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	Title:	STRUCTURAL MODELING OF THE CATALYTIC SUBUNIT-REGUALTORY SUBUNIT DIMERIC COMPLEX OF THE CAMP-DEPENTENT PROTEIN KINASE
	Author(s):	Chang-Shung Tung, T-10, LANL Stephen C. Gallagher, B-Division, LANL Donal A. Walsh, UC Davis Jill Trewhella, B-Division, LANL
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G A :	Froup ddress	Theoretical Biology and Biophysics Group Los Alamos National Laboratory Los Alamos, New Mexico 87545
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STRUCTURAL MODELING OF THE CATALYTIC SUBUNIT-REGULATORY SUBUNIT DIMERIC COMPLEX OF THE CAMP-DEPENDENT PROTEIN KINASE. C.S. Tung(1), S.C. Gallagher(2), D.A. Walsh(3), J. Trewhella(2). (1)Theoretical Division, LANL (2) Bioscience, LANL, Los Alamos, NM 87545, (3)Dept of Biological Chemistry, UC Davis, CA 95616.

Abstract: The cAMP-dependent protein kinase (PKA) is a multifunctional kinase that serves as a prototype for understanding second messenger signaling and protein phosphorylation. In the absence of a cAMP signal, PKA exists as a dimer of dimers, consisting of two regulatory (R) and two catalytic (C) subunits. Based on experimentally derived data (i.e., crystal structures of the R and C subunits, mutagenesis data identifying points of subunit-subunit contacts), the neutron scattering derived model subunit-subunit contacts), the neutron scattering derived model for the heterodimer (Zhao et al., 1998) and using a set of computational approaches (homoiogy modeling, Monte Carlo simulation), we have developed a high-resolution model of the RII α -C α dimer. The nature of the subunit-subunit interface was studied. Our model reveals an averaged size dimer interface (2100 Angstrom 2) that is distant from the pseudo-substrate bunding size on the C subunit. The additional contacts substrate donains size on the C substitut. In a additional contacts made by the pseudosubstrate increases the stability of the dimeric complex. Based on a set of R-C dimer structures derived using a simulated annealing approach, specific interactions (hydrogen bonds) between the two subunits were identified.

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Vedefing the regulatory subtinit: The crystal structure of the X1-80 horize Risk shows of the eAM-dependent protein latence (REGS in PDB), which is the only 3D structure of a resolution of the regulatory subsequences, we used as the integrate. The dependence of the regulatory subsequences are also as the regulator of the resolution of the regulatory subsequences are also as the regulator of PAA horizoftance and bolograms, we the target segments. Its segments has been reported to recention numbers 2425 th Gordball. This form of BU contains the product-structure are closely beening domains that the one target segments. The segments has been reported to the an defition of 2% concentrative readies and structure provide a total of elevant additional readiess. The alignment of the tanget segmences are 0.55, knows with an additional readiess. The alignment of the tanget and any sequences is form a readies of the readiest sequences are observed as the segment of the target sequences are closely been readies and the structure of the target sequences in the target sequences in the target sequences are the second on the target and the sequence is the probability of the many sequences and the sequence of the sequence is and the second on the horizon of the modernic contribution of the sequence are observed as the second on the horizon of the target analysis of the sequence are the probability of the second on the horizon of the target analysis of the sequence are the second on the target second on the horizon of the target analysis of the second of the target second on the target second of the target analysis is second on the target second of the targ date structure to deter ruct the R-C interesting the target







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Figure 3. Four different RJC betweediner structures derived from a course docking procedure. The neutron derived model of the betweediner is shown at the left.

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 $Q_1 = C_1 * (d(i)-d0)^2$ if d(i) > 8.0,

C is a constant arbitrary chosen to be 100 KonkinstAngatom² for the ion pair and 10 KonkinstAngatom² for the six residues while 60 & 8 Angatom Pe2 is a parameter to de-the positions of Rivbetween the structure and the neutron model. Pe2 is defined to be the number of reductors in the structure that are retermal to the workers model.

A second accreating is performed with the limits of the three parameters per al 1.000, 200, and 200, respectively. Only of dimer denotance restores, the case has the best versatility of them the the structure for the complex and down in Fig. 4... The atomic model of the between the the structure for the complex and down in Fig. 4... The atomic model of the between the constructed based on this chosen structure. The atomic model of the between the minimization wing AMBRR. The energy minimizate structure is down in Fig. 4...



Figure 4. Structure of the R/C heterodinser derived using a grid-sampling method. Structure of the dimer with only the C-sipha atoms is shown in 4a and the energy-minimized all-atom structure is shown in 4b.

