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Intermolecular Contacts in Various Crystal Forms Related to the Open and Closed Conformational States of the Catalytic Subunit of cAMP-Dependent Protein Kinase

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Abstract

All hitherto solved crystal structures of the catalytic (C) subunit of cAMP-dependent protein kinase can be classified into two groups, those with a closed and those with an open conformation of the ATP-binding lobe. The molecules with the closed conformation are all related by a crystallographic 2_1 axis that connects them into an infinite-chain motif. The motif has only one large contact region that involves many residues, several of them in the ATP-binding lobe, embedded in an extensive network of water molecules. The dominant feature of this region is the hydrophobic interaction between Trp196 and Arg133, Arg134. This motif has been found so far in three different crystal forms, two correspond to ternary enzyme–inhibitor–ATP complexes with mammalian and recombinant C, and one to a binary enzyme–inhibitor complex with recombinant C. The open conformation has been found in two closely related crystal structures, both of cubic symmetry, of the apoenzyme and a binary complex of the mammalian catalytic subunit. In this cubic structure of the binary complex, the hydrogen-bonded intramolecular contacts between Arg18 of the inhibitor and the ATP-binding lobe of the binary and ternary complexes of the recombinant enzyme are missing due to a strong hydrophobic intermolecular contact involving the diiodinated Tyr7. In solution, no crystal contacts prevent these hydrogen bonds involving Arg18 from forming so that it is likely that the binary complex with Tyr7 of the peptide inhibitor iodinated or not, can assume the closed conformation in solution. While the closed structure very likely represents a stable conformation in solution, there is no evidence to suggest that the open conformation represents a unique stable conformational state of the enzyme in solution.

1. Introduction

The catalytic Cα subunit of cAMP-dependent protein kinase (cAPK) is one of the smallest and simplest members of the protein kinase family. It is also the first protein kinase crystal structure to be solved, initially at 2.7 Å (Knighton, Zheng, Ten Eyck, Ashford *et al.*, 1991; Knighton, Zheng, Ten Eyck, Xuong *et al.*, 1991) and later at 2.0 Å resolution (Knighton *et al.*, 1993). The initial structure was a binary 1:1 complex of the mouse recombinant enzyme and a 20 amino-acid peptide inhibitor PKI(5–24) consisting of residues 5–24 from the heat-stable protein kinase inhibitor (PKI) (Walsh, Perkins & Krebs, 1968).

Several different crystal structures of the recombinant as well as the mammalian enzyme have now been solved. Most of them are summarized in Table 1. Each crystal structure has been given a three-letter mnemonic symbol that is used throughout the text and in the Tables. The first letter refers to the type of complex (A for apo, B for binary, T for ternary), the second letter to the crystal form (M for monoclinic, O for orthorhombic, H for hexagonal, C for cubic) and the third letter to the source of the enzyme (R for recombinant, M for mammalian). For instance BOR refers to the orthorhombic (O) crystal structure of the binary (B) complex of the recombinant (R) enzyme. The mammalian porcine enzyme is myristylated at the amino-terminal Gly in contrast to the recombinant mouse enzyme. Mainly due to the low resolution of the X-ray data, only the molecular-replacement solutions of the crystal structures of the cubic apoenzyme (ACM) and the hexagonal ternary complex (THM) were obtained. Tyr7 of the peptide inhibitor is iodinated at positions 3 and 5 of the phenyl ring in the binary complex of the cubic structure (BCM).

The crystal structures in Table 1 can be classified into two groups, those with a closed and those with a relatively open conformation of the ATP-binding lobe of the enzyme. The latter was found in crystals of the apoenzyme (ACM) and its binary complex (BCM) with the iodinated inhibitor. The transition between the closed and open conformation can be accomplished by a concerted motion of helix *B*, helix *C* and the β-structure in the ATP-binding lobe, which shift 4.4, 1.3 and 2.8 Å, and rotate 12.2, 11.8

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Table 1. *Summary of six crystal structures containing cAMP-dependent protein kinase*

V_m is the Matthews number. Standard deviations (e.s.d.'s) for bond distances and angles are given. MR indicates that only the molecular-replacement solution exists. The conformation of the small lobe is open (O) or closed (C).

