Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary crystallographic results of apo and complex forms of human dehydroepiandrosterone sulfotransferase

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Received 5 February 2001
Accepted 29 June 2001

Dehydroepiandrosterone sulfotransferase converts dehydroepiandrosterone (DHEA) and some other steroids to their sulfonated forms. The human enzyme has been crystallized in the presence of substrate (DHEA) alone, in the presence of substrate and non-sulfated cofactor analogue (PAP) and in the absence of both substrate and PAP in our laboratory, with data sets collected at a synchrotron source. The crystals of the uncomplexed form belong to the orthorhombic space group $C222_1$, with unit-cell parameters $a = 85.26$, $b = 87.69$, $c = 108.20$ Å and data 99.2% complete to 2.35 Å resolution. The DHEA complex crystallizes in the orthorhombic space group $P2_12_12$, with unit-cell parameters $a = 74.46$, $b = 127.49$, $c = 44.59$ Å and data 92.9% complete to 2.15 Å resolution. The ternary complex crystallizes in the orthorhombic space group $P2_12_12_1$, with unit-cell parameters $a = 62.25$, $b = 87.28$, $c = 138.86$ Å and data 98.6% complete to 2.50 Å resolution. Preliminary molecular-replacement solutions indicate significant variations in dimer formation.

1. Introduction

In humans, the adrenal gland secretes large amounts of dehydroepiandrosterone sulfate (DHEA-S) and dehydroepiandrosterone (DHEA) throughout adult life (Labrie, 1991). Although DHEA-S and DHEA levels are higher than any other steroids except cholesterol, they do not have intrinsic biological activity. After circulation to peripheral target tissues, they are first converted to androstenedione (Δ4-dione) and then to either active androgens or estrogens (Adams, 1985; Labrie *et al.*, 1985). The Δ4-dione can either be transformed into androgenic testosterone (T) or dehydrotestosterone (DHT) or into estradiol (E_2) depending upon the levels of 17β-hydroxysteroid dehydrogenase (17β-HSD), 3β-hydroxysteroid dehydrogenase (3β-HSD), 5α-reductase and aromatase activities present in the different peripheral tissues. In post-menopausal and young women, 80–100% of androst-5-ene-3β,17β-diol (Δ5-diol), another important estrogen, is converted directly from DHEA by 17β-HSD. Thus, DHEA-S and DHEA as well as Δ4-dione are considered to be precursor sex steroids. It is estimated that about 30–50% of androgens in men and the majority of estrogens in women (75% and close to 100% for those post-menopause) are produced in peripheral tissues from precursor steroids of adrenal origin (Labrie, 1991).

There is increasing interest in steroid sulfonation studies, especially the production of DHEA-S. Sulfonation generally results in the detoxification of chemical compounds, as it decreases the hydrophobicity of the steroids (Jakoby *et al.*, 1980). DHEA-S, which is the main circulating form of DHEA, has also been shown to be much more stable than DHEA (Longcope, 1996). The enzyme dehydroepiandrosterone sulfotransferase (DHEA-ST; E.C. 2.8.2.2.) catalyzes the sulfonation of DHEA on the 3α oxygen with 3′-phosphoadenosine-5′-phosphosulfate (PAPS) as a cofactor. DHEA-ST has been characterized from different sources; for example, from rodent, guinea pig, bovine and human liver, and from bovine and human adrenal tissues (Gura *et al.*, 1990; Demyan *et al.*, 1992; Oeda *et al.*, 1992; Driscoll *et al.*, 1993; Wilborn *et al.*, 1993; Luu-The *et al.*, 1996.) The function and the regulation of human DHEA-ST have been extensively studied (Falany *et al.*, 1995; Luu-The *et al.*, 1996; Parker *et al.*, 1995, 1998). The crystal structure of the human form of this enzyme has been solved by X-ray crystallography (Pedersen *et al.*, 2000). Although the enzyme crystallized in the presence of both the desulfonated cofactor (PAP) and the preferred substrate, the substrate was excluded from the active site by protein-loop conformations. We began the study before the structure was published and continue with the intention of more closely examining substrate binding and the relationship between cofactor and substrate. The three-dimensional structure of a related enzyme estradiol sulfotransferase

complexed with substrate and cofactor analogues has been solved by X-ray diffraction (Kakuta *et al.*, 1997).