Summary: SASMODEL (Zhao et al., 1998) was used with the the x-ray scattering data of Turning y Security is a second se



Fagure 5. The energy-minimized structure of the R/C complex (the stick structure) is superimposed with the relieved scattering-derived model (the dailed structure).

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Figure 6. Ten structures of the dimeric complex derived using an aunealing procedure involving MD simulation and energy-minimization.

Based on these energy-minimized structures, hydrogen-bonds (H-bond) between the two submits of the R-C complex were identified. Those H-bonds between the submits bonder residences are those identified to be important in the formation of the dimension complex. These residues involved in the H-bond using the main-chain carboxy corgen-er isolicated with (O) in the table 1, 1y-2 of the C-minimi forms a H-bond alternating between Glaz-299 and Sex-300 of the R-submit while Arg-194 of the C-submit forms a H-bond and summaling between Ass-139 and Law 140 (O) of the K-submit forms a

H-bood alternating between Ass-159 and Les-140 (1) of the K-exbann. One of the methods for evaluating binding strength between two maknikis in a complex is to acloubte the contact surface area, in general, the larger the contact surface area, the tronger the binding. The contact surface area is defined to the the difference between the straight the binding. The contact surface area is defined to the the difference between the straight the surface areas were exclusied using. NACCESS (Ribbard and Thornton, 1993). The interface surface areas were exclusied using. NACCESS (Ribbard and Thornton, 1993). The interface surface areas were exclusied using. NACCESS (Ribbard and Thornton, 1993). The interface surface areas were exclusied using. NACCESS (Ribbard and Thornton, 1993). The interface surface areas were exclusied using. NACCESS (Ribbard and Thornton, 1993). The interface surface areas were accessed using the domain and the C-atigning area. Surface Sci 3-333, the interface surface areas between the domain and the C-atigning area. C-submit for the surface areas is to result of the domain in the C-atigning area. C-submit for the surface areas is the surface surface areas between the domain and the C-atigning area. C-submit for the surface areas is the surface surface areas between the domain and the C-atigning area. C-submit for the surface areas is the surface surface areas between the finding based on a binding study by Huang and Taylor (1998). In their study, redices 94-169 and 263-244 were identified as primary and secondary suffices for C-subwalt binding. Our model structure of the complex (Fig. 7) blows behavior the sufficient (1-fo) (depicted in yellow) and residues 256-244 are interacting with the C-subenti (shown in cyan).

H-bonds between R/C submits





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Abstract: The cAMP-dependent protein kinase (PKA) is a multifunctional kinase that serves as a prototype for understanding second messenger signaling and protein phosphorylation. In the absence of a cAMP signal, PKA exists as a dimer of dimers, consisting of two regulatory (R) and two catalytic (C) subunits. Based on experimentally derived data (i.e., crystal structures of the R and C subunits, mutagenesis data identifying points of subunit-subunit contacts), the neutron scattering derived model for the heterodimer (Zhao et al., 1998) and using a set of computational approaches (homology modeling, Monte Carlo simulation), we have developed a high-resolution model of the **RII** α -C α dimer. The nature of the subunit-subunit interface was studied. Our model reveals an averaged size dimer interface (2100 Angstrom²) that is distant from the pseudosubstrate binding site on the C subunit. The additional contacts made by the pseudosubstrate increases the stability of the dimeric complex. Based on a set of R-C dimer structures derived using a simulated annealing approach, specific interactions (hydrogen bonds) between the two subunits were identified.

experiments that provided the structures of the PKA heterodimer and holoenzyme were done using bovine $C\alpha$ subunit. As the template for homology modeling of the structure of this C-subunit form we chose the crystal structure (1CDK in the Protein Data Bank, PDB) of the porcine $C\alpha$ subunit of PKA. The sequence of the target protein has been determined (accession number X67154, GenBank). The sequence identity between the target bovine $C\alpha$ -subunit and the porcine $C\alpha$ -subunit template is extremely high with no insertions or deletions and with the two proteins differing by only by a single conservative amino acid change (Fig. 1, upper panel). Modeling the target structure based on the template structure was therefore simple and straightforward. The porcine crystal structure is for the Csubunit PKI-peptide complex with C in its "closed" conformation. Since the binding of the R-subunit pseudosubstrate domain to the C-subunit also results in a closed conformation, this model is the best choice for constructing the R-C heterodimer. The homology modeled structure of the target C-subunit, as well as the structure of the template, are shown in the lower panel of Fig. 1.