Symbol	Space group	Cell constants $a\ b\ c$ (Å) β (°)	Resolution (Å)	R value (%)	V_m (Å^3 Da^{-1})	E.s.d.'s (Å, °)	Conformation	Reference
BOR	$P2_12_12_1$	73.8 75.8 81.0	2.0	18.6	2.6	0.013/2.3	C	Knighton *et al.* (1993)
TOR*	$P2_12_12_1$	73.6 76.3 80.6	2.2	19.9	2.6	0.013/2.2	C	Zheng *et al.* (1993)
THM	$P6_122$	80.2 80.2 297.2	3.4	33.8†	3.2	MR	C	Karlsson *et al.* (1993)
BCM	$P4_132$	171.5	2.9	19.2	4.9	0.022/4.3	O	Karlsson *et al.* (1993)
ACM	$P4_132$	168.7	3.9	31.0‡	4.9	MR	O	Karlsson *et al.* (1993)
TMM§	$C2$¶	107.6 80.6 110.1 88.6	2.0	19.4		0.037/5.1	C	Bossemeyer *et al.* (1993)

* TOR and BOR are isomorphous.
† 7.0–3.6 Å, cell constants refined.
‡ 7.0–3.9 Å, cell constant refined.
§ Also 107.5 80.6 109.4 Å 88.8° given as cell dimensions.
¶ Coordinates given a non-standard space group.

and 15.4°, respectively (Table 3 in Karlsson, Zheng, Xuong, Taylor & Sowadski, 1993). One of our specific aims is to find out whether there is any support for the hypothesis that the open form represents a major conformational state of the apoenzyme. Alternatively, can the open conformation be a result of the molecular packing? Specifically, has the iodination of the peptide influenced the molecular packing?

To this end we have chosen to study the molecular contacts (summarized in Table 2) of the six crystal structures given in Table 1. The first five structures have one protein molecule per asymmetric unit and were obtained under identical crystallization conditions. From this study have emerged some interesting observations concerning the crystal environment of the closed and open forms.

2. The crystallographic environment of the closed form

The hexagonal and orthorhombic crystal forms in Table 1 represent the closed conformation. The enzyme complexes in these structures are related by a crystallographic 2_1 axis which connects the molecules strongly into an infinite-chain motif with a repetitive length of 80.2–81.0 Å. The 2_1 axis is in the c direction in the orthorhombic crystal form and in the a or b direction in the hexagonal crystal form.

This chain motif in the crystal structure also exists in a monoclinic form of the ternary complex of the mammalian enzyme (which also represents the closed conformation) (Bossemeyer, Engh, Kinzel, Ponstingl & Huber, 1993) with the molecules related by the crystallographic 2_1 axis (b = 80.6 Å). The monoclinic structure contains two independent parallel-chain motifs separated approximately by (1/2, 0.336, 0) with the chains running in the same direction. In the orthorhombic structures, BOR and TOR, the two crystallographically related chain motifs are, of course, antiparallel.

Molecular packing within the motif

The motif contains one massive contact region (Table 2, Figs. 1*a*, 1*b*) involving 48 intermolecular contacts (<4 Å) between 54 residue atoms (the description refers to BOR since it has the highest resolution of 2.0 Å). There are just five hydrogen bonds (contacts between N and O atoms <3.3 Å are considered as hydrogen bonds here) between neighboring residues in adjacent molecules, all common to the ternary structure (TOR). The presence of 25 water molecules in the contact region, where each water forms at least two hydrogen bonds, adds another 65 hydrogen bonds, of which 20 are between waters and 45 are between waters and residues. In the ternary structure only 17 waters were found of which 11 are in common within 0.96 Å with the binary structure. Otherwise, the contact regions in the binary and ternary complexes are essentially identical. A dominant feature in this contact region is the largely hydrophobic interaction between Trp196 and Arg133, Arg134 (Fig. 1*b*).

