Domain Swapping of CD4 Upon Dimerization

Yves-Henri Sanejouand*

Laboratoire de Physique, Ecole Normale Supérieure, 46 allées d'Italie, 69364 Lyon Cédex 07, France

ABSTRACT It has recently been shown that disulfide bond Cys130—Cys159 in domain 2 of monomeric CD4 is involved in the formation of CD4 disulfide-bonded dimers on cell surfaces and that it can influence the permissiveness of cells to HIV infection. Because this disulfide bond is buried in the monomer, a large conformational change must take place in order to allow for such disulfide exchange. Using standard optimization techniques, whose efficiency was first checked in the well-documented CD2 case, we have shown that 3D domain swapping is a likely candidate for the conformational change, the hinge loop, or linker, being loop E—F. Indeed, as a consequence of domain swapping, because Cys130 and Cys159 belong to β-strands C and F, respectively, two disulfide bonds become established between Cys130 in one monomer and Cys159 in the other one. Such a disulfide exchange has already been observed when the nuclear magnetic resonance (NMR) structure of the prion protein was compared to the crystallographic, dimeric one. In both cases, domain swapping implies disulfide exchange because the linker is located in the sequence between two disulfide-bonded cysteines. As in the CD2 case, the proposed configuration of the CD4 dimer is found as a pair of neighboring monomers in the crystallographic unit cell. Moreover, because in this configuration the epitope of monoclonal antibody MT151, which does not compete with Gp120 for CD4 binding, is in the cleft between the pair of CD4 monomers, it is suggested that MT151 achieves its HIV-blocking activity by interfering with the formation of CD4 domain-swapped dimers on cell surface. Proteins 2004;57: 205–212. © 2004 Wiley-Liss, Inc.

© 2004 Wiley-Liss, Inc.

Key words: CD2; crystallographic neighbor; disulfide bond; disulfide exchange; immunoglobulin domain; molecular dynamics; MT151; optimization

INTRODUCTION

Human CD4 is the primary receptor for HIV-1. Its extracellular part is made of four immunoglobulin-like domains, designated D1 to D4. HIV-1 binds to the D1 domain, via Gp120, as was shown previously by site-directed mutagenesis or with neutralizing antibodies that map to D1.[1–5] Recently, it was also shown that CD4 on cell surfaces can form dimers, that the dimers are disulfide bonded,[6,7] and that the disulfide bonds are established between cysteines belonging to the D2 domain.[7] Moreover, although reduction of the cysteines of D2 does not significantly affect binding of Gp120 to CD4, it blocks the entry of HIV-1 into CD4$^+$ cells. This strongly suggests that disulfide exchange in D2 is required for HIV-1 entry.[7]

As a matter of fact, in monomeric CD4, Cys-130 and Cys-159, the two cysteines of D2, are involved in an atypical disulfide bridge. While in standard immunoglobulin domains the disulfide bridge links the two β-sheets of the sandwich-like structure, each cysteine belonging to one sheet, in D2 of CD4 it links two neighboring β-strands belonging to the same sheet. The geometry and strain of the disulfide bond are also unusual: the disulfide bond is right- rather than left-handed and it has a high dihedral strain energy.[7] Indeed, $\chi_3 = 109°$,[8,9] while theoretical studies on small molecules have shown that electronically and sterically preferred values of χ_3 are close to $\pm 83°$.[10,11]

However, in monomeric CD4, the disulfide bridge of D2 is buried in the core of the domain (see top of Fig.1).[8,9] As a consequence, a large conformational change is required in order to allow for the formation of disulfide bonds between two D2 domains in dimeric CD4. Although such conformational changes, during which a part of the core of an immunoglobulin-like domain becomes exposed, have not been observed so far, 3D domain swapping, namely the exchange of β-strands between pairs of monomers belonging to a dimer, has been evidenced in the case of the N-terminal, immunoglobulin-like domain of CD2.[12]

