Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary crystallographic analysis of a partial extracellular fragment of a sperm membrane protein YWK-II/APPH related to the Alzheimer βA4-amyloid precursor protein

Maojun Yang,[a] Binbin Liu,[b] Xiaodong Zhang,[a] Weihong Zhou,[b] Feng Gao,[b] Hai Pang,[b] Shiying Miao,[a] Linfang Wang[a] and Zihe Rao[b,c]*

[a]National Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Peking Union Medical College, 5 Dong Dan San Tiao, Beijing 100005, People's Republic of China, [b]MOE Protein Science Laboratory and Laboratory of Structural Biology, Department of Biological Science and Biotechnology, Tsinghua University, Beijing 100084, People's Republic of China, and [c]National Human Genome Center, Beijing, People's Republic of China

Correspondence e-mail: raozh@xtal.tsinghua.edu.cn

Received 21 June 2002
Accepted 1 October 2002

Crystals of a partial extracellular fragment of a human sperm membrane protein YWK-II/APPH have been grown at 291 K using PEG 4000 as precipitant. The diffraction pattern of the crystal extends to 2.8 Å resolution at 100 K using Cu $K\alpha$ radiation. The crystals belong to space group $P2_12_12_1$, with unit-cell parameters a = 46.009, b = 67.387, c = 149.241 Å, $\alpha = \beta = \gamma = 90°$. The presence of two molecules per asymmetric unit gives a crystal volume per protein mass (V_M) of 3.51 $Å^3$ Da^{-1} and a solvent content of 64.6% by volume. A full set of X-ray diffraction data were collected to 2.8 Å resolution from the native crystal.

1. Introduction

Spermatogenesis is a series of highly ordered processes involving the expression of many genes under precise temporal and spatial regulation. To better understand the molecular mechanism involved in fertilization, we have initiated a study to elucidate the expression and function of genes encoding specific sperm proteins during various stages of spermatogenesis. In our previous studies, a novel cDNA (RSD-2; GenBank accession No. M31322) coding a rat sperm membrane protein, designated YWK-II protein, was screened from a rat testis cDNA expression library with a monoclonal antibody against human sperm membrane protein (Yan *et al.*, 1986, 1987, 1990). The deduced polypeptide contained a segment with high homology (70.6% identity) to the transmembrane-cytoplasmic domains of the βA4-amyloid precursor protein (APP) found in brain plaques of Alzheimer's disease patients. Using the RSD-2 insert in combination with rapid amplification of cDNA ends (RACE), the corresponding human gene (HSD-2; GenBank accession No. AF168956) was isolated from a human testis cDNA expression library. The human testis cDNA, HSD-2, consists of 3654 bp with an open reading frame of 2289 bp, encoding 763 amino acids. Northern blotting of various human tissue RNAs using the HSD-2 cDNA as a probe showed that the gene is transcribed ubiquitously. The YWK-II cDNA shares high homology with human placenta amyloid precursor protein homology (APPH), rat amyloid precursor-like protein 2 (APLP2) and murine centromeric DNA element I (CDEI) binding protein (CDEBP), which are apparently species-specific forms of the same component. Of the members of the APP superfamily, A4-amyloid precursor protein (APP) is the most illustrious component. The role of APP in the pathogenesis of Alzheimer's disease has been underscored by the discovery of mutations within its sequence (Goate, 1998). Information on the function of YWK-II protein is meagre compared with that on APP. Nonetheless, the high homology in their sequences suggests similar functional activities. For example, APLP2 exhibits neurite-outgrowth-promoting activity comparable to that of APP isoforms (Cappai *et al.*, 1999), APLP2 binds collagen type I, heparin and zinc(II) as adhesion molecules (Beher *et al.*, 1996; Bush *et al.*, 1994) and the cytoplasmic domain of YWK-II protein interacts with Go protein (Huang *et al.*, 2000).