Molecular packing between motifs

The molecular contacts between chain motifs are much less extensive. In the orthorhombic forms (Table 2), the chains are connected by two different contacts in the a direction, one with 13 the other with four van der Waals contacts. The contact between molecules in the b direction is weak and may not be reliable due to large atomic temperature factors. Thr348 forms three contacts with OE2 Glu13 in BOR but forms just one contact with CG Glu17 in TOR.

In the hexagonal structure (THM) the chain motifs are held together by four distinct but very weak contacts (Table 2). It is interesting that the hydrogen bond between NZ Lys63 and its symmetry-related atom is bisected by the twofold axis. This information is obtained from the molecular-replacement solution of THM. Efforts to obtain a

structure analysis of THM have proved difficult due to disorder in various parts of the structure. For instance, helix *A* is not seen before residue 23, and no phosphate group of ATP has been found in difference Fourier syntheses; the adenosyl group is, however, clearly seen but its orientation cannot be accurately determined. The inner part of the large domain is well defined but some regions on its surface, which are relatively well defined in TOR, show disorder. Natural flexibility of the protein may be reflected in this disorder possibly resulting from weak intermolecular contacts.

The possibility of a solid crystal contact in one direction (the chain direction) and weaker contacts in the other direction(s) might have been inferred from variations in cell dimensions between different crystals. For the orthorhombic form, a strong contact in the *c* direction and a weak contact in the *b* direction are reflected in the recording of cell dimensions of more than 20 crystals used for data collection after being soaked in the presence of low concentrations of various heavy-metal salts. The *a*, *b* and *c* axes varied within the ranges 73.00–73.96, 75.36–77.61 and 80.44–80.89 Å,* respectively (D. Knighton, personal communication).

In the hexagonal form, the long 6_1 axis was found to vary (Knighton, Zheng, Ten Eyck, Ashford *et al.*, 1991). In the monoclinic form, TMM (Table 1), two values for the *c* axis were reported, 109.4 and 110.1 Å.

3. An analysis of the apoenzyme structure

In order to make a comparison between the two cubic crystal structures of the mammalian apoenzyme ACM and the inhibitor complex BCM (Table 1) as accurate as possible without a full solution of the 3.9 Å resolution structure of the apoenzyme, the molecular packing was analyzed first by refining the six molecular-replacement parameters together with the cell constant by the least-squares method (Karlsson, unpublished programs) using the enzyme model of BCM. This showed $a = 168.7$ Å at $R = 0.31$ for all 5231 non-zero data in the resolution range 7–3.94 Å. The difference in orientation was less than 1°. The molecular centers defined as the average position of all non-H atoms excluding the peptide inhibitor were (0.2858, 0.4305, 0.2198) in BCM and (0.2923, 0.4372, 0.2222) in ACM.

Table 2. *Summary of residues involved in crystal contacts with intermolecular distances* <4 Å

No water molecules are included in this Table. *L* is the identification number for the contact region. Residues of the peptide inhibitor are preceded by *P*. *S* is the symmetry operation on the first molecule. The direction of symmetry axis is indicated if not evident. *N* is the number of distances that involve *M* atoms. *H* is the number of hydrogen bonds.

Structure symbol	*L*	Contacts between molecule 1 and molecule 2	*S*	*N*/*M*	*H*
BOR.	1	(86,89,93,349):(*P*5,*P*8 *P*9), (189-192):	$2_1 c$	48/54	5
TOR		(*P*11 13), 192:*P*16, 194:328, 196:(133 134)		40/44*	8*
BOR	2	(63 67):(81 82)	$2_1 a$	11/13	1
TOR	2	(63 67):(81 82,*P*23)	$2_1 a$	23/19	4
TOR	3	254:298 300	$2_1 a$	4/5	0
TOR	4	17:348	$2_1 b$	1/2	0
BOR	5	13:(348,35)	$2_1 b$	2/3	0
THM	1	As TOR, $L = 1$	$2_1 a$	39/45	8
THM	2	260:345	6_5	5/5	1
THM	3	141:341	6_5	2/3	0
THM	4	299:36	6_5	1/2	0
THM	5	63 64:63 64	2	3/4	1
BCM	1	(135,137):317, 299:(307 309), (139–141,144):(176 177,315)	3	32/35	3
BCM	2	(65 68,122,124,176):(257,259–263,266,270)	3	37/37	5
BCM	3	27:31, 31:190, 86:(194,196), (192 194):(345 346,349)	2	38/32	6
BCM	4	(51,330):(*P*8–*P*9), (233,*P*7):327, (258–259,*P*6):328, (*P*17 *P*18): (*P*12,*P*14,*P*16)	2	55/44	6

* TOR.