	10	20	30	40	50	60
1cdk	GNAAAAKKGSEQES	VKEFLAKAKE	DFLKKWENP	AQNTAHLDQFI	RIKTLGTGSH	GRVMLV

x67154	GNAAAAKKGSEQES	VKEFLAKAKE	DFLKKWENP	AQNTAHLDQFI	RIKTLGTGSI	GRVMLV
	10	20	30	40	50	60
	70	80	90	100	110	120
lcdk	KHKETGNHFAMKIL	DKOKVVKLKO	IEHTLNEKR:	LOAVNFPFLV	KLEYSFKDNS	SNLYMVM
x67154	KHMETGNHYAMKIL	DKOKVVKLKO	IEHTLNEKR:	ILQAVNFPFL	KLEFSFRDNS	SNLYMVM
	70	80	90	100	110	120
	130	140	150	160	170	180
1cdk	EYVPGGEMFSHLRR	IGRFSEPHAR	FYAAQIVLTI	FEYLHSLDLIY	RDLKPENLLI	DQQGYI
			1111111111			
x67154	EYVPGGEMFSHLRR	IGRFSEPHAR	FYAAQIVLTI	PEYLHSLDLIY	RDLKPENLL	DOOGAI
	130	140	150	160	170	180
	190	200	210	220	230	240
lcdk	QVTDFGFAKRVKGR	TWTLCGTPEY	LAPEIILSKO	3YNKAVDWWA1	GVLIYEMAAG	XPPFFA
x67154	QVTDFGFAKRVKGR	TWTLCGTPEY	LAPEIILSKO	JYNKAVDWWA I	GVLIYEMAA	SYPPFFA
	190	200	210	220	230	240
	250	260	270	280	290	300
1cđk	DQPIQIYEKIVSGK	VRFPSHFSSD	LKDLLRNLL	QVDLTKRFGN	KDGVNDIKN	IKWFATT
			********	**********		
x67154	DQPIQIYEKIVSGK	VRFPSHFSSD	LKDLLRNLL	QVDLTKRFGNI	KNGVNDIKN	ikwfatt
	250	260	270	280	290	300
	310	320	330	340	350	
lcdk	DWIAIYQRKVEAPF	TPKFKGPGDT	SNFDDYEEE	EIRVSINEKC	}KEFSEF	
		********	*******			
x67154	DWIAIYQRKVEAPF	IPKFKGPGDI	SNFDDYEEE	EIRVSINEKCO	KEFSEF	
	310	320	330	340	360	



Figure 1. The sequence of the bovine C-subunit (the target) of the cAMP-dependent protein kinase is aligned with that of the porcine C-subunit (the template). The homology modeled structure of the C-subunit (the molecule on the right) and the crystal structure of the C-subunit (the molecule on the left) are shown in the lower part of the figure.

RIα subunit of the cAMP-dependent protein kinase (1RGS in PDB), which is the only 3D structure so far resolved for any PKA regulatory subunit, was used as the template. The homologous murine $\Delta(1-91)$ RIIa subunit, as used for previous neutron scattering experiments of PKA heterodimer and holoenzyme, was the target sequence. Its sequence has been reported (accession number J02935 in GenBank). This form of RII contains the pseudo-substrate and cAMP- binding domains but not the dimerization domain and thus in combination with Csubunit produces an R-C heterodimer. The target and the template sequences are 43% identical with an additional 22% conservative residue substitution. Three insertions provide a total of eleven additional residues. The alignment of the template and target sequences is shown in Fig. 2, upper panel. The homology modeled structure of the target molecule, as well the structure of the template molecule, are shown at the lower panel of Fig. 2 (right and left images). The three insertions in the target molecule are shown on the plot in dark gray. The low resolution neutron scattering-resolved structure of the heterodimer, coupled to mutagenesis studies, show that none of the target R-subunit insertions are involved in the binding of the C-subunit. The C-subunit binding surface on the R-subunit is located on the lower back side of the molecule (as depicted in Fig. 2); further conformation of this binding site is provided by this current study. Our past x-ray scattering data have shown that there is no prominent conformational difference between R in the presence and absence of cAMP, indicating that this is a suitable template structure to determine the target RII-subunit structure; upon which to then construct the R-C heterodimer.