When domain swapping occurs, the 3D structures of each half of the domain-swapped dimer, namely the functional units,[13] are nearly identical to the parent monomer, with the exception of the conformation of the so-called 'hinge-loop,' or linker, which allows for domain exchange.[14] When the linker is located in the protein sequence between two cysteines involved in an intramolecular disulfide bridge, domain swapping implies disulfide exchange, and the same disulfide bridge becomes an intermolecular one. For instance, while the nuclear magnetic resonance (NMR) structure of the prion protein is monomeric, its crystal structure was obtained as a disulfide-bonded swapped dimer.[15] Because in this later case loop 190–199 is the linker, the intramolecular disulfide bridge established between Cys-179 and Cys-214 is rear-
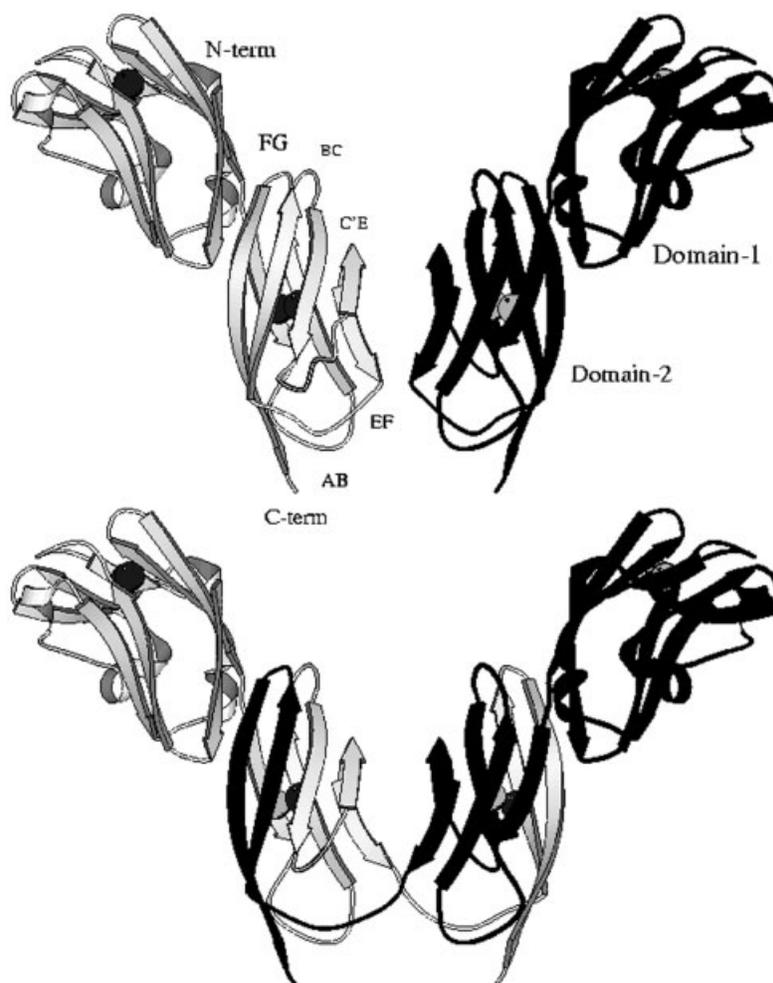
Fig. 1.   Domain swapping of CD4. Top: the contact dimer, as observed in the 1g9m crystal structure[17] of the two N-terminal domains of CD4 (D2 loops are labeled). Bottom: the swapped dimer, when loop E-F of domain 2 is assumed to be the linker. The sequence of loop E-F is: SQLELQDSG. Sulfur atoms, which are involved in disulfide bonds, are depicted as Van der Waals spheres. In the swapped-dimer conformation, they are covalently linking the pair of monomers.

ranged into an intermolecular one, as a consequence of the 3D swapping of helix 3.[15] Note that the transition from the monomer to the domain-swapped dimer involves the reduction and reformation of two disulfide bonds. However, it occurs spontaneously, with no reducing agent added to the buffer or crystallisation solution.[15]

The purpose of the present study is to show that domain swapping is likely to occur in D2 of CD4, to identify which among its loops is the linker, the one that 'jumps' from one molecule to the other, and to propose a model for the 3D structure of the swapped dimer.

MATERIALS AND METHODS
Crystallographic Structures Considered

Protein Data Bank (PDB) codes of the studied structures are as follows: 1hng[16] for the contact dimer of the N-terminal domain of CD2, 1g9m[17] for the contact dimer of the two N-terminal domains of CD4, 1jl4[18] and 3cd4[8] for other structures with extensive contacts between pairs of

CD4 D2 arranged in a nearly parallel configuration. For each of them, crystallographic neighbors were generated using the WHAT IF 5.0 Web Interface.[19]

Loop limits were obtained from PDB files and confirmed by visual inspection of the structures, using the VMD program[20]. When various loop limits were found, in different PDB files for instance, those corresponding to the longest loops were retained. Thus, for the N-terminal domain of CD2, loop names and limits are as follows: A-B, 10–14; B-C, 17–27; C-C′, 35–37; C′-D, 44–55; D-E, 58–63; E-F, 66–74; F-G, 83–87. For domain 2 of CD4, they are as follows : A-B, 103–113; B-C, 120–126; C-C′, 132–138; C′-E, 141–143; E-F, 147–155; F-G, 163–166. Loop names come from standard nomenclature of immunoglobulin folds, where one β-sheet of the sandwich-like structure contains strands A, B, D and E, and the other sheet contains strands C, C′, F and G. In domain 2 of CD4, strand D is missing, and the disulfide bond is established between Cys 130 in strand C and Cys 159 in strand F, while in standard

immunoglobulin folds it is established between a pair of cysteines in strands B and F.

Figures were drawn with the Molscript program.[21]

Optimization of Putative Swapped Dimers

Starting from a contact dimer configuration, all possible domain-swapped conformations were built; that is, each loop was considered to be the linker, one after the other. From a practical point of view, this means that the chosen loop was cut after the ith amino acid in both monomers, the ith amino acid in one monomer becoming bonded to the ith + 1 amino acid in the other monomer. Because at this point the coordinates of the amino acids were still the same as they were in the contact dimer, large amounts of energy strain are thus introduced into the linker. So, for each of the putative swapped dimers, the following optimization was undertaken, using the CHARMM program[22] and EEF1[23]. Note that EEF1 refers not only to the implicit solvation model but also to the specific modifications and nonbonded options used in CHARMM. EEF1 was recently shown to correctly discriminate native protein structures from decoys.[24]

Because when the actual linker is chosen only its conformation remains unknown, all calculations were performed with harmonic restraints on C_α atoms, except for those belonging to the putative linker, with a 100 kcal mol^{-1} Å^{-1} force constant. Specifically, at each step of the optimization process, restrained atoms were (implicitly) rotated and translated, a best fit being performed so as to minimize restraint energy.

