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Molecular modelling of co-receptor CD8 $\alpha\alpha$ and its complex with MHC class I and T-cell receptor in sea bream (*Sparus aurata*)

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Abstract T-cells are the main actors of cell-mediated immune defence; they recognize and respond to peptide antigens associated with MHC class I and class II molecules. In this paper, we investigated by molecular modelling methods in the teleost sea bream (*Sparus aurata*) the interaction among the molecules of the tertiary complex CD8/MHC-I/TCR, which determines the T-cell-mediated immunological response to foreign molecules. First, we predicted the three-dimensional structure of CD8 $\alpha\alpha$ dimer and MHC-I, and, successively, we simulated the CD8 $\alpha\alpha$ /MHC-I complex. Finally, the 3D structure of the CD8/MHC-I/TCR complex was simulated in order to investigate the possible changes that can influence TCR signalling events.

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Introduction

T-cell-mediated immunity is one of the main lines of defence that vertebrates rely on for eliminating microbial pathogens. While antibodies recognize intact antigens, T-cells distinguish foreign material from self through presentation of fragments of the antigen by the MHC cell surface receptors. Only if an MHC molecule presents a specific antigenic peptide will a cellular immune response be triggered. The recognition and signalling events require

the activities of several T-cell receptor (TCR) associated molecules, including co-receptors CD3, CD8 or CD4, and other costimulatory receptors [1].

In the cellular immune response, the antigens, generally peptides, are displayed to T-cells in complex with class I or class II MHC molecules. The peptide binding site is constituted by $\alpha 1$ – $\alpha 2$ domains of MHC-I molecules and $\alpha 1$ – $\beta 1$ domains of MHC-II molecules. These molecules present the peptides to TCR in an extended conformation via a vice-like groove, comprising two α -helices and a floor composed of anti-parallel β -strands [2–5]. T-cell receptors are cell surface heterodimers consisting of either disulphide-linked α - and β - or γ - and δ -chains. Each TCR chain is composed of a variable and a constant Ig-like domain, followed by a transmembrane domain and a short cytoplasmic tail [2,3].

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The $\alpha\beta$ TCRs bind MHC through complementary-determining regions (CDR loops) present in variable domains and are oriented approximately diagonally relative to the long axis of the MHC peptide-binding groove [6,7]. The co-receptors CD8 and CD4 stabilize the interaction of the T-cell receptor with MHC-I [8]. In particular, CD8 molecules are expressed as homo- or heterodimers and both chains are composed of an extracellular Ig Vdomain, a membrane-proximal hinge region, a transmembrane domain and a cytoplasmic tail [9] responsible for interaction with a lymphocyte specific kinase (p56lck) [10–12]. CD8 exerts its activity of TCR co-receptor by interacting with MHC-I and β_2 -microglobulin (β_2 -m) during TCR-mediated MHC recognition [4,13,14]. Structural studies have shown that CD8 $\alpha\alpha$ associates with β_2 -m and the $\alpha 2$ and $\alpha 3$ domains of MHC using the A and B strands and CDR loops within its V domain. Protein crystallographic studies on human or mouse molecules have investigated the interactions in these binary complexes but no complete system complex, i.e. CD8 $\alpha\alpha$ /MHC-I/TCR β , has been yet experimentally characterized.

In teleost fishes CD8, MHC-I and TCR molecules have been recently evidenced. In particular, in sea bream (*Sparus aurata*) the CD8 α [8], MHC-I α [15], MHC-II α [16] and TCR β [17] genes have been cloned. This gives us the possibility to investigate by molecular modelling the 3D structure of CD8 $\alpha\alpha$, MHC-I and the complexes formed by CD8 $\alpha\alpha$ /MHC-I and CD8 $\alpha\alpha$ /MHC-I/TCR. The interaction among the molecules in the complexes has been analysed in order to study the molecular basis that influences recognition and signalling events.

Methods

Modelling of sea bream CD8 $\alpha\alpha$ homodimer

The three-dimensional model of two sea bream CD8 $\alpha 1$ and CD8 $\alpha 2$ (EMBL entry: AJ878605.3) chains (see Results and Discussion for motivation) was performed according to the comparative modelling strategy using the template structures of human and murine CD8 $\alpha 1$ (PDB code: 1AKJ, chain D, and 1BQH, chain H) and CD8 $\alpha 2$ (PDB code: 1AKJ, chain E, and 1BQH, chain G), respectively [4,5].