In order to estimate differences in conformation of the ATP-binding (small) lobe between ACM and BCM, the rigid-body parameters of five molecular segments, which were considered to move as rigid entities, were refined using the new cell constant. The five segments, all in the small lobe, were residues 31–41, the β-structure (residues 42–75 and 107–120), helix *B* (residues 76–82), helix *C* (residues 84–97) and the C-terminal segment (residues 343–350). The β-structure and the C-terminal part, which lies on top of the β-structure, both moved 0.70 Å relative to the large lobe [the old cell constant gave a larger shift (Karlsson *et al.*, 1993)]. These were the only movements that were significantly larger than their standard deviations. Due to the small relative movements of the segments, it was considered correct not to have removed any side chains during refinement.

4. The molecular packing in the cubic crystal forms

In the crystals of cubic symmetry, the molecules are held together by a three-dimensional network with

* *Note added in proof*: Only one crystal of the ternary complex (TOR) with a larger *b* axis ($a = 73.1$, $b = 80.1$, $c = 80.5$ Å) was found and the structure has been solved to 2.4 Å resolution. The crystal contacts remain essentially the same as in TOR except those few that connect the molecules in the *b*-axis direction (contacts 3 and 4, Table 2). Contact 4 has disappeared but in contact 3, NZ Lys254—O Ala298, where a water molecule bridges the N and O atoms, the N—O distance decreases from 3.79 to 2.58 Å to form a direct hydrogen bond. In addition, there is a conformational change of 180° about the Lys254 C_δ—C_γ bond. A water molecule still forms hydrogen bonds to each of the N and O atoms. This crystal contact is likely to be influenced by metal ions due to the solvent-exposed amino group of Lys254. This could explain why no useful heavy-atom derivative could be obtained by soaking.

very extensive intermolecular contacts (Table 2) with at least twice as many protein atoms and hydrogen bonds as in the orthorhombic structures of the ternary and binary complexes. The structure can be described as trimers (Fig. 2*a*) with 45.27 (BCM) and 45.31 Å (ACM) between the molecular centers. Neighboring trimers are held together by two types of contacts with twofold symmetry (BCM, contacts 3 and 4 in Table 2). The distances between the molecular centra related by the twofold axes are 41.36 and 38.97 Å in BCM. The corresponding distances for the apoenzyme are 41.28 and 35.30 Å. The twofold axis that corresponds to the second shorter distances and to contact 4, passes through the crystallographic threefold (trimer) axis (shown in Fig. 2*a*) at (3/8, 3/8, 3/8) bringing two trimers on top of each other. When the iodinated peptide inhibitor, which dominates contact 4, is removed to obtain the apoenzyme, one of the trimers is shifted along the threefold axis and moves 3.7 Å closer to the other trimer.

We assume from the above results that the crystal packing as well as the open conformations of ACM and BCM are essentially the same.