The alternative splicing patterns of YWK-II protein gene isoforms and APP gene family members are also similar (Sprecher *et al.*, 1993; Sandbrink *et al.*, 1994*a*,*b*; von der Kammer *et al.*, 1994; Huang *et al.*, 2000). There are seven conservative regions of YWK-II protein: cysteine-rich growth factor-like region, negative region, KPI region, α-helix-rich region, coil-rich region, transmembrane region and cytoplasmic region (Fig. 1). The human YWK-II protein is immunolocalized on the surface of the midpiece, tail and equatorial regions of human sperm heads (Yan *et al.*, 1987). Components in these regions are considered to participate in the interaction between spermatozoa and oocytes and in the subsequent fusion of the membranes during fertilization. This proposal is supported by the findings that YWK-II antibodies blocked the

© 2003 International Union of Crystallography
Printed in Denmark – all rights reserved

penetration and fertilization of zona-free hamster eggs and/or arrested the growth of embryos at the two-cell stage but not at more advanced developmental stages (Wang *et al.*, 1987; Vanage *et al.*, 1992; Koide *et al.*, 2000; Kamada *et al.*, 2001; Takikawa *et al.*, 2001). A yeast two-hybrid system was used to screen a rat ovary cDNA library for potential ligands capable of interacting with the YWK-II protein (HSD226 region). Müllerian-inhibiting substance (MIS) was found to interact with the extracellular domain of YWK-II protein. The recombinant Müllerian-inhibiting substance can significantly increase the viability and longevity of human spermatozoa *in vitro*, presumably through binding to the YWK-II protein on the sperm membrane. The results of this study indicate that the extracellular domain of YWK-II sperm membrane protein may function as a receptor for Müllerian-inhibiting substance (Tian *et al.*, 2001).

In the present study, the crystallization and preliminary crystallographic analysis of a partial extracellular fragment of YWK-II protein are reported. The structure of this protein may be helpful in illustrating the function of YWK-II protein.

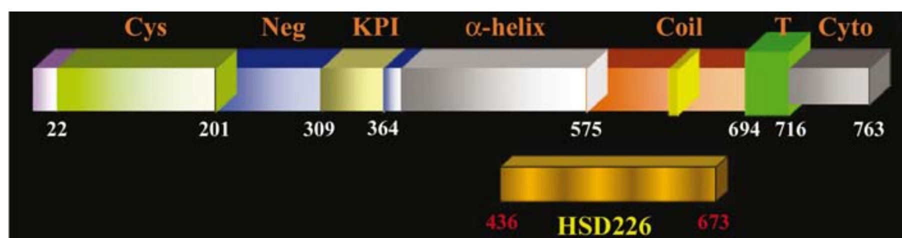

Figure 1
The conserved regions of YWK-II protein and the HSD226 region.

2. Materials and methods

2.1. Protein expression and purification

The expression, purification and crystallization of a partial extracellular fragment of the human sperm membrane protein YWK-II/APPH (HSD-2 cDNA 1306–2020 bp, without the splicing region 1837–1872 bp, encoding a total of 226 amino-acid residues; the protein coded was named HSD226) were performed according to established protocols. A PCR product containing the coding sequence of HSD226 was generated from the recombinant pUC19 plasmid carrying the HSD-2 gene. Two PCR primers, 5′-CAG CCA TGG TTA AAA GCT TTA GAG-3′ and 5′-CCT CGA GTT AGC CTC CAA CTC TCT CGG C-3′, were designed. The PCR product was restricted with *Nco*I and *Xho*I and purified and ligated into *Nco*I- and *Xho*I-restricted sites of the pET30a(+) vector (Novagen) with T_4 DNA ligase. A further transformation into *Escherichia coli* DH5α competent cells was performed and the positive clones with an insert of the right size were identified by double digestion with *Nco*I and *Xho*I. The sequence of the insert was verified by sequencing. The expected recombinant plasmid was transformed into *E. coli* strain BL21 (DE3) and the transformants were selected on LB agar plates containing 25 μg ml^{-1} kanamycin. The cells were then cultured at 310 K in LB medium containing 50 μg ml^{-1} kanamycin. When the culture density reached 0.6–0.7 (A_{600}), the culture was induced with 0.5 m*M* IPTG and grown for an additional 2.5 h before the cells were harvested.

The bacterial cell pellet was resuspended in lysis buffer (20 m*M* Tris–HCl pH 8.0, 500 m*M* NaCl, 10 m*M* imidazole, 1 m*M* PMSF, 1 m*M* β-mercaptoethenol) and was homogenized by sonication. The lysate was centrifugated at 20 000*g* for 25 min to remove the cell debris. The supernatant was applied to an Ni^{2+}-chelating column (1 ml Ni^{2+}-NTA agarose) and the contaminant protein was washed out with lysis buffer. The target protein was eluted with eluting buffer (20 m*M* Tris–HCl pH 8.0, 500 m*M* NaCl, 200 m*M* imidazole, 1 m*M* PMSF, 1 m*M* β-mercaptoethenol). The eluant was exchanged into buffer *A* (50 m*M* Tris–HCl pH 8.0, 100 m*M* NaCl, 1 m*M* β-mercaptoethenol) using a G-25 resin column and the gel-filtration chromatography method and applied to a Q-Sepharose High-Performance ion-exchange column (Pharmacia) (buffer *A* was 50 m*M* Tris–HCl pH 8.0, 100 m*M* NaCl, 1 m*M* β-mercaptoethenol and buffer *B* 50 m*M* Tris–HCl pH 8.0, 1.0 *M* NaCl, 1 m*M* β-mercaptoethenol). The protein was concentrated using an Ultrafree 10 000 NMWL filter unit (Millipore) and Superdex-75 (Pharmacia) (buffer *A*). The purified protein was analyzed on SDS–PAGE.