As the sequence identities between the sea bream chains and the homologous template models were lower than 30%, we used a procedure, already applied and described in previous similar works [17–22], to search for the best alignment of sequences. In detail, the multiple alignment of many CD8 sequences was made and few manual refinements were added to account for the position of secondary structures. This protocol allows us to improve the alignment of the template and target protein sequences, as well as to avoid the presence of gaps and insertions in secondary structure elements, which are often responsible for wrong models.

The MODELLERv7 module [23] implemented in Insight II program (Accelrys, San Diego, CA) was used to build 10 full-atom models of both CD8 $\alpha 1$ and CD8 $\alpha 2$ chains by setting 4.0 Å as root mean square deviation (RMSD) among initial models and by full model optimization. To select the best models, we used the Prosall program [24] to check the fitness of the sequences relative to the obtained

structures and to assign a scoring function, and the PRO-CHECK program [25] to evaluate their stereochemical and structural packing quality. Secondary structures were assigned by the DSSP program [26]. Search for structural classification was performed on the CATH database [27,28].

The structure of sea bream CD8 $\alpha\alpha$ dimer was assembled by superimposing the two best modelled chains of sea bream CD8 $\alpha 1$ and CD8 $\alpha 2$ to the corresponding murine CD8 chains, in order to obtain the same relative orientation of the two subunits. The CVFF force field within the Discover module of Insight II was used to assign potentials and charges, and a mild energy minimization was applied, by performing 500 steps under the conjugate gradient algorithm, in order to optimise the interaction between the two sea bream protein chains and avoid sterical clashes according to the procedure used in our previous similar work [29].

3D modelling of sea bream MHC-I

The modelling of sea bream MHC-I N-terminal region (20–200 amino acid region, accession number: DQ211541.1) was performed using as template the human and murine experimental structures of MHC-I complexed with TCR β (PDB code: 2GJ6, chain A, and 1G6R, chain H) [2,3]. The modelling of the C-terminal region (201–295 amino acid region, accession number: DQ211541.1) was instead based on the experimental human and murine structures of MHC-I complexed with CD8 $\alpha\alpha$ (PDB code: 1AKJ chain A, and 1BQH chain A) [4,5]. The sequence identity percentages between sea bream MHC-I and man/mouse resulted in 31% and 34%, respectively. The 3D structure of sea bream MHC-I was modelled and analysed with the same procedures and software described above for CD8.

Simulation of sea bream CD8 $\alpha\alpha$ /MHC-I and CD8 $\alpha\alpha$ /MHC-I/V β complexes

The sea bream CD8 $\alpha\alpha$ /MHC-I and CD8 $\alpha\alpha$ /MHC-I/V β complexes were created starting from the reference experimental models of murine CD8 $\alpha\alpha$ /MHC-I [5] and V β /MHC-I [4], respectively. In detail, we created the sea bream CD8 $\alpha\alpha$ /MHC-I and V β /MHC-I complexes by superimposing the sea bream coordinates on those of murine corresponding molecules in the two available experimental complexes. For the V β (i.e. TCR variable beta) region of the sea bream T-cell receptor (21–240 region, accession number: AM490437) we used the three-dimensional model already described in our recent paper [17]. Moreover, to simulate the sea bream CD8 $\alpha\alpha$ /MHC-I/V β complex we superimposed the identical MHC-I chains present in the two sea bream CD8 $\alpha\alpha$ /MHC-I and V β /MHC-I complexes. The complexes were minimized using the same procedure described in previous similar works [15,19,29–32]. To compare the protein-protein interaction in the complexes, the “Protein-Protein Interaction Server” [33] and the program NACCESS [34] were used to evaluate the interface surface area and to identify the amino acids at the protein-receptor interface. H-bonds were calculated with the Hbplus program [35]. Moreover, the binding free energy between the different chains was calculated by using the DCOMPLEX program [36].

Figure 1 Alignment of CD8 α 1 (A) and CD8 α 2 (B) in human, mouse, sea bream, sea bass, *Paralichthys*, *Salmo trutta*, *Salmo salar*, trout, and *Danio rerio*. Amino acids in beta-strands are evidenced in grey and those in the 3_{10} helix are reported in a box. The labels indicate the β -strands and CDR loops.

