Structural role of a detergent molecule in retinoic acid nuclear receptor crystals

Bruno P. Klaholz and Dino Moras*

Laboratoire de Biologie Structurale, Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS/INSERM/ULP, 1 Rue Laurent Fries, BP 163, F-67404 Illkirch CEDEX, France

Correspondence e-mail:
moras@igbmc.u-strasbg.fr

The human nuclear receptor of retinoic acid hRARγ is a ligand-dependent transcription regulator. The presence of a completely ordered dodecyl-α-D-maltoside molecule in the crystal structure of the hRARγ ligand-binding domain (LBD) refined at 1.3 Å resolution is reported. The non-ionic detergent is required for stabilization and crystallization of the hRARγ LBD and mediates a crystal contact in the region where coactivator proteins bind. Its dodecyl moiety is buried in a hydrophobic channel, whereas the maltoside head group is hydrogen bonded to water molecules and polar residue side chains.

1. Introduction

Detergents are usually employed for solubilization and functional reconstitution of membrane proteins in order to replace the heterogeneous natural lipids of the membrane by a homogeneous detergent environment prior to crystallization assays (Ostermeier & Michel, 1997). However, detergents have also been necessary for stabilization and crystallization of the nuclear receptor hRARγ LBD (human retinoic acid receptor ligand-binding domain; Rochel et al., 1997; Renaud et al., 1995; Klaholz et al., 2000), a nuclear protein with an antiparallel α-helical sandwich fold that regulates transcription in a ligand-dependent manner (for a review, see Moras & Gronemeyer, 1998). In vivo, several protein partners can interact with RAR and protect parts of solvent-exposed hydrophobic surfaces: the heterodimerization partner RXR (9-cis retinoic acid receptor) covers 980 Å^2 of the RAR LBD (Bourguet et al., 2000) and coactivator proteins that mediate transcription activation can bind to a hydrophobic cleft of the nuclear receptors (Feng et al., 1998); a peptide of the coactivator SRC-1 (steroid receptor coactivator) covers ~710 Å^2 of the RAR-homologous protein PPARγ (peroxisome proliferator-activated receptor; Nolte et al., 1998). Crystallization of the monomeric hRARγ LBD was carried out with the non-denaturing zwitterionic detergent 3-(3-cholamidopropyl)-dimethyl-ammonio-1-propanesulfonic acid (CHAPS) and the mild non-ionic detergent dodecyl-α-D-maltoside (Fig. 1a). The latter was used at concentrations close to the critical micelle concentration (CMC = 0.15–0.18 mM). The crystal structures of several retinoids bound to the hRARγ LBD have been reported at resolutions between 2 and 2.5 Å (Renaud et al., 1995; Klaholz et al., 1998). Recently, high-resolution (1.3–1.5 Å) diffraction data collected at liquid-nitrogen temperature have revealed a completely ordered molecule of dodecyl-α-D-maltoside in the hRARγ LBD crystals (Klaholz et al., 2000). This is remarkable as visualization of detergents is often limited by their intrinsic flexibility, which leads to disorder, low occupancies and high B factors, as reported e.g. for n-octyltetraoxyethylene (Kreusch et al., 1994), octyl-β-glucoside (Egloff et al., 1995) and N,N-dimethyldo-N-oxide (Deisenhofer et al., 1995). In this paper, we will focus on a crystal contact that leads to an unusual interaction pattern between RARγ and the detergent molecule trapped at the coactivator-binding site.
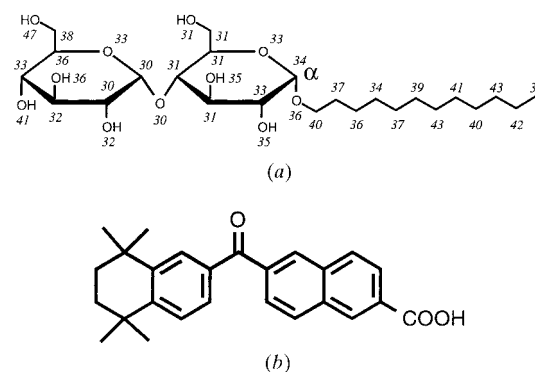

Figure 1
(a) Chemical structure of dodecyl-α-D-maltoside; numbers indicate average values for individually and anisotropically refined B factors of the detergent molecule bound in the crystal of the hRAR γLBD/CD564 complex. (b) Chemical structure of the RARβ,γ-selective agonist retinoid CD564 (Delescluse et al., 1991).