Following the cloning and characterization of the DHEA-ST gene (Luu-The *et al.*, 1995), we have overexpressed it in *Escherichia coli* and purified DHEA-ST to homogeneity (Luu-The *et al.*, 1995; Chang *et al.*, 2001). Here, we report the crystallization and preliminary X-ray diffraction results of this enzyme in an apo form and co-crystallized with DHEA alone and as a DHEA–PAP–enzyme ternary complex.

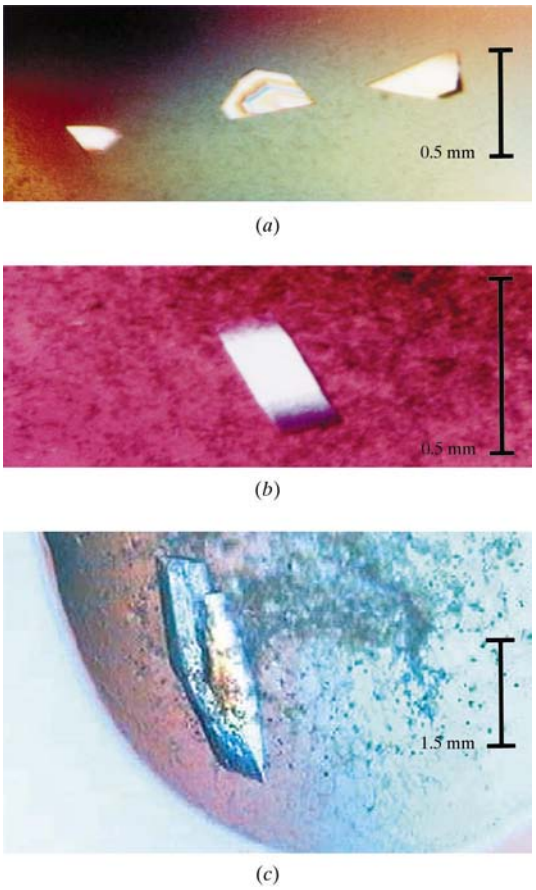

Figure 1
DHEA-ST crystals were obtained in a hanging drop by vapor diffusion. (*a*) Crystals of DHEA-ST. Equal volumes of enzyme stock at 20 mg ml^{-1} and reservoir solution containing 250 m*M* $(NH_4)_2SO_4$ and 27.5% PEG 4000 pH 8.5 were mixed to initiate the crystallization. The crystals appeared in 2–3 d and grew to dimensions of 0.25 × 0.25 × 0.25 mm after 7–10 d. (*b*) Crystal of DHEA-ST complexed with its substrate DHEA. The reservoir contained 1.2 m*M* $CoCl_2$ and 170 m*M* $(NH_4)_2SO_4$ pH 7.5. The protein sample was at 12 mg ml^{-1} with 0.5 m*M* DHEA. The hanging drop was initiated by mixing of protein and reservoir solution in a 2:3(*v*/*v*) ratio. Crystals appeared after 2–3 d and matured in 7–10 d. (*c*) Crystal of DHEA-ST complexed with its substrate DHEA and PAP. The reservoir contained 8% PEG 4000, 0.1 *M* sodium acetate pH 4.6. The protein sample consisted of 14 mg ml^{-1} DHEA-ST and 4 m*M* PAP in 10 m*M* Tris pH 7.5, 0.5 m*M* EDTA and 0.01% β-OG. The hanging drop was initiated by combining 1.5 µl of protein and reservoir solutions followed by the addition of 0.225 µl 5 m*M* DHEA. The crystals grew to their full size in 3–4 d.

2. Materials and methods

Human DHEA-ST was purified in our laboratory. Briefly, recombinant human dehydroepiandrosterone sulfotransferase expressed as a glutathione sulfotransferase fusion protein in *E. coli* was purified using glutathione Sepharose 4B affinity chromatography, a Factor Xa cleavage step and Q-Sepharose Fast Flow column chromatography (Chang *et al.*, 2001). The substrate DHEA, the detergent *n*-octyl β-D-glucopyranoside (β-OG) and chemicals such as trizma base (Tris), ammonium sulfate, lithium sulfate and cobalt chloride were purchased from Sigma–Aldrich Ltd (Ontario, Canada). Polyethylene glycol (PEG) 4000 was from Fluka. The sparse-matrix screening kits were from Hampton Research (California, USA). The Bio-Rad protein-assay dye reagent concentrate was from Bio-Rad Ltd (Ontario, Canada). Centricon 30K was from Millipore, Inc. (Ontario, Canada).