Results and discussion

Modelling of sea bream CD8 $\alpha\alpha$ dimer

The sequence of sea bream CD8 α resulted similar to 20 protein sequences, defined as "CD8", from different organisms. The e-values computed by means of the BLAST software resulted in all $<10e^{-6}$ and this confirms a significant similarity of these sequences with sea bream CD8 α . The percentage of sequence identity between CD8 in sea bream and the other sequences ranged from 59% to 36% for teleost fish sequences, and from 33% to 26% for mammal sequences. In Fig. 1, we show the alignment of human, mouse, sea bream and other six fish sequences. Human and mouse sequences are of particular interest in our study because the three-dimensional structure of these proteins has been experimentally solved. On the basis of this knowledge, it is possible to build a theoretical model for the homologous protein from sea bream, by applying the homology modelling strategy or comparative modelling. The reliability of the theoretical models obtained by this strategy is considered very high, but it requires a careful application of a number of software and validation tools as described in the Materials and Methods section. In this case, the experimental three-dimensional structures of human and murine CD8 α 1 (PDB code: 1AKJ, chain D, and 1BQH, chain H) and CD8 α 2 (PDB code: 1AKJ, chain E, and 1BQH, chain G) have been used for comparative modelling of sea bream CD8 α 1 and CD8 α 2. We constructed two CD8 models because these two chains have an identical sequence but may show some conformational differences as reported for experimental murine and human structures. Starting from the alignment of the CD8 sequences (Fig. 1), we created 10 structural models for the 22–128 region of two sea bream CD8 α chains and selected the best models on the basis of stereochemical and energy parameters (see Methods). Both models present 10 beta-strands (ABCC'C'D'EFGH), involving about 50% of the sequence and define the global structure as an immunoglobulin-like beta-sandwich made of two anti-parallel sheets. Three loop regions are present between B and C strands (CDR1), between C' and C'' strands (CDR2) and between F and G strands (CDR3). The two cysteines present in B and F strands (Cys 17-Cys85) are at a suitable distance to form an S-S bond (see Fig. 2), as happens in the template human and mouse structures [4,5]. These cysteines are conserved in all CD8 sequences from teleost fishes as reported in a recent paper [15]. Moreover, the sea bream CD8 α 1 and CD8 α 2 models were compared by structural superimposition obtaining an RMSD value of 0.85 Å. The comparison of secondary structures shows that all β -strands are quite conserved with some little differences. These evaluations suggest that these two chains have a similar tertiary structure and the differences are located at the level of CDR loops.

The CD8 $\alpha\alpha$ dimer was analysed in terms of interaction residues and interchain H-bond number. The two sea bream CD8 α chains form a dimer that has pseudodyad symmetry according to human and murine dimers. In the CD8 $\alpha\alpha$ dimer one hydrogen bond is formed among Glu115 of CD8 α 1 and Thr74 of CD8 α 2 and the interface is constituted by 17 and 19 amino acids of CD8 α 1 and CD8 α 2, respectively. In detail,

Figure 2 3D model of sea bream CD8 $\alpha\alpha$ dimer. The backbone ribbon is reported in blue for CD8 α 1 and in red for CD8 α 2. β -strands are indicated with yellow arrows and helices with red cylinders. Green and yellow sticks indicate the presence of two putative intrachain disulphide bonds.

the dimer interaction in sea bream CD8 $\alpha\alpha$ is mediated by the CDR3-like domain, the C-C' loop and the G and C' strands as in the human and mouse [37]. Both the G and C' strands of this immunoglobulin-like domain contain highly conserved beta-bulges that are found at the dimer interface and are believed to play an important role in facilitating dimerization [38,39]. The conserved sequence motifs for the C' strand bulge (i.e. ProThrPheLeuLeu and ValVal in human and mouse CD8 $\alpha\alpha$, respectively), and for the G strand bulge (i.e. PheSerHisPhe and SerSer in human and mouse CD8 $\alpha\alpha$, respectively) are not conserved in sea bream. Moreover, the sea bream CD8 $\alpha\alpha$ homodimer burying aromatic residues, i.e. Trp50, Tyr105 and Tyr111, in the dimer interface shares, as expected, the same dimerization features of human and murine CD8 $\alpha\alpha$ [4,5].