2.2. Crystallization

The purified protein was dialyzed into crystallization buffer (100 m*M* NaCl, 5 m*M* Tris–HCl pH 8.0, 5 m*M* DTT, 1 m*M* EDTA) and was concentrated to 20–30 mg ml^{-1} using an Ultrafree 10 000 NMWL filter unit. Protein concentrations were estimated spectroscopically by absorbance at 280 nm, assuming an A_{280} of 0.230 for a 1.0 mg ml^{-1} solution. Crystallization was performed using the hanging-drop vapour-diffusion method at 291 K in 16-well plates. Conditions were screened using Crystal Screen reagent kits (Hampton Research). Typically, 3.8 μl droplets were prepared on siliconized cover slips by mixing 2 μl protein solution and 1.8 μl reservoir solution consisting of 35% PEG 4000 in 100 m*M* sodium citrate buffer pH 6.0 containing 8% ethylene glycol as an additive and the mixture was vapour-equilibrated against 500 μl reservoir solution. Small needle-like crystals appeared after two weeks.

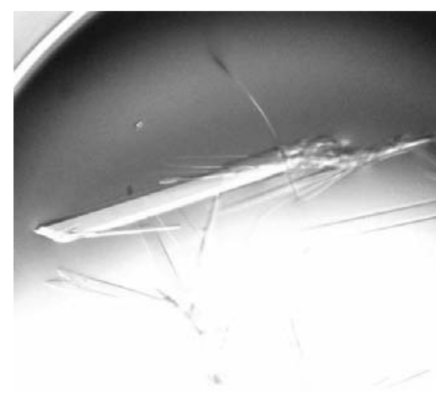

Figure 2
Crystals of HSD226. The size of the largest crystal is 0.1 × 0.2 × 2 mm.

2.3. X-ray crystallographic studies

Preliminary diffraction data sets were collected at room temperature in-house on a Rigaku RU-2000 rotating-copper-anode X-ray generator operated at 48 kV and 98 mA (Cu *K*α; λ = 1.5418 Å) with a MAR 345 mm image-plate detector. The beam was focused by an Osmic mirror. For a more detailed analysis, flash-cooled crystals were used. Crystals were immersed in a freezing solution for 5–10 s, picked up in a loop and then flash-cooled in a stream of nitrogen gas cooled to 100 K. The reservoir solution was used as the cryoprotectant. All intensity data were indexed, integrated and scaled with the *HKL* programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

Table 1
Intensities of systematic absences.

h	k	l	Intensity	σ	I/σ
0	0	3	1.4	5.3	0.3
0	0	7	9.1	47.1	0.2
0	0	9	−17.6	61.5	−0.3
0	0	11	183.6	50.0	3.7
0	0	13	−31.4	61.8	−0.5
0	0	15	75.7	74.9	1.0
0	0	17	91.0	93.4	1.0
0	0	19	−12.4	95.5	−0.1
0	0	21	31.2	104.4	0.3
0	0	23	−203.3	125.6	−1.6
0	0	25	−36.6	172.0	−0.2
0	0	27	85.2	132.9	0.6
0	0	29	−179.4	145.1	−1.2
0	0	31	204.9	221.9	0.9
0	0	33	78.2	240.4	0.3
0	0	35	94.2	261.9	0.4
0	0	37	268.1	281.5	1.0
0	0	39	−310.2	306.1	−1.0
0	0	41	−210.1	337.0	−0.6
0	0	45	336.0	354.8	0.9
0	0	47	360.9	329.6	1.1
0	0	51	−213.9	365.7	−0.6
0	5	0	71.4	77.9	0.9
0	7	0	139.9	110.4	1.3
0	9	0	164.8	98.0	1.7
0	11	0	−75.1	218.8	−0.3
0	13	0	−86.1	193.1	−0.4
0	15	0	−334.4	170.1	−2.0
0	17	0	−376.2	265.2	−1.4
0	19	0	−224.6	304.5	−0.7
0	21	0	147.3	243.3	0.6
0	23	0	−277.0	255.4	−1.1
5	0	0	52.7	114.1	0.5
7	0	0	97.1	167.7	0.6
9	0	0	151.4	221.1	0.7
11	0	0	311.8	344.5	0.9
13	0	0	450.2	363.5	1.2
15	0	0	−307.9	352.0	−0.9

Table 2
Data collection and processing statistics.