2. Material and methods

Protein purification and crystallization procedures have been described elsewhere (Rochel *et al.*, 1997; Klaholz *et al.*, 2000). Briefly, the histidine-tagged hRARγ LBD was overproduced using an *Escherichia coli*/T7 system and purified by nickel-chelate

hRARγ$_{sym}$ hRARγ

(a)

(b)

affinity chromatography and gel filtration before addition of the RARβ,γ-selective retinoid CD564 (Delescluse *et al.*, 1991; Fig. 1*b*). Detergents were added at the gel-filtration step: the gel-filtration buffer contained 500 m*M* NaCl, 10 m*M* Tris–HCl pH 7.0, 5 m*M* DTT, 4% glycerol, 2 m*M* CHAPS (Boehringer Mannheim; CMC = 7.4 m*M*) and 0.15 m*M* dodecyl-α-D-malto-side (Sigma). Crystallization, X-ray diffraction data collection and the detailed statistics of the data processing (R_{sym} = 3.1%, completeness = 92.6% for 1.30–18 Å resolution data, space group $P4_12_12$, one molecule per asymmetric unit, unit-cell parameters $a = b = 59.85$, $c = 155.16$ Å) and structure refinement [R_{cryst} = 13.3%, R_{free} = 16.4%, root-mean-square deviation (r.m.s.d.) for bond lengths = 0.014 Å; r.m.s.d. for bond angles = 2.40°, anisotropic *B*-factor refinement with *SHELXL*; Sheldrick & Schneider, 1997) are described elsewhere (Klaholz *et al.*, 2000). The dodecyl-α-D-maltoside molecule was detected at the beginning of the refinement, fitted to a σ_A-weighted $F_o - F_c$ omit map (Fig. 2*c*; Read, 1986) and included in subsequent refinement cycles (final average *B* factor = 35.8 Å^2, full occupancy; average protein *B* factor = 24.1 Å^2; estimated average *B* factor from the Wilson plot = 19.1 Å^2). The superposition of the crystal structures of the hRARγ LBD/CD564 complex and of the ternary complex PPARγ LBD/SRC-1 peptide/BRL49653 (rosiglitazone) using the

subunit monomer that contains the L*xx*LL motif 2 (residues 690–694, sequence LHRLL) was obtained by a least-squares fit in *O* (Jones *et al.*, 1991) including the structurally conserved parts of the LBDs (r.m.s.d. on C$^\alpha$ atoms = 0.85 Å).

3. Results and discussion

The dodecyl-α-D-maltoside molecule is tightly bound between two symmetry-related protein molecules (hRARγ and hRARγ$_{sym}$; Fig. 2*a*); helices H3, H4 and H12 of hRARγ are in contact with helices H9, H10 and with the loop between H8 and H9 (L8/9) of hRARγ$_{sym}$. Upon binding of agonist ligands, in the present case the synthetic retinoid CD564 (Delescluse *et al.*, 1991; Klaholz *et al.*, 2000), the C-terminal transactivation helix H12 adopts the so-called agonist position that together with H3 and H4 generates a surface for coactivator binding (Moras & Gronemeyer, 1998). H9, H10 and L8/9 of hRARγ$_{sym}$ correspond to the heterodimer interface within RAR/RXR complexes (Bourguet *et al.*, 2000). Thus, the crystal contact covers hydrophobic side chains of residues from both the dimer interface and the coactivator binding. This generates a cavity of appropriate size for the α-isomer of dodecyl-D-maltoside (Figs. 2*a* and 2*b*). The β-isomer would be difficult to fit to the cavity; other detergents with shorter aliphatic chains and polar heads such as octyl-β-glucoside would provide fewer contacts.

Binding of the detergent molecule in the cavity increases the interface between symmetry-related protein molecules from 730 to 1010 Å^2 (+38%). The resulting stronger crystal contact may contribute to the good diffraction potential of the crystals. 300 Å^2 of the detergent surface covers 42% of the coactivator binding site, whereas 350 Å^2 covers 36% of the heterodimer interface of the symmetry-related molecule. Thus, 83% of the total detergent molecule surface (785 Å^2) is buried, the maltoside head being oriented towards the solvent (Fig. 2*b*).