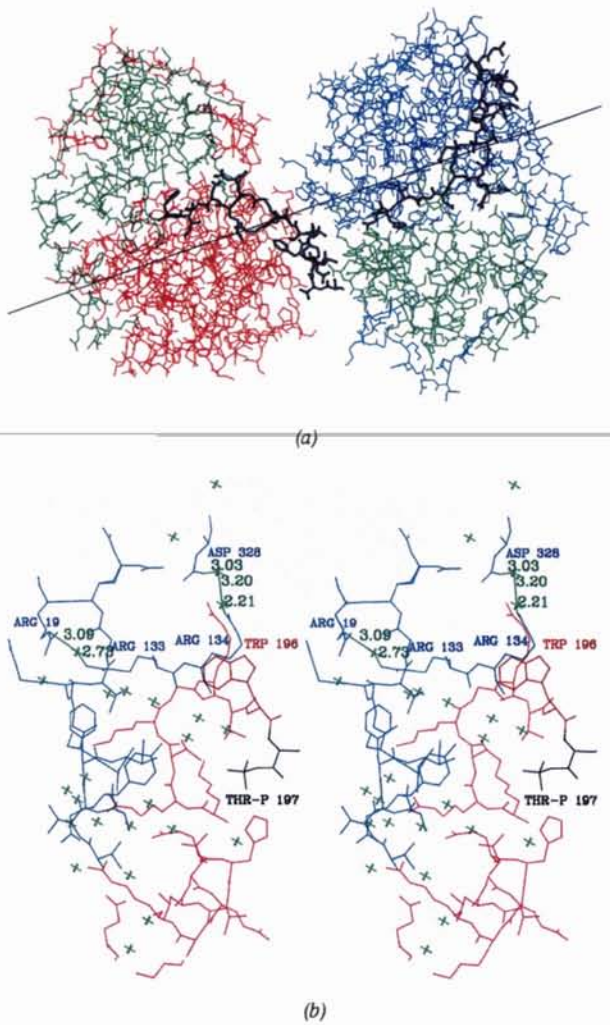


Fig. 1. Intermolecular contacts in the orthorhombic crystal forms. (*a*) The molecular packing in the chain motif with the 2_1 axis indicated. Peptide inhibitor is in black, large domains are in red and blue and small domains (residues 7–128) in green. Trp196 of the right molecule (blue) is seen close to the molecular interface and the 2_1 axis. (*b*) The hydrophobic contact region with Trp196 (red), Arg133 (blue), Arg134 (blue) and water molecules (green crosses).

Iodination

Iodination of tyrosine is a modification that causes a significant increase in its size and hydrophobicity making it more prone to a hydrophobic environment. Consequently, BCM contains the strongest hydrophobic intermolecular interaction in all six crystal forms, where the phenyl rings of Tyr7 PKI(5–24) and Phe327 are stacked together (Fig. 2*b*). Residues 327–330 in this otherwise poorly defined region (318–342) were, possibly because of this interaction, rather well located. Direct substitution of the iodines in BOR would cause overcrowding. There is less than 20° difference in all dihedral angles involving the $C\alpha$ and $C\beta$ atoms of Tyr7 PKI(5–24) between BCM and BOR but a large conformational change at Thr6 of the peptide that shifts the positions of first two peptide residues, Thr5 and Thr6. The conformation angles of Thr6 PKI(5–24) in BCM (BOR) are $\psi = 173°$ ($-66°$), $\varphi = 150°$ ($-41°$).

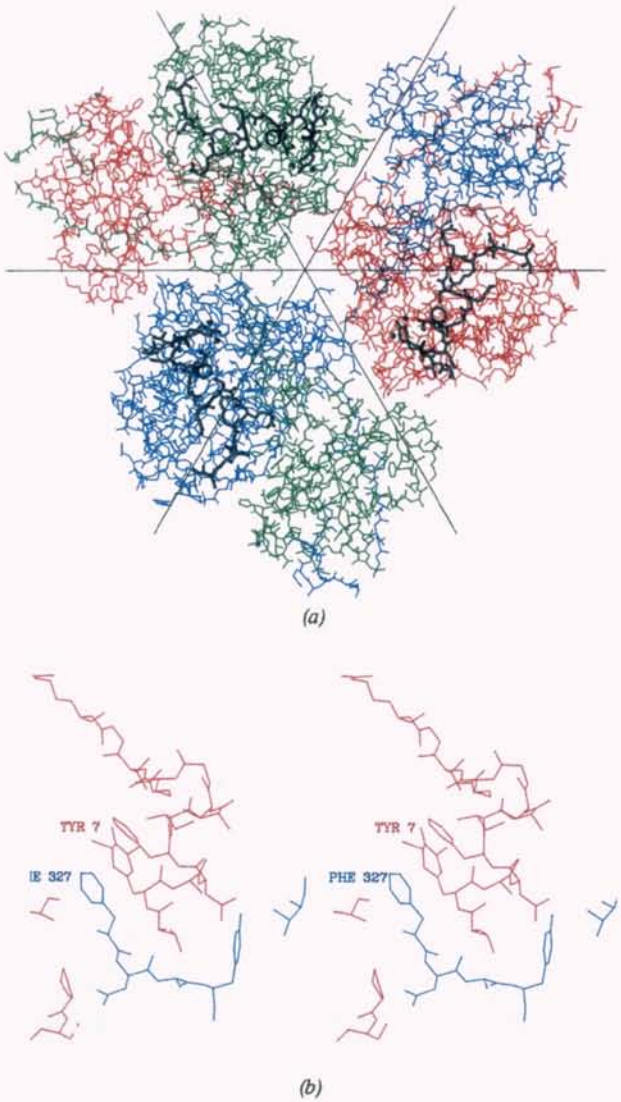
5. Intramolecular hydrogen bonding related to the transition between open and closed conformations