After purification, human DHEA-ST was concentrated to 20–25 mg ml^{-1} (determined by the Bio-Rad protein assay method; Bradford, 1976) in a buffer containing 10 m*M* Tris pH 7.5, 0.5 m*M* EDTA and 0.1% β-OG using a Centricon 30K. The concentrated enzyme can be stored at 277 K for at least one month. Crystallization of DHEA-ST and its complexed forms were carried out in parallel using the hanging-drop method of vapour diffusion (McPherson, 1999) with the sparse-matrix kits I and II (Jancarik & Kim, 1991) for condition screening. The initial conditions for the three forms were found with the first attempt, *i.e.* sparse-matrix screening kit I No. 17 for DHEA-ST alone, sparse matrix II No. 25 for DHEA-ST–DHEA and sparse matrix II No. 37 for DHEA-ST–DHEA–PAP. The conditions were then optimized.

The X-ray diffraction analyses of the three crystal forms were carried out at the X8C beamline at the National Synchrotron Light Source, Brookhaven National Laboratory, Upton, New York, USA using a Quantum-IV CCD imaging-plate detector. The data sets were collected under cryoconditions. All crystal forms used glycerol and mineral oil as cryoprotectant.

Molecular replacement and map calculation were carried out with the *CNS* software package (Brunger *et al.*, 1998). Models and maps were visualized with *O* (Jones *et al.*, 1991). The human DHEA-ST structure in complex with PAP (Pedersen *et al.*, 2000) was used as the search model.

3. Results

DHEA-ST crystals were obtained by mixing 2.4 µl of enzyme solution with 2.4 µl reservoir solution in the hanging drop. The enzyme stock concentration was 20 mg ml^{-1} and the reservoir contained 100 m*M* Tris pH 8.5, 250 m*M* $(NH_4)_2SO_4$ and 27.5% PEG 4000. The crystals appeared in 2 d and grew to dimensions of 0.25 × 0.25 × 0.25 mm (Fig. 1*a*) after 7–10 d. Crystals were moved through mineral oil before being flash-frozen on the beamline. These trapezoidal crystals are *C*-centered orthorhombic, with unit-cell parameters a = 85.26, b = 87.69, c = 108.20 Å. Data were collected from a single crystal 180 mm from the detector. Since the mosaicity was determined to be approximately 1°, an oscillation angle of 2° for each image was chosen. A total of 92 images were collected and processed using the *HKL* package (Otwinowski & Minor, 1997). Although the data were nearly 100% complete at 2.22 Å, with the last resolution shell having an $I/\sigma(I)$ value greater than 2, the R_{sym} of the last resolution shell was too high. The resolution was lowered to 2.35 Å, as the R_{sym} value for this shell was more reasonable. The final R_{sym} was 6.3% with data 99.2% complete. Based on systematic absences, the space group was determined to be $C222_1$. The data collection is summarized in Table 1. V_M was calculated to be 2.99 Å^3 Da^{-1} (Matthews, 1968), assuming one subunit in the asymmetric unit and a calculated subunit mass of 33 794 Da. The solvent content of the crystal was therefore calculated to be 59%. The molecular-replacement translation function gave a single solution 3.7σ greater than the second peak. This solution corresponded to the strongest peak of the rotation function.

The DHEA-ST was also cocrystallized with DHEA in a hanging-drop method by vapor diffusion. The reservoir contained 100 m*M* Tris pH 7.5, 1.2 m*M* $CoCl_2$ and 170 m*M* $(NH_4)_2SO_4$. The protein stock contained 12 mg ml^{-1} (0.35 m*M*) DHEA-ST and 0.5 m*M* DHEA in the buffer described in §2. The hanging-drop crystallization was initiated by adding 1.8 µl of protein to 2.7 µl

Table 1
Data-processing statistics.

Values in parentheses refer to the highest resolution shell.