The σ_A-weighted $F_o - F_c$ omit map clearly delineates the complete detergent molecule (Fig. 2*c*). Both glucose monomers exhibit the low-energy chair-like conformation and the hydroxyl groups occupy the equatorial positions. The bent shape of the detergent molecule is a result of its adaptation to the cavity. Flexibility is essentially a consequence of the conformational freedom of the dodecyl moiety and to a lesser extent of the rotation of the methylenehydroxyl
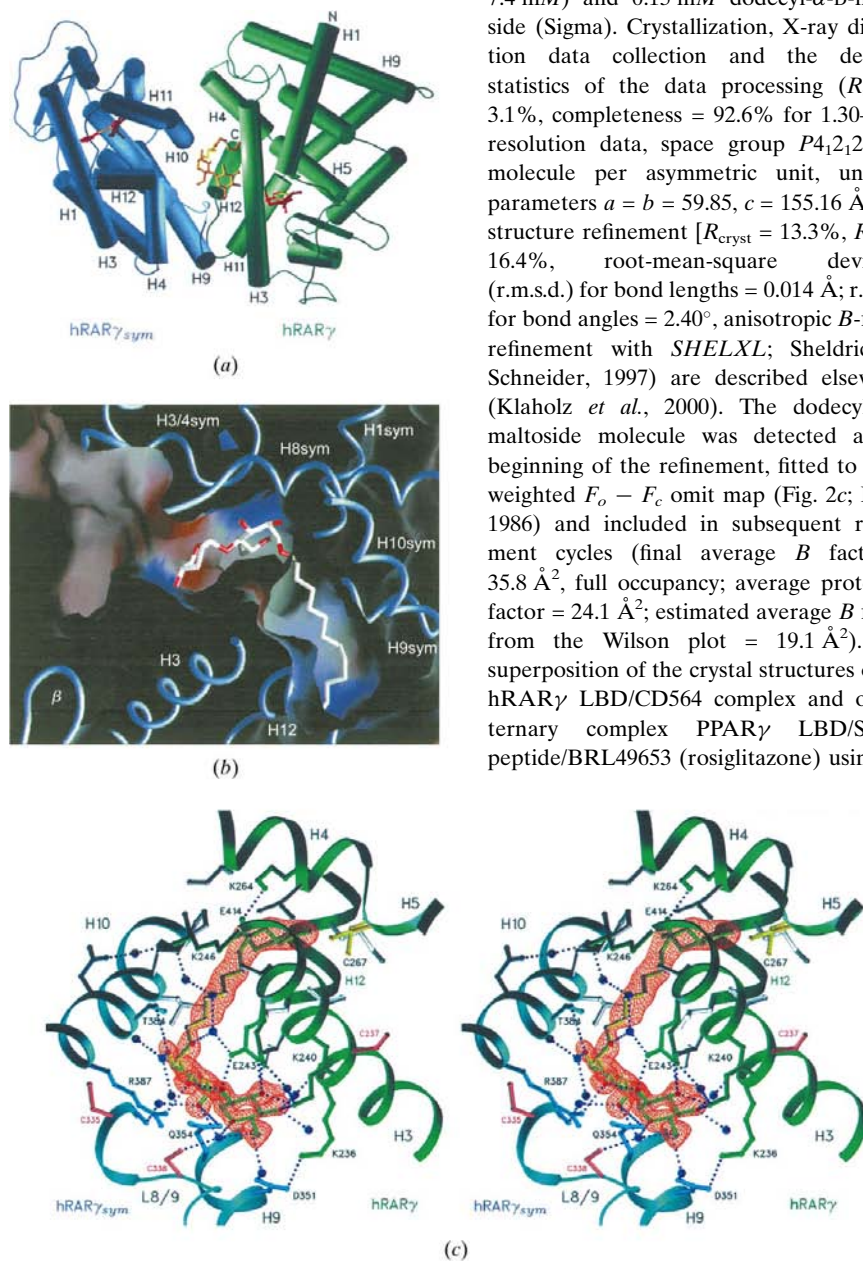

(c)