The flexibility of the small lobe that allows the transition between the open and closed conformations must not be prevented by intramolecular hydrogen bonding with the exception of the hydrogen bonding to the peptide inhibitor. The flexible parts of the small lobe, helices *B* and *C*, the β-structure and residues 318–350, the latter part lying on top of the β-structure, have few hydrogen bonds in common with the large lobe or between themselves that could prevent this flexibility. The most important contacts between N and O atoms in BOR, TOR and BCM, some of which could be hydrogen bonds, are contained in Table 3. Contacts in TMM corresponding to those of TOR in Table 3 are very similar.

The hydrogen bonds between the guanidine group of Arg18 PKI(5–24) and the side-chain O atoms of the small-domain residues Thr51, Asp328 and Tyr330 were found to be missing in BCM (Table 3). In BOR, two possible positions of the guanidine group of Arg18 PKI(5–24) were found and distances in parentheses in Table 3 correspond to a position of 34% occupancy. The hydrogen bond between NE2 His87 and the phosphate group of Thr197 in BOR and TOR also needs to be broken in order to

accomplish this transition. The distance between NE2 His87 and O Gly186 (BCM) is 3.41 Å. This is too long for a direct hydrogen bond, so the two atoms could be linked over water molecules. Thus, hydrogen bonds to NE2 His87 must be considered less likely to stabilize or act as a lock for the open conformation in contrast to the closed conformation.

The alternative N-atom assignment of the imidazole ring of His87 is not likely, since the transition would require a 180° rotation of the ring and a disruption of the hydrogen bonds to both ND1 and NE2 His87 (Table 3, values in parentheses).



(a)

(b)

Fig. 2. Intermolecular contacts in the cubic crystal form. (*a*) The trimer perpendicular to the threefold axis with iodinated peptide in black. The larger domains are in blue, green and red and their corresponding smaller domains are in green, red and blue. The indicated twofold axes, perpendicular to the threefold axis, bring two trimers on top of each other. The second dimer is not drawn. (*b*) Part of the hydrophobic contact region between the two trimers related by the twofold axes indicated in (*a*). The figure shows the phenyl rings of Phe327 (blue molecule) and Tyr7 of the peptide stacked together.

Table 3. *Selected intramolecular distances* (Å) (*distances* >4.5 Å *or not given*) *between* N *and* O *atoms in BOR, TOR and BCM of the flexible parts of the small domain* (*β-structure, helix B, helix C, residues* 318–350) *and the rest of the structure*

Thp197 indicates phosphorylated Thr197. Distances in parentheses for BCM refer to the alternative assignment of the ND1 and NE2 atoms in His87 and for BOR to the alternative position of the side chain of Arg18 PKI(5–24) is indicated as ArgP18.