3D modelling of sea bream MHC-I

The 3D MHC-I model (Fig. 3) in sea bream has a classical organization in three distinct domains. Two G- α 1 [D1] and G- α 2 [D2] domains are classified as "alpha-beta" and each consists of an alpha helical region and four strands of beta sheets in an anti-parallel orientation. The third domain (C-like [D3]) has a "mainly beta" fold and is characterized by an immunoglobulin-like beta-sandwich made of two anti-parallel sheets, each consisting of three main strands and few shorter strands, organized in greek-key motifs. In the sea bream MHC-I model four cysteine residues are located in the same positions of the human and murine templates and they should form two intrachain disulfide bonds in the second and third domains, respectively. The sea bream MHC-I model was compared by structural superimposition with the experimental structures used as templates even if the presence of gaps in the alignment made it difficult to perform a complete structural comparison of the three models. RMSD values obtained comparing the N-terminal regions of human/murine templates complexed with V β and sea bream MHC-I model resulted in 0.79 and 0.63 Å respectively, whether those obtained for the C-terminal regions of human/murine

Figure 3 3D model of sea bream MHC-I/CD8 $\alpha\alpha$ complex. The backbone ribbon is reported in green for MHC-I, in blue for CD8 α 1 and in red for CD8 α 2. Secondary structure topology is shown: yellow arrows represent β -strands and red cylinders represent α -helices. Green and yellow sticks indicate the presence of disulphide bonds. The labels indicate the CD loop in MHC-I and three CDR-like loops in the CD8 $\alpha\alpha$ dimer.

Table 1 Analysis of the three complexes in terms of interface surface area ($\text{\AA}^2$), interchain H-bonds and number of interaction residues

	Interface surface area ($\text{\AA}^2$)	Interchain H-bonds	Interaction residues
<i>MHC-I/CD8$\alpha\alpha$ complex</i>			
MHC-I	605.86	6	22
CD8 α 1	645.08	6	18
MHC-I	665.78	9	18
CD8 α 2	654.95	9	18
<i>MHC-I/CD8$\alpha\alpha$/Vβ complex</i>			
MHC-I	609.98	6	18
CD8 α 1	652.80	6	16
MHC-I	686.43	9	16
CD8 α 2	684.24	9	14
MHC-I	634.81	7	17
V β	657.61	7	19
<i>MHC-I/Vβ complex</i>			
MHC-I	622.88	4	18
V β	640.17	4	18

templates complexed with CD8 and our sea bream model resulted in 0.58 and 0.75 $\text{\AA}$, respectively. These values indicated that our model keeps the typical architecture of MHC-I in agreement with the structural classification

Table 2 The list of interaction residues between MHC-I, CD8 $\alpha\alpha$ and V β in the three complexes

MHC-I/CD8 $\alpha\alpha$ complex	Interaction residues
MHC-I	N110,Q114,D121,D126,L127,I133,A134,P135,H208 R209,A210,S211,V213,E220,H222,V225,H227,G228 E229,F230,M240,Q261
CD8 α 1	E24,V25,K37,H40,P43,G44,S45,M46,I48,S65 S66,N67,Q88,L110,Y111,K112,G113,L116
MHC-I	T193,P194,S195,S196,V213,V214,E219,E220,L221 H222,E223,E224,V225,D226,H227,K245,L246,S247
CD8 α 2	P43,G44,S45,M46,I47,I48,F60,S65,S66,N67,M69 P70,K85,T109,L110,Y111,K112,G113
<i>MHC-I/CD8$\alpha\alpha$/Vβ complex</i>	
MHC-I	Q114,L127,I133,P135,H208,R209,A210,S211,V213 E220,H222,V225,H227,G228,E229,F230,M240,Q261
CD8 α 1	E24,K37,H40,P43,G44,S45,M46,I48,S65 S66,N67,Q88,L110,Y111,K112,G113
MHC-I	P194,S195,S196,V213,V214,E219,E220,L221 H222,E223,E224,V225,D226,H227,K245,L246
CD8 α 2	P43,G44,S45,M46,S65,S66,N67,P70,K85 T109,L110,Y111,K112,G113
MHC-I	G69,Q72,T73,K75,A76,D79,Q83,I141,K145,W146 D147,N148,N149,K150,G151,L152,L155
V β	K46,T48,S49,Y50,Y52,T71,Q72,S73,P74,P75 Q77,E79,K92,D93,A94,G119,E120,Y101,E122
<i>MHC-I/Vβ complex</i>	
MHC-I	G69,Q72,T73,K75,A76,I78,D79,Q83,I141,K145 W146,D147,N148,N149,K150,G151,L152,L155
V β	K46,T48,S49,Y50,Y52,T71,Q72,S73,P74 P75,Q77,E79,A94,G119,N120,T121,H124