Values in parentheses are for the outermost resolution shell.

Space group	$P2_12_12_1$
Unit-cell parameters (Å, °)	a = 46.0, b = 67.4, c = 149.2, $\alpha = \beta = \gamma$ = 90.0
Matthews coefficient (Å^3 Da^{-1})	3.51
Resolution (Å)	50–2.8
Total observations	63355
Unique reflections	11884 (1170)
Redundancy	5.33 (5.35)
Average $I/\sigma(I)$	13.21 (7.03)
$R_{\rm merge}$† (%)	12.2 (30.0)
Data completeness (%)	99.7 (99.8)

† $R_{\rm merge} = 100\sum|I_i - \langle I\rangle|/\sum I_i$, where I_i is the intensity of the ith observation.

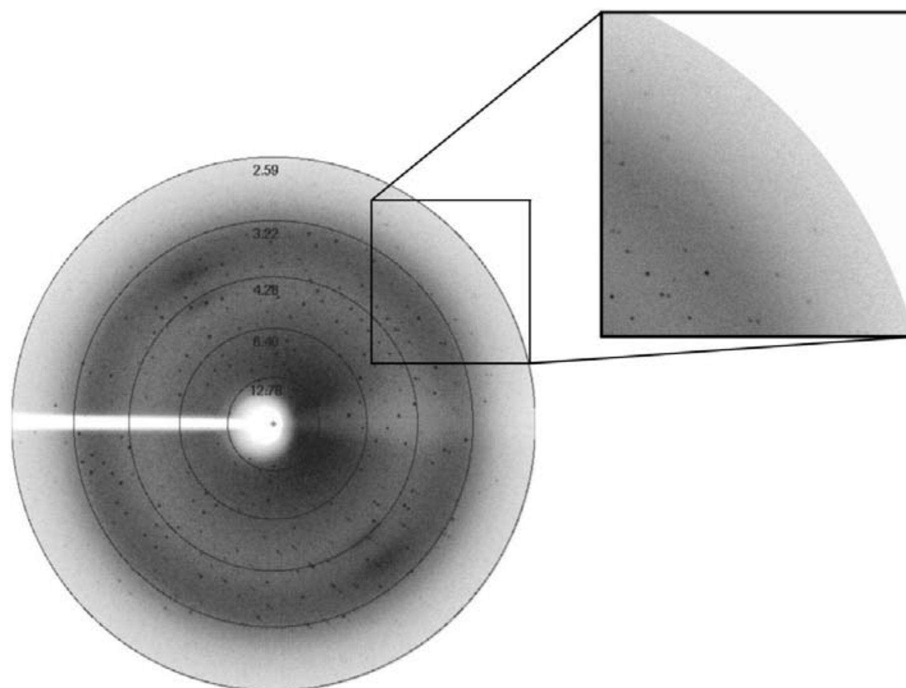

Figure 3
A typical diffraction pattern of HSD226 crystals. The detector edge corresponds to 2.59 Å resolution and an enlarged image of the indicated area is shown. The exposure time was 500 s, the detector distance was 174 mm and the oscillation range per frame was 1°.

3. Results

Crystallization trials were conducted at 291 K in 16-well plates using the hanging-drop vapour-diffusion method. The screening reagents supplied in Hampton Research kits (Riverside, CA, USA) were used for initial screening. Drops consisting of 1 µl protein solution and 1 µl of reservoir solution were equilibrated against 200 µl of reservoir solution. The crystallization of HSD226 was unsuccessful. Therefore, we screened PEG conditions in the pH range 5.0–8.0. Eventually, we found that the protein could be crystallized in a solution containing 29–35% PEG 4000 in 100 mM sodium citrate buffer pH 6.0, but the crystals were twinned and were not suitable for X-ray diffraction. Further optimization was performed and better crystals were obtained using 35% PEG 4000 in 100 mM sodium citrate buffer pH 6.0 and 8% ethylene glycol (Fig. 2). When the crystals were exposed to X-rays, diffraction spots were observed to a Bragg spacing of 2.59 Å (Fig. 3). A set of data was collected from this crystal. The crystals belong to space group $P2_12_12_1$ (intensities of systematic absences are shown in Table 1), with unit-cell parameters a = 46.009, b = 67.387, c = 149.241 Å, $\alpha = \beta = \gamma = 90°$. Scaling and merging of the crystallographic data resulted in an overall $R_{\rm merge}$ of 12.2% and an $R_{\rm merge}$ in the highest resolution shell (2.90–2.80 Å) of 30.0%. The value of the Matthews coefficient (Matthews, 1968) is 3.51 Å^3 Da^{-1} for two molecules in the asymmetric unit and the estimated solvent content is 64.6%. Complete data-collection statistics are given in Table 2.