			BOR		TOR	BCM
NZ	Lys72 —OE	Glu91	2.58		3.63	3.66
NZ	Lys72 —OD2	Asp184	3.35		3.66	4.49
NE2	His87 —O	Gly186	—		—	3.41 (2.80)
NE2	His87 —OE3	Thp197	2.74		2.79	—
ND1	His87 —NE2	Gln84	2.69		2.78	2.73 (3.73)
ND2	Asn90 —O	Ala188	3.50		3.50	3.48
OE2	Glu91 —N	Phe185	3.57		3.76	3.37
NE	ArgP18—OE1	Glu127	2.83	(3.63)	3.37	2.93
NE	ArgP18—OE2	Glu127	3.55	—	2.82	2.87
NH1	ArgP18—OE1	Glu127	—	(2.37)	—	3.62
NH2	ArgP18—OE2	Glu127	2.87	—	2.94	3.32
NH2	ArgP18—O	Thr51	3.71	—	2.93	—
NH1	ArgP18—O	Thr51	2.89	—	3.14	—
NH1	ArgP18—OH	Tyr330	3.16	—	3.50	—
NE	ArgP18—OH	Tyr330	3.76	(3.06)	3.41	—
NH2	ArgP18—OD1	Asp328	—	(2.62)	—	—
NH2	ArgP18—OD2	Asp328	—	(3.34)	—	—

The hydrogen bonding involving the side chains of the invariant Lys72, Asn90 and the invariant Glu91 seem to be less rigid in BCM due to the inherent flexibility of their side chains and the possible participation of water molecules. Thus, they are less likely to be an obstacle to this transition.

6. Summary and concluding remarks

A relatively open conformation of the ATP-binding lobe has been found in cubic crystals of the apoenzyme (ACM) and its complex (BCM) with the iodinated inhibitor. The two cubic structures, although not isomorphous, are very similar and there is no large difference between the open conformations of the apoenzyme and the binary complex. The structures can best be described as trimers held together by two types of dimeric contacts. Owing to the lack of the peptide in the apoenzyme structure only one of the dimeric contacts is different.

Thus, the open conformation cannot be uniquely related to the iodination of Tyr7 of the peptide as initially suspected since the same conformation exists in the structure of the apoenzyme. One may still argue though, whether the open conformation is a product of intermolecular interactions or whether the ATP-binding lobe exists in two distinct conformations that are in equilibrium in solution, *i.e.* in the absence of crystal contacts.

The small lobe is assumed to be rather flexible since few hydrogen bonds seem to prevent its flexi-

bility. In fact in the transition from the closed to the open conformation few hydrogen bonds need to be broken (Table 3). The most important bond is between NE2 His87 and the phosphate group of Thr197. In BCM, NE2 His87 forms no hydrogen bond directly to any backbone N or O atom. In the binary complexes, this transition would require a breakage of the intramolecular hydrogen bonding between the guanidine group of Arg18 PKI(5–24) and Thr51, Asp328 and Tyr330 that may help to hold the small domain in the closed conformation. The hydrogen bonding between Arg18 and Glu127, a residue that contributes to peptide recognition (Taylor *et al.*, 1993), appears to be somewhat strengthened in BCM (Table 3).

The open form is stabilized by several intermolecular contacts. Particularly interesting is the strong hydrophobic intermolecular stacking of the phenyl rings of the iodinated Tyr7 of the peptide and Phe327 which is close to Asp328. However, no intramolecular hydrogen bond seems to be formed that could act as a lock for, or even stabilize, the open conformation. In solution, where no lattice contact prevents Arg18 binding to the small domain, the binary complex is likely to exist in the closed conformation with or without an iodinated Tyr7 of the peptide.

This study has not found any convincing evidence that the particular open conformation found in BCM and ACM can be stabilized in the absence of crystal contacts. It is likely, therefore, that other open conformational states exist in solution in addition to the one already found in the two cubic crystal structures. A very open conformation of the apoenzyme is indicated by small-angle X-ray scattering experiments (Olah, Mitchell, Sosnick, Walsh & Trewhella, 1993).

From this point of view, a solution of the hexagonal crystal structure of the cAMP-dependent enzyme from yeast (Kuret & Pflugrath, 1991) could be of interest.

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