reported by the CATH database [27,28] for the crystallographic structures of human and murine MHC-I molecules. Moreover, the comparison of the secondary structures evidenced that helices and beta-strands are well conserved along the sequence, with just some residue which may increase or decrease their length.

Simulation of sea bream CD8 $\alpha\alpha$ /MHC-I complex

On the basis of the crystallographic structure of the murine CD8 $\alpha\alpha$ /MHC-I complex we simulated the interaction between CD8 $\alpha\alpha$ and MHC-I in sea bream (Fig. 3). For this complex, we evaluated the interaction residues, the

number of interchain H-bonds and the interface surface area (Tables 1 and 2). MHC-I and CD8 α 1 chains may form six H-bonds at their surface of interaction, while MHC-I and CD8 α 2 nine H-bonds. The interaction regions between MHC-I and CD8 α 1/CD8 α 2 in mammalian and sea bream complexes are well conserved (Fig. 4) even if only few amino acids are conserved between fish and mammalian molecules. The CD8 α 1 and CD8 α 2 subunits make interactions through their CDR-like loops (CDR1, CDR2 and CDR3) with the α 3 domain (C-like [D3]) of MHC-I. Moreover, CD8 α 1 interacts also with the α 2 domain (G- α 2 [D2]) of MHC-I. The major contribution of MHC-I α 3 is provided from the protruding loop region (Glu220-Asp226) interacting with

		G- α 1 [D1]	
human		GSHSMRYFFTSVSRPGRGEPFRFIAVGIVDDTQFVRFDSDAASQRMERAPWIE---QEG	
mouse		GPHSLRYFVTAVSRPGLGEPYMEVGYVDDTEFVRFDSDAENPRYEFRAWME---QEG	
sea bream		VTHSLKYFYTASSG-VPNFPEFVIIVGLVNEVEMFHYDSNTT--KAEPKQDWMSRVTADD	
Salmosalar		VTHSLKYFYTASSE-VPNFPEFVVSMVDGVQMVHYDSNSQ--RAVPKQDWMNKAEEAL	
Salmotrutta		VTHSLKYFYTASSE-VANFPEFVAVGMVDGVQMFHYDSNSQ--RAVPKQDWMNKATETL	
Trout		VTHSLKYFYTASSE-VPNFPEFVVGMVDGVQMVHYDSNSQ--RAVPKQDWMNRAAETL	
		G- α 1 [D1]	G- α 2 [D2]
human		PEYWDGETRKVKAHSQTHRVDLGLRGYYNQSEAGSHTVQRMYGCDVGSQDWRFLRGYHQ	
mouse		PEYWERETQKAKGNEQSFRVLDLRLGLGYYNQSKGGSHTIQVISGCEVGSQDGRLLRGYQQ	
sea bream		PQYWQRQTEQSVGAQQTFFKANIDIAKQRFNQ-TGGVHVFFQWMYGCEWDDDETQEVNGFMQ	
Salmosalar		PQYWDIETGKFLGSHQSFKANIDICKQRFNQS-GGVHVFFQKMYGCEWDDDEAGATEGLTQ	
Salmotrutta		PQYWERETGNCKGSQQNFKANIDIVQQRFNQS-GGVHVFFQNMVYGCEWDDDEAGVTEGFYQ	
Trout		PQYWESQTGNFKGTQQTYSKANIDIVKQRFNQS-GGVHIFQRMCGCEWDDDEAGVTEGFNQ	
		G- α 2 [D2]	
human		YAYDGDYIALKEDLRSWTAADMAAQTTHKKWEAA-HVAEQLRAYLEGTCEWELRRYLE	
mouse		YAYDGDYIALNEDLKTWTAADMAALITKKWBOA-GEAERLRAYLEGTCEWELRRYLK	
sea bream		YGYDGEDFIAFDLKTTLTWIAPKQQAIVITKKWNNKGLRELKRTYLTDCGDWVKKYVD	
Salmosalar		YGYDGEDFIAFDLKTTSXIAPTQSLITKLKWDSDTAYNEQEKNYLTQICIEWLKKYVD	
Salmotrutta		FGYDGDYIAFDLKTTLKWTAPTQQAIVITKLKWDSDNMAFNEQKNYLTQICIEWLKKYVD	
Trout		YGYDGEDFIALDLKTTKWIAPTQQAIVITKLKWDSDNTANTYWKNYLTQTCIEWLKKYVD	
		G- α 2 [D2]	C-like [D3]
human		NGKETLQRTDAPKTHMTHHAVSDHEATLRCWALSFPYPAEITLTWQDGEDQTDTELVE	
mouse		NGNATLLRTDSPKAHVTHHSRPEDKVTLCWALGFYPADITLTWQNGEELIQDMELVE	
sea bream		YGRSVLLRTELPSVSLQKTP---SSPVSLATGTFYPHRASLVWRKDEGLHEEVDHGE	
Salmosalar		YGKSTLMRTVPPSVSLQKTP---SSPVTCHATGFYPSGVMVSWQKDGQDHHDVEYGE	
Salmotrutta		YGKSTLMRTVPPSVSLQKTP---SSPVTCHATGFYPSDVMVSWQKDGQDHHDVEYGE	
Trout		YGKSTLMRTVPPSVSLQKAP---SSPVTCHATGFYPRDVMVSWQKDGQDHHDVEYGE	
		C-like [D3]	
human		TRPAGDGTFOKWAADVVPSPG---QEQRVYCHVQHEGLPKPLTLRWEP	
mouse		TRPAGDGTFOKWASVVVPLG---KEQYVYCHVYHQGLPEPLTLRW	
sea bream		FLPNHDGTFOMSVDLKLSSVTPEDWTRYDCVFQLSGVNEDIITKLDK	
Salmosalar		TLQNDDGTFOKSSHL---TVTPEEWKNNKYQCVVQVTKGKEDFIKVL	
Salmotrutta		TLPNDDGTFOKSIRL---TVTPEEWKNNKYQCVVQVTKGKEDFIKVL	
Trout		TLPNDDGTFOKSSHL---TVTPEDRKNNKYQCVVQVTKGKEDFIEVL	