> 20 10 30 40 50 RKVIPKDYKTMAALAKAIEKNVLFSHLDDNERSDIFDAMFPVSFIAGETVIQQGDEGDNF 1rqs j02935 RVVHPKTDEQRCRLQEACKDILLFKNLDQEQLSQVLDAMFEKIVKTDEHVIDQGDDGDNF 20 40 50 70 80 90 100 YVIDQGEMDVYVNNEWAT-SVGEG---GSFGELALIYGTPRAATVKAKTNVKLWGIDRDS 1rgs j02935 YVIERGTYDILVTKDNQTRSVGQYDNRGSFGELALMYNTPRAATIIATSEGSLWGLDRVT 80 90 100 70 110 130 140 150 160 170 YRRILMGSTLRKRKMYEEFLSKVSILESLDKWERLTVADALEPVQFEDGQKIVVQGEPGD 1ras j02935 FRRIIVKNNAKKRKMFESFIESVPLFKSLEMSERMKIVDVIGEKIYKDGERIIAOGEKAD 170 130 140 150 160 180 180 190 200 210 220 1rgs EFFIILEGSAAVLOR-----RSENEEFVEVGRLGPSDYFGEIALLMNRPRAATVVARGP 1 1 j02935 SFYIIESGEVSILIRSKTKSNKNGGNQEVEIAHCHKGQYFGELALVTNKPRAASAYGVGD 190 200 210 220 240 250 260 LKCVKLDRPRFERVLGPCSDILKRNIQQYN-SFVSL 1rgs j02935 VKCLVMDVQAFERLLGPCMDIMKRNISHYEEQLVKM 250 260 270



Figure 2. The sequence of the murine regulatory-subunit (the target) of the cAMP-dependent protein kinase is aligned with that of the bovine regulatory subunit (the template). The homology modeled structure of the R-subunit (molecule on the right) and the crystal structure of the R-subunit (molecule on the left) are shown in the lower part of the figure.

Modeling the heterodimer: Modeling the complex structure of the R/C dimer is essentially a docking problem. We have divided the docking procedure into two steps. The first being the coarse docking of the two subunits into the modeled complex derived from neutron scattering study (Zhao et al., 1998). The coarse docking involves the matching of centers–of–mass and principal–moment–axes between the two homology modeled subunits and those in the neutron model. Due to rotational symmetries, each of the subunits can be matched in four different configurations giving a total of 16 complex conformations. To reduce the number of possible complex conformations, we use the observed ion pair between Glu–143 of the R–subunit and Lys–213 of the C–subunit (Gibson et al., 1997) as a structural constraint. For each of the subunits, two configurations are having the residue that form the observed ion–pair located at the far side of the complex interaface, therefore must be discarded. This leaves 4 different conformations of the R/C complex as the potential solution (see fig. 3).



Figure 3. Four different R/C heterodimer structures derived from a coarse docking procedure. The neutron derived model of the heterodimer is shown at the left.

The second step of the docking involves a detailed search in the conformational space around the 4 heterodimer structures derived from the coarse docking procedure. Each of the subunit structure is sampled at a conformational grid associated with the six degrees of freedom (2 Angsrom grid for translational, 10 degree grid for rotational). Taking the size and shape into consideration, we will sample 3,600 configurations for the R subunit and 6,000 configurations for the C subunit, giving a total of almost 22 million conformations to be sampled for each of the four initial heterodimer structures. To speed up the calculation, we will use a reduced--coordinate representation that includes an extended atom (at C-alpha) per residue. Each of the subunits is treated as a rigid body in the docking procedure. In addition to the ion pair, six residues (Asp-141(R), Lys-247(R), Thr-195(C), Trp-196(C), Thr-197(C), Lys-217(\hat{C})) were identified to be important to the complex formation of the dimer, therefore should be physically close to the corresponding subunit. Using van der Waals exclusion (no pairwiase C-alpha distance from the two subunits should be less than 3 Angstrom) and close contact rule (the minimum distance (d(1-6))) between the close-contact residues and the corresponding subunits should be less than 12 Angstrom while the distance between the two residues that form the ion-pair (d(0)) should be less than 10 Angstrom) as constraints, the initial screening shows that the grid sampling produces no satisfying dimer structure from initial structures 1 and 2 (see Fig. 3). The same screening produces 2642 and 10631 dimer conformations from initial structures 3 and 4 respectively.

To further delineate these structures, we define three empirical parameters (Pvdw, Pc1, Pc2). Pvdw is a measure of the 6–12 vdw energy with parameters corresponding to vdw radii of 4.0 Angstrom and a depth of 0.12. Pc1 is a constraint associated with the residues in close contact upon the complex formation. Pc1 can be calculated according to:

$$Pc1 = \sum_{i} Q_{i},$$

where

 $Q_i = C_i^* (d(i)-d0)^2$ if d(i) > 8.0, = 0. if d(i) < = 8.0,

C i is a constant arbitrary chosen to be 100 Kcal/molAngstrom² for the ion pair and 10 Kcal/molAngsrom² for the six residues while d0 is 8 Angstrom. Pc2 is a parameter to describe the goodness of fit between the structure and the neutron model. Pc2 is defined to be the number of residues in the structure that are external to the neutron model.