	DHEA-ST apo	Enzyme–DHEA complex	Enzyme ternary complex
No. of images	92	94	84
Oscillation range (°)	2.0	1.0	1.0
Space group	$C222_1$	$P2_12_12$	$P2_12_12_1$
Unit-cell parameters (Å)	a = 85.26, b = 87.69, c = 108.20	a = 74.46, b = 127.49, c = 44.59	a = 62.25, b = 87.28, c = 138.86
Mosaicity (°)	1.01	0.44	0.48
Resolution range (Å)	20–2.35 (2.43–2.35)	20–2.15 (2.23–2.15)	50–2.50 (2.59–2.50)
No. of measured reflections	118952 (11112)	86055 (8489)	83482 (7962)
No. of unique reflections	17184 (1692)	22121 (2234)	25906 (2577)
Redundancy	6.9 (6.6)	3.9 (3.8)	3.2 (3.1)
R_{sym}† (%)	6.3 (51.0)	4.9 (29.5)	6.7 (43.4)
Completeness (%)	99.2 (99.9)	92.9 (94.9)	98.6 (98.9)
$\langle I/\sigma(I)\rangle$	24.4 (3.5)	19.4 (3.3)	10.7 (2.2)

† $R_{sym} = \sum|I - \langle I\rangle|/\sum I$.

of reservoir solution. The crystals appeared after 2–3 d and matured in 7–10 d. 80% glycerol (1.5 µl) was added to the drop before the crystals were passed through mineral oil and flash-frozen on the beamline. These crystals have a thick plate shape (Fig. 1*b*) and are primitive orthorhombic, with unit-cell parameters a = 74.46, b = 127.49, c = 44.59 Å. Data were collected from a single crystal 160 mm from the detector. The mosaicity of these crystals was determined to be approximately 0.4° and therefore a 1° oscillation angle was chosen. A total of 94 images were collected and processed using the *HKL* package (Otwinowski & Minor, 1997). Data were processed to 2.15 Å with an R_{sym} of 4.9% and a completeness of 92.9%. Using a resolution cutoff based on a minimum $I/\sigma(I)$ of 2 for the last resolution shell, a slightly higher resolution value was possible with good completeness and a reasonable R_{sym} for the last resolution shell. Based on systematic absences, the space group was determined to be $P2_12_12$. The data collection is summarized in Table 1. V_M was calculated to be 3.13 Å^3 Da^{-1} (Matthews, 1968), assuming one subunit in the asymmetric unit. The solvent content of the crystal was therefore calculated to be 61%. The molecular-replacement translation function gave a single solution 6.4σ greater than the second peak. The solution corresponded to the strongest rotation peak.

DHEA-ST was also cocrystallized as a ternary complex with DHEA and the non-sulfonated cofactor analogue (PAP) by the hanging-drop method of vapour diffusion. The reservoir contained 100 m*M* sodium acetate pH 4.6 and 8% PEG 4000. The protein stock contained 14 mg ml^{-1} DHEA-ST and 4 m*M* PAP in the buffer described in §2. The crystallization in hanging drops was initiated by mixing 1.5 µl of protein with 1.5 µl of reservoir solution and then adding 0.225 µl of 5 m*M* DHEA. The crystals reached full size in 3–4 d. As cryoprotectant, 80% glycerol was added to the drop to a final concentration of 20%, after which the crystals were passed through mineral oil and flash-frozen on the beamline. These crystals have a thick irregular shape (Fig. 1*c*) and are primitive orthorhombic, with unit-cell parameters a = 62.25, b = 87.28, c = 138.86 Å. Data were collected from a single crystal 180 mm from the detector. The mosaicity of these crystals was determined to be approximately 0.5° and therefore a 1° oscillation angle was chosen. A total of 84 images were collected and processed using the *HKL* package (Otwinowski & Minor, 1997). Data were processed to 2.50 Å with an R_{sym} of 6.7% and a completeness of 98.6%. Using a resolution cutoff based on a minimum $I/\sigma(I)$ of 2 for the last resolution shell gave good completeness and a reasonable R_{sym} for the last resolution shell. Based on systematic absences, the space group was determined to be $P2_12_12_1$. The data collection is summarized in Table 1. V_M was calculated to be 2.79 Å^3 Da^{-1} (Matthews, 1968), assuming two subunits in the asymmetric unit. The solvent content of the crystal was therefore calculated to be 56%. Two molecular-replacement translation-function solutions (3.0 and 2.7σ greater than the third peak) corresponding the two subunits in the asymmetric unit were obtained. These solutions corresponded to the two strongest rotation-function peaks.

4. Discussion

Human DHEA-ST is the enzyme involved in the sulfonation of the steroid DHEA to form DHEA-S. The latter is more stable, binds better to albumin and has a higher hydrophilicity than its non-sulfonated counterpart. In the transport of this important precursor steroid from adrenal origin to periphery tissue, DHEA-S is the major circulating form in the blood.