First, interactions between the two linkers (one per monomer) were ignored, using the BLOCK feature of CHARMM, so that they could cross each other, while the energy was minimized through 5000 steepest descent steps followed by adopted basis Newton–Raphson steps, until a root mean square (RMS) gradient of 0.001 kcal mol^{-1} Å^{-1} was reached (typically, after 10000–15000 steps). Next, interactions between them were restored, as was their interaction with the water environment, through the EEF1 energy term. Then, energy minimization was performed again, followed by a 100 ps molecular dynamics (MD) simulation at 300K, with the temperature controlled by a Langevin thermal bath, using a 10 ps^{-1} friction constant for all atoms. Finally, fifty points from the corresponding trajectory were energy minimized, and the lowest-energy one was retained for further analysis.

Recognition of Actual Swapped Dimer Conformation

In order to recognize the actual swapped dimer, several criteria were considered. First, as mentioned above, the total energy, including the solvation free energy. However, because swapped dimers do not seem to be frequent competitors for standard, contact dimers, other criteria were required in order to assess the likehood of domain swapping. In the present study, it was assumed that domain swapping is possible only if the linker can adopt a low-energy conformation. So, in order to evaluate how constrained the linker is, the following criteria were used.

L_s, the linker strain, was calculated as

$$L_s = \frac{E_b + E_a + E_\phi}{n_l}$$

where E_b, E_a and E_ϕ are, respectively, the energy of bonds, angles and dihedrals (including improper ones[22]) in which the n_1 atoms belonging to the two linkers (one per monomer) are involved.

L_i, the interaction energy of the linkers was calculated as the total energy per linker atom, with the solvation free energy not taken into account and all non-linker atoms kept fixed. Thus, it represents the sum of the linker strain and the electrostatic and Lennard–Jones inter-linker and linker–non-linker interaction energies.

RESULTS AND DISCUSSION

First, the case of the N-terminal domain (D1) of CD2 was considered, in order to check that C′-D, the experimentally known linker,[12] could be singled out using our optimization protocol when the configuration of the standard, contact dimer is known. Indeed, when the structure of the domain-swapped dimer of CD2 D1 was determined,[12] it was found that amino acids 1–43 of one monomer and 56–99 of the other monomer, those apart from loop C′-D, could be superimposed to monomeric D1[16] with a 1.2 Å C_α-RMS. Moreover, the two structural domains thus defined could be superimposed to a pair of adjacent D1 domains previously observed in a crystallographic structure of the monomeric form with a 1.0 Å C_α-RMS.[12] Note that this latter contact dimer, made with neigboring monomers found in the crystallographic unit cell, is atypical, with a largely hydrophilic interface.[12]

Starting from the contact dimer, all possible domain-swapped conformations were built; that is, each of the seven loops of D1 was considered to be the actual linker, one after the other. Then, the structure of each putative swapped dimer was optimized, using the CHARMM force field,[22] the EEF1 implicit solvent model[23] and a combination of energy minimization and MD techniques for the optimization process (see Materials and Methods).

Because a loop length cannot be unambiguously determined from a crystallographic structure, for each putative swapped dimer, fifteen different calculations were performed. From one calculation to another, the length of the linker was varied, one residue being included or excluded at one end, or at both. Moreover, three different options were considered for the initial conformation of the linker, corresponding to three different ways to cut and paste the swapped domains, namely by cutting the linker at its beginning, at its end, or in the middle. Then, among the fifteen structures obtained as a result of the optimization processes, the ten lowest energy ones were retained for further analysis.

As shown in Table I, which details results for the three best low-energy swapped dimers thus obtained, in the case of CD2 D1, the actual experimentally determined conformation of the swapped dimer was singled out. Indeed, when the linker was assumed to be loop C′-D, it had by far the lowest average strain (0.4 kcal/mol per atom), as well as

**TABLE I. Lowest Energy Swapped Dimers
of Domain 1 of CD2**

Linker	Linker Strain[a]	Interaction Energy[b]
C'-D	0.41 ± 0.04 (0.34)	−2.3 ± 0.1 (−2.5)
F-G	3.03 ± 1.54 (1.16)	1.1 ± 2.4 (−1.8)
B-C[c]	2.81 ± 1.26 (1.37)	2.2 ± 2.6 (−0.6)

Each dimer is identified by its linker, the loop that jumps from one monomer to the other. For each, averages are given for the ten lowest energy conformations found, while the best values are shown in parantheses. Energies are in kcal/mol and per loop atom.
[a]L_s, the strain of the linker, is the sum of the energies of bonds, angles and dihedrals in which atoms belonging to the linker are involved.
[b]L_i, the interaction energy of the linker, is the sum of its internal energy plus its interaction energy with other protein atoms.
[c]Six conformations have an overall energy lower than −4000 kcal/mol. Only those are considered for the averaging.