A second screening is performed with the limits of the three parameters set at 1,000, 200., and 200. respectively. Only 7 dimer structures around the initial structure 4 are selected from the second screening. Out of these 7 dimer structures, the one has the best overall fit is chosen to be the structure for the complex and shown in Fig. 4a. The atomic model of the heterodimer is constructed based on this chosen structure. The atomic model is subjected to energy minimization using AMBER. The energy minimized structure is shown in Fig. 4b.



Figure 4. Structure of the R/C heterodimer derived using a grid—sampling method. Structure of the dimer with only the C–alpha atoms is shown in 4a and the energy—minimized all–atom structure is shown in 4b.

Summary: SASMODEL (Zhao et al., 1998) was used with the the x-ray scattering data of the R/C dimeric complex to generate a refined model structure (the dotted structure shown in Fig. 5) that reveals more information with regard to the shape of the complex. The energy minimized structure (the stick model) when docked onto this refined scattering-derived model shows a good agreement as depicted in Fig. 5.



Fogure 5. The energy-minimized structure of the R/C complex (the stick structure) is superimposed with the refiened scattering-derived model (the dotted structure).

To obtain the detailed R-C interaction, the structure of the heterodimer was allowed to relax at room temperature (300 K). This procedure is accomplished by subjecting the complex to a 100 pico second run of molecular dynamics using AMBER. During the simulation, C-alpha atoms on both subunits, except those located on the proximity of the interface were constraint to their original positions. This procedure allows for those residues at the interface to relax and sample more of the conformational space while maintaining relative orientation of the two subunits. Structures at every 10 pico second during the simulation were selected and energy-minimized. The resulting 10 energy-minimized structures are shown in Fig. 6.



Figure 6. Ten structures of the dimeric complex derived using an annealing procedure involving MD simulation and energy-minimization.

Based on these energy-minimized structures, hydrogen-bonds (H-bond) between the two subunits of the R-C complex were identified. Those H-bonds between the subunits existing in the simulation at least 80% of the time are tabulated in Table I. The boxed residues are those identified to be important in the formation of the dimeric complex. Those residues involved in the H-bond using the main-chain carbonyl oxygen are indicated with (O) in the table. Lys-23 of the C-subunit forms a H-bond alternating between Glu-299 and Ser-300 of the R-subunit while Arg-194 of the C-subunit forms a H-bond alternating between Asn-139 and Leu-140 (O) of the R-subunt.

One of the methods for evaluating binding strength between two subunits in a complex is to calculate the contact surface area. In general, the larger the contact surface area, the stronger the binding. The contact surface area is defined to be the difference between the total surface areas of the two individual subunits and the surface area of the complex. The surface areas were calculated usning NACCESS (Hubbard and Thornton, 1993). The interface surface area of the R/C complex is 2010 square Angstrom, a value compatible to the surface area of a typical protein complex (Conte et al., 1999). When the R-subunit is divided into two structurally distinct domains (A: residues 114–250; B: residues 251–383), the interface surface areas between the domain and the C-subunit are 1584 and 423 square Angstrom respectively for A and B. This result of A-domain being the C-subunit high affinity binding domain is consistent with the findings based on a binding study by Huang and Taylor (1998). In their study, residues 94–169 and 236–244 were identified as primary and secondary sufaces for C-subunit binding. Our model structure of the complex (Fig. 7) shows both residues 114–169 (depicted in yellow) and residues 236–244 are interacting with the C-subunit (shown in cyan).

H-bonds between R/C subunits

R	C	
E-143	K213	100%
K-244(0)	K-217	1.00%
К-247	N-283	1.00%
D-141	S-212	100%
N-139 L-140(0)	R-194	100%
E-299 S-300	K23	1.00%
D-281	K-28	90%
K-329	E-24	90%
к-244	E-208	80%
E-276	K-192	80%



Figure 7. The interaction of A and B domain of the Rsubunit (molecule on the right) with the C-subunit (molecule on the left).

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H-bonds between R/C subunits

R	С	
E-143	K213	100%
K-244(0)	K-217	100%
K-247	N-283	100%
D-141	S-212	100%
N-139 L-140(0)	R-194	100%
E-299 S-300	K23	100%
D-281	K-28	90%
K-329	E-24	90%
K-244	E-208	80%
E-276	K-192	80%



Figure 7. The interaction of A and B domain of the Rsubunit (molecule on the right) with the C-subunit (molecule on the left).