Figure 2
(*a*) The dodecyl-α-D-maltoside molecule (in yellow) mediating a crystal contact between two molecules of the hRARγ LBD/CD564 complex (space group $P4_12_12$; symmetry operators $x - \frac{1}{2}$, $-y + \frac{3}{2}$, $-z + \frac{3}{4}$). The retinoid CD564 is shown in red. H1 to H12 denote helices and N and C denote the N- and C-termini of the protein, respectively. (*b*) *GRASP* representation of the cavity. 83% of the detergent molecule surface are buried within the crystal contact. Calculations of excluded areas were performed with *GRASP* (Nicholls *et al.*, 1991). (*c*) The σ_A-weighted $F_o - F_c$ omit map (coloured orange; stereo representation) at 1.30 Å resolution is contoured at 2.5σ and reveals the bent shape of the dodecyl-α-D-maltoside molecule. The dodecyl moiety is buried in a hydrophobic channel generated by helices H3, H4 and H12 of hRARγ (coloured green) and loop L8/9 and helices H9 and H10 of hRARγ$_{sym}$ (coloured blue). The O atoms of the detergent are coloured red and the C atoms grey. Hydrophobic side chains are indicated in grey, water molecules in blue and the mercury-binding cysteines in pink. The double conformation of Cys267 is shown in yellow. The positions of Lys246, Lys264 and Glu414 can be compared with those described in Fig. 3. Figs. 2(*a*), 2(*c*) and 3 were created with *SETOR* (Evans, 1993).
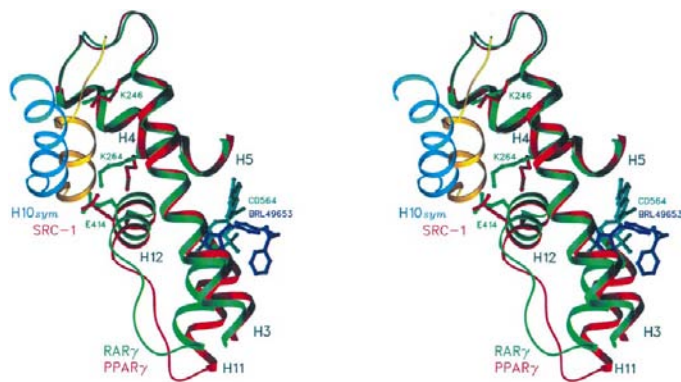
Figure 3
Stereo representation of the superposition of the hRARγLBD/CD564 complex and the ternary complex PPARγ LBD/SRC-1 peptide/BRL49653 as obtained by a least-squares fit of the crystal structures illustrates that the dodecyl-α-D-maltoside molecule binds at the coactivator-binding site of RAR; the PPAR protein part is coloured red. H10 of hRARγ$_{sym}$ (blue) exhibits the same orientation as the SRC-1 helix (yellow).

groups providing optimal geometries for hydrogen bonds. All but one of the O atoms from the hydroxyl and acetal groups of the maltoside moiety are linked to the protein by at least one hydrogen bond (Fig. 2c). This explains why the dodecyl-α-D-maltoside molecule is well ordered: the temperature factors of the maltoside group are in the range 30–36 Å^2 for the sugar skeleton (Fig. 1a) and vary from 34 to 43 Å^2 along the dodecyl moiety, with a maximum where the chain exhibits a slight kink towards Cys267 (Fig. 2c). The average B factor of residues in the cavity is 16–18 Å^2 (C$^\alpha$ atoms), 17–18 Å^2 (side chains close to the dodecyl moiety), 20–30 Å^2 (side chains close to the maltoside head) and ~40 Å^2 (water molecules in the vicinity of the maltoside head).

The detergent molecule fits the cavity with an astonishing complementarity between its amphipathic character and the hydrophobic or polar protein side chains. The dodecyl moiety is surrounded by a number of hydrophobic side chains, whereas the hydroxyl moieties of the polar maltoside head form numerous direct and water-molecule-mediated hydrogen bonds to the side chains of residues Lys236, Lys240 and Glu243 of hRARγ and Cys338, Asp351, Gln354, Arg387 and Thr384 of hRARγ$_{sym}$ (Fig. 2c). Note that Glu243 adopts two distinct side-chain conformations providing alternative hydrogen bonds.

Interestingly, the detergent protects Cys267 from the reaction with p-chloro-mercuribenzylsulfonate that was used as a derivative for phasing of the data from the hRARγ LBD/all-trans retinoic acid complex

(Renaud et al., 1995). In contrast, the three cysteine residues in proximity to the maltoside moiety are accessible and provided mercury-binding sites (Cys237, Cys335 and Cys338; Fig. 2c).

The superposition of the structures of the hRAR γLBD/CD564 complex and the ternary complex of a short peptide of the coactivator SRC-1 bound to PPARγ LBD/rosiglitazone (BRL49653) reveals that the dodecyl-α-D-maltoside molecule occupies the coactivator-binding site (Fig. 3). Helix H10 of the symmetry-related molecule hRARγ$_{sym}$ and the helix of the SRC-1 peptide exhibit the same orientation, but with a marked shift of H10 (3–4 Å between C$^\alpha$ atoms of the LxxLL motif 2 of SRC-1 and H10 that contains the sequence PRMLM). The general crystal packing and the detergent molecule prevent H10 from specific interactions with residues Lys246, Lys264 and Glu414, whereas the corresponding residues in PPARγ form a 'charge clamp' (Nolte et al., 1998) with the backbone of the SRC-1 peptide (Figs. 2c and 3). This crystal contact provides a further example of the interaction of amphipathic helices with the coactivator-binding site, as reported for sequences from nuclear receptors (helix H12), coactivators and corepressors related to the LxxLL motif (Nolte et al., 1998; Perissi et al., 1999; Nagy et al., 1999).

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