**TABLE II. Ten Lowest Energy Conformations of CD2
Swapped Dimer, Found After Fifteen Independent
Optimizations, Starting from Contact Dimer**

Energy (kcal/mol)	Linker Strain	Interaction Energy	Linker RMSD (Å)[a]
−6156.1	0.43	−2.54	1.8
−6135.0	0.37	−2.34	1.8
−6133.5	0.46	−2.41	2.0
−6126.2	0.34	−2.22	1.9
−6096.2	0.36	−2.12	4.7
−6094.5	0.42	−2.14	4.0
−6084.8	0.46	−2.18	4.9
−6078.5	0.39	−2.02	4.6
−6075.6	0.44	−2.32	4.8
−6072.7	0.41	−2.21	4.7

[a]$C\alpha$ RMSD (with respect to the crystal domain-swapped conformation) are given for amino acids belonging to the linker, the C'-D loop.

**TABLE III. Lowest Energy Contact Dimers of Domain 1 of
CD2, Obtained Starting from Two Different Domain-
Swapped Conformations**

Initial Domain Swapped Form	Linker Strain	Interaction Energy
Crystallographic	0.32 ± 0.02 (0.30)	−2.3 ± 0.2 (−2.5)
Calculated	0.40 ± 0.03 (0.37)	−2.3 ± 0.1 (−2.5)

Averages are given for the ten lowest-energy conformations found, while the best values are shown in parantheses. Energies are in kcal/mol and per loop atom.

the largest favorable interaction energy (−2.3 kcal/mol per atom). This latter point shows that, in the swapped-dimer conformation, the linker does not make steric clashes or unfavorable interactions with the rest of the protein. Moreover, all ten conformations selected as a result of the fifteen independant optimization processes have very similar linker strain and interaction energy (fluctuation values of less than 0.1 kcal/mol per atom). This was not observed when other loops were chosen as putative linkers (then, fluctuation values were larger than 1.0 kcal/mol per atom). This probably reflects the fact that conformations with low strain and large interaction energy are more numerous in the case of the actual linker. Indeed, as shown in Table II, such conformations were found even when the optimization process did not succeed in reaching the lowest-energy conformations. However, when it did, when the total energy was lower than −6100 kcal/mol, the conformation of the linker was found to be quite close to the one it has in the crystallographic structure of the domain-swapped form (PDB code 1hng), with a C_α RMS difference of less than 2.0 Å (note that loop C'-D is 11 amino acids long). So, our optimization protocol appears to be efficient enough, for the purpose of the present study. However, shorter protocols without MD simulations might be sufficient.

Indeed, good quality results were also obtained prior to MD simulations. At this stage of the optimization process, average strain and interaction energy of the linker were already quite low (0.52 ± 0.08 and −2.0 ± 0.2 kcal/mol per atom, respectively). However, the linker was found to be less than 2.0 Å away from its crystallographic conformation in only two of the ten retained conformations (the two lowest-energy ones), compared to four when the complete protocol was used (see Table II), indicating that in this case MD simulations do help in finding conformations closer to experimentally determined ones. Although more extensive studies are required in order to confirm this point, the long optimization protocol, with MD simulations, was used throughout this work.

Calculations can also be performed the other way around, starting from a domain-swapped conformation, either the crystallographic or the lowest-energy conformation found using the previously described calculations (see Table II). In both cases, in the lowest-energy conformation of the contact dimer obtained as a result of the optimization process, the linker is also found less than 2.0 Å away from the crystallographic one. Moreover, as shown in Table III, the average linker strain and interaction energy are found to be very similar in the domain-swapped (see Table I) and in the contact dimer.

In order to confirm that our optimization protocol is efficient enough for the purpose of the present study, tests on other well-known cases, such as the prion protein[15] or cystatin[27] would be welcome. However, to our knowledge, CD2 D1 remains the only case for which the 3D structures of both contact and domain-swapped dimers are known. Indeed, a protein is considered to be a genuine exemple of 3D domain swapping only if both the monomer and the dimer exist in stable forms, in which the dimer adopts a domain-swapped conformation.[13] Usually, probably because the domain interface is not large enough, the contact dimer is not observed; that is, it is less stable than the domain-swapped one. However, as domains continue to swap,[13] other cases in which both crystal structures of contact and domain-swapped dimers are known should become available.

Nevertheless, in order to check that linker strains and interaction energies obtained for the domain-swapped conformations of CD2 are likely to have typical values, the following calculation was undertaken. First, using our optimization protocol, starting from a domain-swapped conformation, contact dimers of the well-known RNase A case were obtained. Interestingly, two different types of

TABLE IV. Lowest Energy Swapped Dimers of RNase A, Obtained Starting from Contact Dimer Conformations Generated Using Our Optimization Protocol

Linker	Linker Strain	Interaction Energy
15–22	0.35 ± 0.06 (0.25)	−3.0 ± 0.2 (−3.2)
112–115	0.47 ± 0.05 (0.41)	−2.2 ± 0.1 (−2.3)

Averages are given for the ten lowest-energy conformations found, while the best values are shown in paranthesis. Energies are in kcal/mol and per loop atom.