As DHEA and DHEA-S are the precursors of the steroid hormones such as DHT, T, E_2 and Δ5-diol, dysfunction of DHEA-ST could lead to high levels of DHEA in the plasma and thus lead to dysfunction of the above important steroids. The abnormal regulation of the hormones may lead to sex-hormone-dependent tumours or diseases such as estrogen-related breast cancer and androgen-related prostate cancer. It has been reported that hyperandrogenicity in women is associated with high levels of DHEA arising from non-classical 3β-HSD deficiency. However, there is no evidence of mutations for the type 1 and type 2 3β-HSD genes (Luu-The *et al.*, 1996; Zérah *et al.*, 1994) and therefore dysfunction of DHEA-ST is a potential candidate for causing familial hyperandrogenicity. There is some evidence linking DHEA and DHEA-S to cardiovascular disease (Khaw, 1996) and it has also been reported that reduced hepatic content of DHEA-ST is related to chronic liver disease (Elekima *et al.*, 2000). Structural knowledge of DHEA-ST, especially in relation to substrate binding, will give us an opportunity to study in detail the mechanism of these diseases and the role of this enzyme in regulating sex hormones in the human body.

The structure of the binary complex of DHEA-ST and its cofactor analogue was recently reported by Pedersen *et al.* (2000). Even though these crystals were grown under saturating concentrations of DHEA, there was no substrate found at the active site. This has been attributed to the presence of a loop that penetrates the active site in a conformation stabilized through dimer interactions. Pedersen *et al.* (2000) suggest that it represents the physiological dimer in an inactive state. Although their crystallization conditions are quite different (0.8 *M* citrate, 80 m*M* cacodylate pH 5.75), the space group of the enzyme cocrystallized in the presence of both DHEA and PAP by our group is the same as that achieved by Pedersen *et al.* (2000), differing by an increase of approximately 10 Å in each unit-cell parameter. An examination of the crystal packing showed that the dimer interactions are essentially the same and the initial maps show clear density from the cofactor analogue and the active-site penetrating loop. Even though our crystals were grown in the presence of saturating DHEA

there was no substrate density found in the active site. A possible consideration is that the pre-mixing of the non-cognate cofactor and protein before the addition of DHEA may affect the substrate binding. DHEA-ST crystals obtained through the cocrystallization with DHEA were immediately destroyed when exposed to PAP, suggesting a possible conformational change upon binding of the non-cognate cofactor.

An examination of the packing of the three crystal forms of DHEA-ST described in this paper shows that the dimer arrangement described above for the PAP-containing crystal form ($P2_12_12_1$) is not found in either the DHEA-containing ($P2_12_12$) and apo ($C222_1$) crystal forms. The three-dimensional structure of a related enzyme, estradiol sulfotransferase (EST), complexed with substrate and cofactor analogues has been solved by X-ray diffraction (Kakuta *et al.*, 1997). The overall protein fold is very similar, although the enzymes have different substrate specificities and the sequence identity is quite low at 37.6%. The EST dimer differs from the 'inactive dimer' of DHEA-ST described by Pedersen *et al.* (2000) by a 110° rotation of one of the monomers. It is possible that the latter dimer arrangement (non-crystallographic) may have been a consequence of crystal packing and that the true biological dimer was defined by crystallographic symmetry. A comparison of the DHEA cocrystallized form with the PAP cocrystallized form shows just such a relationship, requiring an approximately 10° rotation in one direction for the two monomers to overlap, although in these cases the degree of interaction is not so strong as seen with the so-called 'inactive dimer'. The apo enzyme crystal form ($C222_1$) would require some translation but little rotation for overlap to occur. This would be far less rearrangement than would be necessary to convert the 'inactive dimer' of DHEA-ST/PAP to the dimer arrangement described in EST (Kakuta *et al.*, 1997). The determination of which arrangement constitutes the biological dimer requires further study.

Our preliminary results indicate that the crystal packing of the DHEA-ST crystallized in the presence of DHEA alone is significantly different from that crystallized in the presence of DHEA and PAP. The dimer interactions, which stabilize the active-site penetrating loop, are not present in the former structure; preliminary examination indicates significant changes in the active site, including the possibility of substrate binding. Work is in progress to refine these structures in order to examine the relationship between substrate and cofactor binding to the monomer and dimer arrangement. A comparison of substrate binding with that seen with estradiol sulfotransferase will lead to a more complete understanding of steroid sulfonation in general. The study of structure–function relationships of DHEA-ST would lead to a more complete understanding of the synthesis of these important precursor steroids and the related function of androgens and estrogens.

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