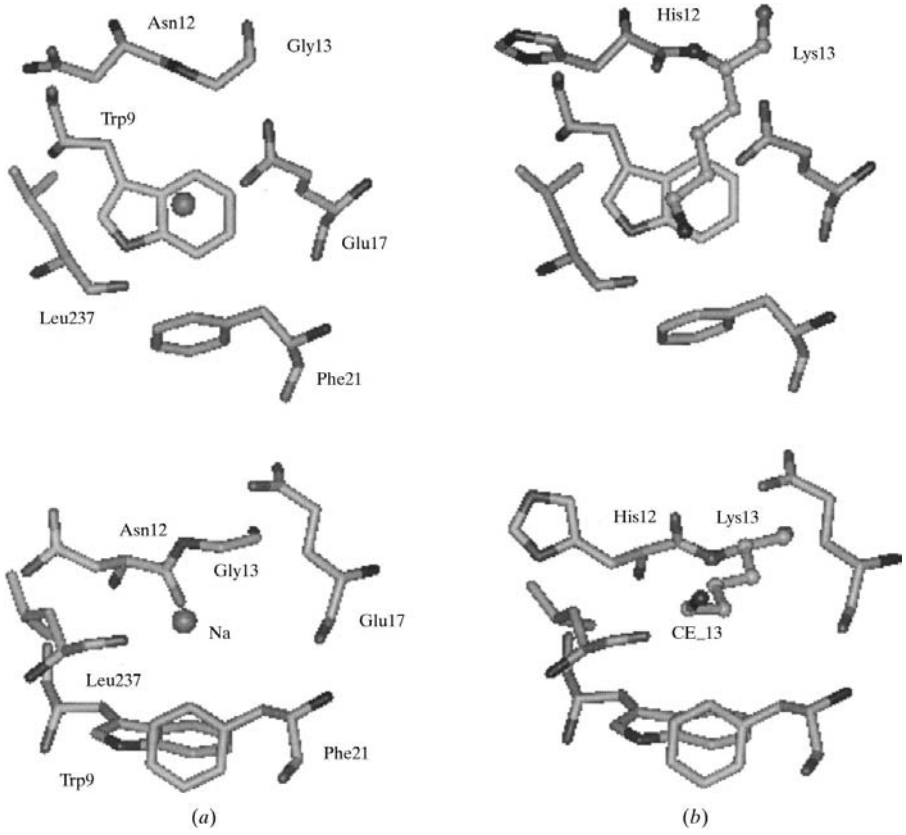

Figure 1
Two perpendicular views (top and bottom) of the environment of Trp9 in the crystal structure of the thermophilic *B. stearothermophilus* TIM: (*a*) H12N/K13G double-mutant structure and (*b*) wild-type structures. The geometry of the cation–π interaction is very similar in both monomers, so only one site (monomer *A*) is discussed. Note the different conformation at the main chain for residues 12–13.

Based on the experimental structure of the sodium cation–π interaction observed in bTIMmut, a theoretical optimization procedure has been established by assessing the influence of a series of parameters (partial charge, dielectric constant and polarizability) on the geometry of cation–π complexes. Because *ab initio* (molecular orbital, density functional theory) methods rapidly become limited by the size of the system to be studied, empirical (force-field) methods were pursued. Existing force fields were adapted by modulating the polarizability contribution in the Lennard–Jones function and fine-tuning the dielectric constant used during the geometry optimization. The resulting procedure was applied with success to the simulation of the cation–π interaction observed in bTIMmut and will be presented in detail elsewhere (Wouters, 2000). This work points to the important role that both electrostatic and polarization terms play in the description of the cation–π interaction.

4. Conclusions and perspectives

Indications for the existence of tryptophan–cation interaction in the crystallographic structure of a thermophilic *B. stearothermophilus* triosephosphate isomerase (H12N/K13G) mutant are presented: a sodium cation faces the lateral chain of Trp9. The distance between the ion and the indole ring (4.1–4.3 Å) is compatible with earlier reports (Wouters, 1998), although it is longer than the mean distance observed in small-molecule structures (cation centroid of the aromatic distance around 3.4 Å). Definite confirmation of the presence of a metal cation–π binding site could be obtained by replacing the Na^+ ion by an Rb^+ ion (Di Cera *et al.*, 1995) or a Cs^+ ion (Kooystra *et al.*, 1988) cation.

It is expected that if procedures to search for cation–π sites are generalized to other crystallographic structures, it is likely that more examples of metal cation–π interactions could be found. Development of screening methods to locate metal ion binding sites in proteins therefore seems promising.

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