TABLE V. Lowest Energy Swapped Dimers of Domain 2 of CD4

Linker	Linker Strain	Interaction Energy
E-F	0.27 ± 0.03 (0.21)	−2.9 ± 0.1 (−3.0)
C-C′	0.46 ± 0.09 (0.32)	−2.5 ± 0.2 (−2.7)
C′-E	0.56 ± 0.33 (0.24)	−1.7 ± 0.6 (−2.3)
A-B	0.74 ± 0.31 (0.45)	−1.9 ± 0.5 (−2.4)

Each dimer is identified by its linker, the loop that jumps from a monomer to the other. For each of them, averages are given for the ten lowest energy conformations found, while the best values are shown in paranthesis. Energies are in kcal/mol and per loop atom.

TABLE VI. Lowest-Energy Contact Dimers of CD4, Obtained Starting from a Domain-Swapped Conformation

Linker in Domain Swapped form	Linker Strain	Interaction Energy
E-F	0.30 ± 0.05 (0.25)	−3.2 ± 0.1 (−3.4)
C-C′	0.43 ± 0.06 (0.34)	−2.6 ± 0.1 (−2.8)
C′-E	0.41 ± 0.19 (0.27)	−2.3 ± 0.2 (−2.6)
A-B	0.43 ± 0.36 (0.22)	−2.4 ± 0.4 (−2.6)

Averages are given for the ten lowest-energy conformations found, while the best values are shown in paranthesis. Energies are in kcal/mol and per loop atom.

domain-swapped dimers of RNase A are known, one in which loop 15–22 is the linker[26] (PDB code 1a2w) and one in which loop 112–115 is the linker[27] (PDB code 1fv0). In both cases, starting from the lowest-energy conformation found for the contact dimer, our optimization protocol yielded low-energy domain-swapped conformations. As shown in Table IV, linker strain and interaction energy thus obtained were found to have values within the range of those obtained in the CD2 case (see Table I).

Next, the configuration of the contact dimer of domain 2 of CD4 was sought, assuming that, as in the CD2 case, it is among available crystallographic data, as a contact between the domain 2 pairs of CD4 molecules in the crystallographic unit cell. To be consistent with dimer formation on a cell surface, the configuration of the pair of D2 domains is likely to be such that their main axes are more or less parallel. Because of this, only three possibilities were retained. At the top of Figure 1, the back-to-back configuration found in structure 1g9m[17] is shown. Another configuration, with more extensive contacts, comes from structure 1jl4,[18] while the most common one, found in structure 3cd4[8] for instance, is a side-by-side configuration of pairs of the two N-terminal domains. As a configuration of a swapped dimer, this latter one is very unlikely, because the two linkers would have to cross each other, one on top of the other, yielding a non-symmetrical dimeric structure. Nonetheless, it was considered, like the two others, as a starting point for the optimization process, performed following the same protocol as in the CD2 case. Swapped dimers found to have low linker strain (less than 1.0 kcal/mol per atom) as well as large favorable interaction energy with the rest of the protein (better than −1.3 kcal/mol per atom) were all obtained starting from the contact dimer shown at the top of Figure 1. Note that this means that enforcing domain swapping, starting from a wrong contact dimer configuration, usually yields swapped dimers with high average linker strain and unfavorable interaction energy with the rest of the protein.

Interestingly, as shown in Table V, the swapped dimers with the three lowest linker strain values as well as those with the two most favorable interaction energy values and the lowest fluctuation values have their linker located in the sequence between Cys-130 and Cys-159 (which are in β-strands C and F, respectively). Thus, they all imply disulfide exchange, as illustrated at the bottom of Figure 1, where loop E-F is assumed to be the linker. This is the best case obtained according to all criteria found to be efficient in the CD2 case. However, here the energy strain of the

linker is lower than that of CD2, while the corresponding interaction energy is larger (see Table I). This means that domain swapping is quite easy to achieve when loop E-F is the linker, starting from the contact dimer configuration shown at the top of Figure 1. The fact that linker E-F is not as clearly singled out as that of CD2 (see Table I), the energy strain of the linker and the corresponding fluctuation being also quite low when loop C-C′ is the linker, means that in the actual conformation of the swapped dimer of CD4 the linker may be longer than the longest one considered in the present study.

Calculations were also performed the other way around, starting from a domain-swapped conformation, the lowest-energy one found in previous calculations (see Table V). As shown in Table VI, linker strain and interaction energy were found to be very similar in the domain-swapped (see Table V) and contact dimer conformations, when either loop E-F or loop C-C′ was taken to be the linker. However, in the former case, in the two lowest-energy contact dimer conformations obtained as a result of the optimization process, the linker was found to be less than 1.8 Å away from its crystallographic conformation, while in the latter case, it was at least 3.9 Å away. This means that when loop C-C′ is assumed to be the linker, our protocol may not be able to reach the actual lowest-energy conformations.