Figure 4 Alignment of MHC-I in human, mouse, sea bream, *Salmosalar*, *Salmotrutta* and trout. Amino acids in beta-strands are evidenced in grey but those in the helix are reported in a box. Amino acids in bold interact with CD8 α 1 and those underlined with CD8 α 2. The residues at the interface with V β are reported in italics and bold. The labels indicate the different structural domains.

the CD8 α subunits by main chain and side chain interactions according to experimental complexes [40,41]. In detail, this relatively rigid loop (indicated as loop CD) protrudes into the antigen-binding-like pocket formed by the six CDR-like loops of the CD8 $\alpha\alpha$ homodimer. A second distinctive feature essential for the interaction between MHC-I and CD8 $\alpha\alpha$ is the AB loop of the MHC α 3 domain, which interacts with the CDR2-loop of CD8 α 2. A third aspect that is characteristic of sea bream MHC-I/CD8 $\alpha\alpha$ complex regards the interaction between the MHC α 3 domain and the N-terminal residues of the CD8 α 1 subunit.

Moreover, the comparison of the specific interaction residues found in sea bream MHC-I with respect to other fish species shows that 35% of the amino acids are conserved as reported in Fig. 4. In the case of CD8 α molecules we can note that some structural interactions are in common only within sea bream and sea bass.

There is no significant change in the CD8 $\alpha\alpha$ homodimer structure between the un-complexed versus MHC-I bound state as for human and mouse [4,5]. Finally, it is important to underline that β 2-m interacts with MHC-I and CD8 α 1 [13,14] and will influence the interaction between these two chains in the complex. However, the sequence of sea bream β 2-m is still unknown, and for this reason we simulated only the interaction between MHC-I and CD8 $\alpha\alpha$. Similarly, the presence of the antigenic peptide in the MHC groove would be useful to simulate the conformation of the MHC molecule when it binds the peptide. Unfortunately, there is no information about the sequence of an antigenic peptide which interacts with MHC class I in sea bream.