Determination of the structure of HSD226 is under way. The structure of this fragment will be helpful in elaboration of the function of the YWK-II protein in spermatogenesis.

This work was supported by the following grants: NSFC Nos. 39870174, 39970155 and 30070364; Project '973' Nos. G1999055901, G1999075602 and G1999011902); Project '863' Nos. 2001AA221131 and 2001AA233011.

References

Beher, D., Hesse, L., Masters, C. L. & Multhaup, G. (1996). *J. Biol. Chem.* **271**, 1613–1620.
Bush, A. I., Pettingell, W. H. Jr, de Paradis, M., Tanzi, R. E. & Wasco, W. (1994). *J. Biol. Chem.* **269**, 26618–26621.
Cappai, R., Mok, S. S., Galatis, D., Tucker, D. F., Henry, A., Beyreuther, K., Small, D. H. & Masters, C. L. (1999). *FEBS. Lett.* **442**, 95–98.
Goate, A. M. (1998). *Cell Mol. Life Sci.* **54**, 897–901.
Huang, P., Miao, S. Y., Fan, H. Y., Sheng, Q., Yan, Y. C., Wang, L. F. & Koide, S. S. (2000). *Reproduction*, **6**, 1069–1078.
Kamada, M., Takikawa, M., Maegawa, M., Yamamoto, S., Yamano, S., Irahara, M., Aono, T., Futaki, S., Ohmoto, Y. & Koide, S. S. (2001). *Arch. Androl.* **47**, 89–96.
Kammer, H. von der, Hanes, J., Klaudiny, J. & Scheit, K. (1994). *DNA Cell Biol.* **13**, 1137–1143.
Koide, S. S., Wang, L. F. & Kamada, M. (2000). *Proc. Soc. Exp. Biol.* **224**, 123–132.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Sandbrink, R., Masters, C. L. & Beyreuther, K. (1994*a*). *Biochim. Biophys. Acta*, **1219**, 167–170.

Sandbrink, R., Masters, C. L. & Beyreuther, K. (1994*b*). *J. Biol. Chem.* **269**, 14227–14234.

Sprecher, C. A., Grant, F. J., Grim, M. G., O'Hara, P. J., Norris, F., Norris, K. & Foster, D. C. (1993). *Biochemistry*, **32**, 4481–4486.

Takikawa, M., Kamada, M., Maegawa, M., Yamano, S., Irahara, M., Aono, T., Futaki, S., Ohmoto, Y. & Koide, S. S. (2001). *Zygote*, **9**, 145–151.

Tian, X. Y., Sha, Y. S., Zhang, S. M., Chen, Y. B., Miao, S. Y., Wang, L. F. & Koide, S. S. (2001). *Reproduction*, **121**, 873–880.

Vanage, G., Lu, Y. A., Tam, J. P. & Koide, S. S. (1992). *Biochem. Biophys. Res. Commun.* **183**, 538–543.

Wang, L. F., Yan, Y. C., Miao, S. Y. & Koide, S. S. (1987). *New Horizons in Sperm Cell Research*, edited by H. Mohri, pp. 409–420. Tokyo: Japan Scientific Societies Press.

Yan, Y. C., Bai, Y., Wang, L. F., Miao, S. Y. & Koide, S. S. (1990). *Proc. Natl Acad. Sci. USA*, **87**, 2405–2408.

Yan, Y. C., Wang, L. F. & Koide, S. S. (1987). *Arch. Androl.* **18**, 245–254.

Yan, Y. C., Wang, L. F., Mitsudo, S. M. & Koide, S. S. (1986). *Immunological Approch to Contraception and Promotion of Fertility*, edited by G. P. Talwar, pp. 231–240. New York: Plenum Publishing.