However, our results show that domain swapping in CD4 D2 is possible, starting from the dimer configuration shown at the top of Figure 1, in the sense that some loops can jump from one monomer to another, at a low-energy cost. When loop E-F was assumed to be the linker, its strain was found to be (slightly) lower in the domain-swapped than in the contact dimer conformation. As negative controls, the fact that no such low-energy linker
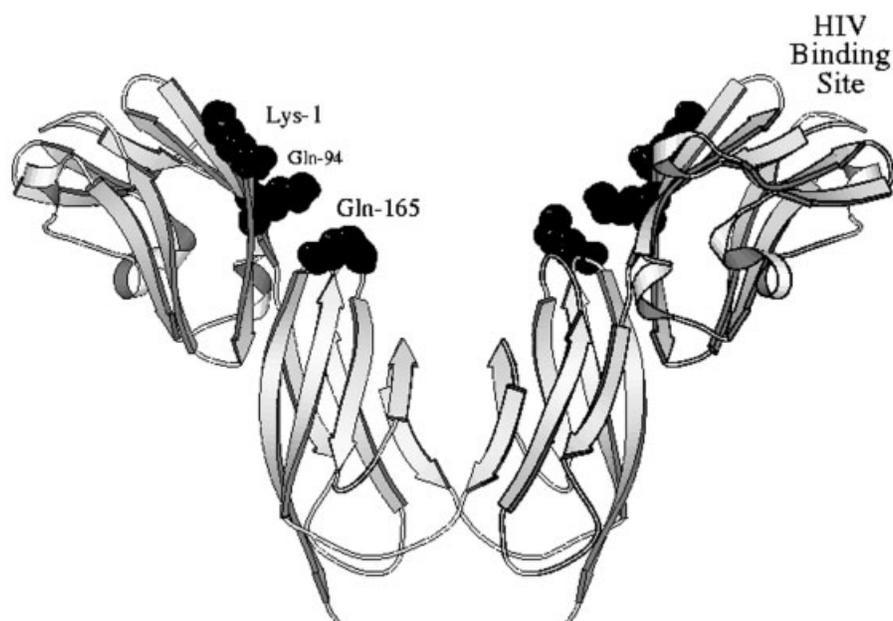
Fig. 2.   Epitope recognized by HIV-blocking monoclonal antibody MT151. Atoms of amino acids Lys 1, Gln 94 and Gln 165, which were shown to belong to the epitope recognized by MT151, are depicted as Van der Waals spheres. The HIV-blocking activity of MT151, which does not compete with Gp120 for CD4 binding, is suggested to be achieved through the inhibition of CD4 domain-swapped dimer formation.

conformation was obtained, starting from two other CD4 dimer configurations found in two other crystallographic structures, shows that it is not particularly easy for CD4 loops to jump from one monomer to the other. Of course, such results do not prove that the domain-swapped conformation shown at the bottom of Figure 1 is as stable as monomeric CD4 (or more so). The ability to compute the free energy difference between these two forms may yield evidence for this. Unfortunately, in the present state of their development, methods for estimating protein–protein equilibrium constants are far from being accurate enough. Thus, our results must only be viewed as a convincing way of interpreting experimental data, of explaning how CD4 dimers can be disulfide bonded, with the two cysteines of CD4 D2 involved in the two corresponding disulfide bridges,[7] while they are known to be deeply buried in the monomer.[8,9]

It was previously proposed that, as a consequence of Gp120 binding, a large overall rigid-body displacement of D1 occurs with respect to D2. This proposal is based on normal mode calculations[28,29] and on the fact that the only mutations of D2 found to lower the affinity of CD4 for Gp120 are located either in loop B-C[2] or in loop F-G,[2] the two loops of D2 involved in the interface between D1 and D2. Moreover, loops B-C and F-G of D2, either one or both, belong to the epitopes recognized by HIV-blocking monoclonal antibodies MT151, OKT4B, OKT4F and 5A8.[1,3–5,30–32] Note that these antibodies do not compete with Gp120 for CD4 binding. The epitope of MT151, which has been studied extensively,[1,3–5,29] is shown in Figure 2. Because it is located in the cleft between the two CD4 monomers, our results suggest that MT151 may inhibit CD4 domain-swapped dimer formation through steric hindrance effects.

They also suggest that this is how MT151 achieves its HIV-blocking activity. Indeed, it has been shown that disulfide exchange in D2 is likely to be required for HIV-1 entry,[7] while our results show that domain swapping is a likely mechanism for the formation of CD4 disulfide-bonded dimers on cell surfaces.

However, other modes of CD4 dimerization are likely to occur, since the OKT4 mAb which maps to the D3 domain and does not significantly inhibit HIV infection or HIV-induced cell-to-cell fusion,[32] has been shown to inhibit homodimerization of soluble recombinant CD4 molecules but not of membrane-bound ones.[6] Because extensive dimeric association through D4 domains has been observed in the crystal structure of recombinant soluble CD4,[33] one can speculate, assuming that the dimer configuration found in the crystal is representative of its configuration in solution, that OKT4 also inhibits CD4 dimer formation through steric hindrance effects. Accordingly, because in the later configuration of dimeric CD4 the N-terminal domains are far away from each other,[33] MT151 is not expected to inhibit homodimerization of soluble recombinant CD4 molecules.