Simulation of sea bream CD8 $\alpha\alpha$ /MHC-I/V β complex

The crystallographic studies on $\alpha\beta$ T-cell receptors bound to MHC-I or MHC-II or to TR co-receptors (CD4 and CD8) have further advanced our knowledge on structural variability of TR/MHC recognition and on the amino acid segments that constitute the TR signalling complex. Despite all these efforts, the structural basis for MHC restriction and signalling remains elusive and no structural features that define a common binding mode or signalling mechanism have yet been determined. In fact, in the absence of a ternary CD8/MHC-I/V β complex structure, no conclusions can yet be drawn to the precise role that the V β /MHC docking geometry plays in co-receptor binding and downstream signalling [1].

For these reasons, we created a model for CD8 $\alpha\alpha$ and V β bound to MHC class I (see Fig. 5). The sea bream V α sequence was not included in the complex because it is still unknown. Certainly its absence could influence the structure of V β /MHC-I complex. However, we modelled the sea bream V β using as template the mammalian V β structure already complexed with the V α chain and this gave us the possibility to take into account the V β influence on the complex [31]. In fact, using this procedure it is possible to simulate the conformational changes occurring when a protein interacts with its receptor.

The binding sites of CD8 $\alpha\alpha$ and V β to MHC-I are spatially separated even if it is known that they require similar orientations with respect to the MHC α 3 domain [1]. In detail, the amino acids of MHC-I at the interface with V β chain are

Figure 5 3D model of sea bream MHC-I/CD8 $\alpha\alpha$ /V β complex. The backbone ribbon is reported in green for MHC-I, in blue for CD8 α 1, in red for CD8 α 2 and in pink for V β .

located mainly in helical regions comprised in G- α 1 [D1] and G- α 2 [D2] domains and those at the interface with CD8 $\alpha\alpha$ are located in loop and beta regions located in G- α 2 [D2] and C-like [D3] domains (see Fig. 4).

We compared the two sea bream complexes simulated in this work (i.e. CD8 $\alpha\alpha$ /MHC-I, and CD8 $\alpha\alpha$ /MHC-I/V β) and the MHC-I/V β recently published [17] in terms of interaction residues, number of interchain H-bonds and interface surface area (Tables 1 and 2). These complexes show little differences in the interface surface area values and number of interaction residues.

The number of interchain H-bonds between MHC-I and CD8 $\alpha\alpha$ is the same in both CD8 $\alpha\alpha$ /MHC-I and CD8 $\alpha\alpha$ /MHC-I/V β complexes but taking into consideration MHC-I and V β this number is higher in the CD8 $\alpha\alpha$ /MHC-I/V β complex with respect to MHC-I/V β (see Table 1). Moreover, for each complex we have evaluated the binding energy (Fig. 6) between the different chains. The binding energy between MHC-I and CD8 $\alpha\alpha$ is similar in CD8 $\alpha\alpha$ /MHC-I and CD8 $\alpha\alpha$ /MHC-I/V β complexes with a difference of only 0.2 kcal/mol, whether this value between MHC-I and V β is higher

Figure 6 Binding free energies for the complexes. The bars represent the binding energies (expressed in kcal/mol) evaluated for MHC/CD8αα (panel a), MHC/Vβ (panel b) and MHC/CD8αα/Vβ (panel c) complexes.

(of about 0.8 kcal/mol) in the CD8αα/MHC-I/Vβ compared Q8 to MHC-I/TRβ.

These results suggest that the binding of sea bream CD8αα to MHC-I should increase the affinity of Vβ for MHC-I and stabilize the MHC-I/Vβ complex. Therefore, the observation that the binding sites of CD8 and TR to MHC-I are spatially distinct but require similar orientations of the MHC α3-domain, is consistent with an avidity-based contribution of the CD8 to the MHC-I-TR binding, as already reported [4].

Conclusions

Despite the impressive increase in the cloning and expression of genes coding in fish for immunoregulatory molecules, the knowledge on “in vivo” and “in vitro” functional immunology of corresponding peptide products is still at the beginning. Structural studies could help in improving our knowledge on the behaviour of these molecules until specific markers for immunoregulatory peptides will be available. Moreover, it may be possible to design some peptides, based on the amino acids present at the interface of the modelled complexes, that can block complex formation and that, therefore, should inhibit T-cell activation in vitro and permit interesting functional analyses, as already performed in mammals [42].

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