Because the interface of the domain-swapped dimer is rather small, one may wonder if it is stable enough, with respect to the monomeric form. As a matter of fact, contacts between pairs of D3 or D4 domains could also contribute to dimer stabilization. Indeed, the C-terminal ends of the D2 domains were found to be close to each other in the proposed swapped-dimer conformation (see Fig.1). However, because loop E-F is involved in the interface between the D2 and D3 domains,[33] its conformational change as a consequence of domain swapping may also destabilize this interface, which has already been shown to

allow for segmental flexibility of soluble CD4.[33] This may induce some large conformational change of the whole extracellular part of CD4, already suspected to be required for HIV-1 entry into cells,[34–37] allowing cell and virus membranes to come closer to each other.[29]

CONCLUSIONS

We have shown that, in the case of the N-terminal domain of CD2, it is possible to identify the experimentally known linker in the domain-swapped conformation,[12] starting from the configuration of the standard contact dimer, using a protocol based on a combination of energy minimization and MD techniques. Interestingly, in the lowest-energy domain-swapped dimers thus obtained, the conformation of the linker is found to be less than 2.0 Å away from its crystallographic conformation.

Applying this protocol to the case of domain 2 of CD4, starting from the CD4 dimer configuration found in crystal structure 1g9m,[17] we have then shown that domain swapping is likely to occur, loop E-F being the linker. This provides a straightforward explanation for the fact that CD4 dimers can be disulfide bonded,[6,7] the two cysteines of CD4 D2 being involved in the two corresponding disulfide bridges,[7] while they are known to be deeply buried in the monomer.[8,9]

In order to confirm that domain swapping does occur in domain 2 of CD4, and that loop E-F is indeed the linker, strategies previously used in order to stabilize domain-swapped conformations may prove to be useful.[13] For instance, shortening the length of the linker of cyanovirin-N yielded an obligate domain-swapped dimer.[38] Engineering such obligate CD4 swapped dimers may pave the way for subsequent studies of the role of CD4 dimers during the first steps of HIV infection.

REFERENCES

1. Sattentau Q, Dalgleish A, Weiss R, Beverley P. Epitopes of the CD4 antigen and HIV infection. Science 1986;234:1120–1123.
2. Clayton L, Hussey R, Steinbrich R, Ramachandran H, Husain Y, Reinherz E. Substitution of murine for human CD4 residues identifies amino acids critical for HIV-Gp120 binding. Nature 1988;335:363–366.
3. Mizukami T, Fuerst T, Berger E, Moss B. Binding region for human immunodeficiency virus (HIV) and epitopes for HIV-blocking monoclonal antibodies of the CD4 molecule defined by site-directed mutagenesis. Proc Natl Acad Sci USA 1988;85:9273–9277.
4. Jameson B, Rao P, Kong L, Hahn B, Shaw G, Hood L, Kent S. Location and chemical synthesis of a binding site for HIV-1 on the CD4 protein. Science 1988;240:1335–1339.
5. Ashkenazi A, Presta L, Marsters S, Camerato T, Rosenthal K, Fendly B, Capon D. Mapping the CD4 binding site for human immunodeficiency virus by alanine-scanning mutagenesis. Proc Natl Acad Sci USA 1990;87:7150–7154.
6. Lynch G, Sloane A, Raso V, Lai A, Cunningham A. Direct evidence for native CD4 oligomers in lymphoid and monocytoid cells. Eur J Immunol 1999;29:2590–2602.
7. Matthias L, Yam P, Jiang X, Vandegraaff N, Li P, Poumbourios P, Donoghue N, Hogg P. Disulfide exchange in domain 2 of CD4 is required for entry of HIV-1. Nat Immunol 2002;3(8):727–732.
8. Wang J, Yan Y, Garret T, Liu J, Rodgers D, Garlick R, Tarr G, Husain Y, Reinherz E, Harrison S. Atomic structure of a fragment of human CD4 containing two immunoglobulin-like domains. Nature 1990;348:411–418.
9. Ryu S, Kwong P, Truneh A, Porter T, Arthos J, Rosenberg M, Dai X, Xuong N, Axel R, Sweet R, Hendrickson W. Crystal structure of an HIV-binding recombinant fragment of human CD4. Nature 1990;348:419–426.
10. Rauk A. Chiroptical properties of disulfides–*ab initio* studies of dihydrogen disulfide and dimethyl disulfide. J Am Chem Soc 1984;106(22):6517–6524.
11. Katz B, Kossiakoff A. The crystallographically determined structures of atypical strained disulfides engineered into subtilisin. J Biol Chem 1986;261(33):15480–15485.
12. Murray A, Lewis S, Barclay A, Brady R. One sequence, two folds: a metastable structure of CD2. Proc Natl Acad Sci USA 1995;92:7337–7341.
13. Liu Y, Eisenberg D. 3D domain swapping: as domains continue to swap. Protein Sci 2002;11:1285–1299.
14. Bennett M, Choe S, Eisenberg D. Domain swapping: entangling alliances between proteins. Proc Natl Acad Sci USA 1994;91(8):3127–3131.
15. Knaus K, Morillas M, Swietnicki W, Malone M, Surewicz W, Yee V. Crystal structure of the human prion protein reveals a mechanism for oligomerization. Nat Struct Biol 2001;8(9):770–774.
16. Bodian D, Jones E, Harlos K, Stuart D, Davis S. Crystal structure of the extracellular region of the human cell adhesion molecule CD2 at 2.5 Å resolution. Structure 1994;2(8):755–766.
17. Kwong P, Wyatt R, Robinson J, Sweet R, Sodroski J, Hendrickson W. Structure of an HIV Gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. Nature 1998;393:648–659.
18. Wang J, Meijers R, Xiong Y, Liu J, Sakihama T, Zhang R, Joachimiak A, Reinherz E. Crystal structure of the human CD4 N-terminal two-domain fragment complexed to a class II MHC molecule. Proc Natl Acad Sci USA 2001;98(19):10799–10804.
19. http://www.cmbi.kun.nl/gv/servers/WIWWWI.
20. Humphrey W, Dalke A, Schulten K. VMD-visual molecular dynamics. J Molec Graphics 1996;14:33–38.
21. Kraulis P. Molscript: a program to produce both detailed and schematic plots of protein structures. J Appl Cryst 1991;24:946–950.
22. Brooks B, Bruccoleri R, Olafson B, States D, Swaminathan S, Karplus M. CHARMM: a program for macromolecular energy, minimization, and dynamics calculations. J Comp Chem 1983;4:187–217.
23. Lazaridis T, Karplus M. Effective energy function for proteins in solution. Proteins 1999;35(2):133–152.
24. Seok C, Rosen J, Chodera J, Dill K. MOPED: method for optimizing physical energy parameters using decoys. J Comput Chem 2003;24(1):89–97.
25. Janowski R, Kozak M, Jankowska E, Grzonka Z, Grubb A, Abrahamson M, Jaskolski M. Human cystatin C, an amyloidogenic protein, dimerizes through three-dimensional domain swapping. Nat Str Biol 2001;8(4):316–320.
26. Liu Y, Hart P, Schlunegger M, Eisenberg D. The crystal structure of a 3D domain-swapped dimer of RNase A at a 2.1 Å resolution. Proc Natl Acad Sci USA 1998;95(7):3437–3442.
27. Liu Y, G G, Libonati M, Eisenberg D. A domain-swapped RNAse A dimer with implications for amyloid formation. Nat Str Biol 2001;8(3):211–214.
28. Sanejouand YH. Normal-mode analysis suggests important flexibility between the two N-terminal domains of CD4 and supports the hypothesis of a conformational change in CD4 upon HIV binding. Prot Engin 1996;9(8):671–677.
29. Sanejouand YH. Rôle du changement de conformation du CD4 lors de la fusion VIH/cellule. C R Acad Sci Paris, Sciences de la vie 1997;320:163–170.
30. Peterson A, Seed B. Genetic analysis of monoclonal antibody and HIV binding sites on the human lymphocyte antigen CD4. Cell 1988;54:65–72.
31. Burkly L, Olson D, Shapiro R, Winkler G, Rosa J, Thomas D, Williams C, Chisholm P. Inhibition of HIV infection by a novel CD4 domain 2-specific monoclonal antibody: dissecting the basis for its inhibitory effect on HIV-induced cell fusion. J Immunol 1992;149(5):1779–1787.
32. Moore J, Sattentau Q, Klasse P, Burkly L. A monoclonal antibody to CD4 domain 2 blocks soluble CD4-induced conformational changes in the envelope glycoproteins of human immunodefi-

ciency virus type 1 (HIV-1) and HIV-1 infection of CD4+ cells. J Virol 1992;66(8):4784–4793.

33. Wu H, Kwong P, Hendrickson W. Dimeric association and segmental variability in the structure of human CD4. Nature 1997;387: 527–530.

34. Healey D, Dianda L, Moore J, McDougal J, Moore M, Estess P, Buck D, Kwong P, Beverley P, Sattentau Q. Novel anti-CD4 monoclonal antibodies separate human immunodeficiency virus infection and fusion of CD4+ cells from virus binding. J Exp Med 1990;172(4):1233–1242.

35. Bachelder R, Bilancieri J, Lin W, Letvin N. A human recombinant Fab identifies a human immunodeficiency virus type 1-induced conformational change in cell surface-expressed CD4. J Virology 1995;69(9):5734–5742.

36. Yachou A, Sekaly R. Binding of soluble recombinant HIV envelope glycoprotein, rGp120, induces conformational changes in the cellular membrane-anchored CD4 molecule. Biochem Biophys Res Commun 1999;265(2):428–433.

37. Murray J, Hu Q, Navenot J, Peiper S. Role of CD4 hinge region in Gp120 utilization by immunoglobulin domain 1. Biochem Biophys Res Commun 2002;292(2):449–455.

38. Kelley B, Chang L, Bewley C. Engineering an obligate domain-swapped dimer of cyanovirin-N with enhanced anti-HIV activity. J Am Chem Soc 2002;124(13):3210-3211.