Acta Cryst. (1998). D**54**, 999–1001

Crystallization and preliminary X-ray crystallographic analysis of the EGF receptor ectodomain

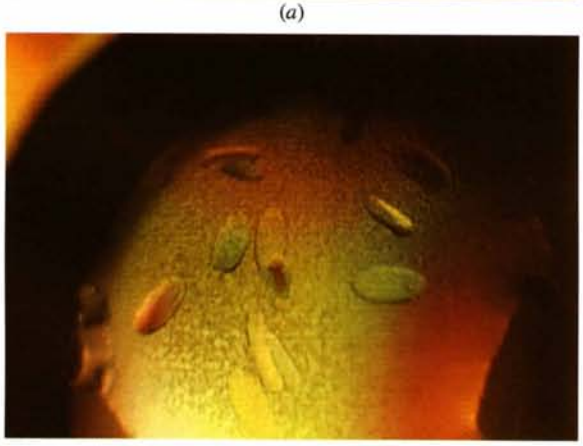
M. Degenhardt,[a] W. Weber,[a] S. Eschenburg,[a] K. Dierks,[b] S. S. Funari,[c] G. Rapp[d] and Ch. Betzel[a]* at [a]*Universitäts-Krankenhaus Eppendorf, Institut f. Phys. Chemie, c/o DESY, Geb. 22a, Notkestrasse 85, 22603 Hamburg, Germany,* [b]*Dierks & Partner, Pinneberger Weg 22-24, 20257 Hamburg, Germany,* [c]*MPI-Colloids and Interfaces, Teltow, Germany, and* [d]*EMBL Outstation, c/o DESY, Notkestrasse 85, 22603 Hamburg, Germany. E-mail: betzel@unisgi1.desy.de*

(*Received 20 October 1997; accepted 30 January 1998*)

Abstract

Crystallization of the hydrophilic ectodomain of the epidermal growth factor (EGF) receptor has been accomplished in the presence of the ligand EGF. Two different crystal forms have been obtained, one of which was suitable for X-ray analysis. The space group of this form has been assigned to $P2_12_12$ with unit-cell dimensions of $a = 207.4$, $b = 113.3$ and $c = 120.4$ Å. A native data set has been recorded and a heavy-atom search is currently under way. Diffraction from these crystals, however, is limited to low resolution and extensive trials to improve crystal quality further have all failed. To analyse the molecular shape and aggregation of the receptor protein in solution, small-angle X-ray diffraction and dynamic light-scattering techniques have been applied. Synchrotron radiation in combination with cryo-techniques is essential for data collection because of the high solvent content and radiation sensitivity.

(*a*)



(*b*)

Fig. 1. (*a*) Example of the needle-like crystal morphology, dimensions 0.15 × 0.15 × 0.5 mm, which does not diffract at all. (*b*) Crystals used for data collection are more ellipsoidal in morphology with dimensions of 0.3 × 0.4 × 0.8 mm.

1. Introduction

The EGF receptor mediates the biological effects of polypeptide mitogens such as EGF and TGF-α, playing an important role in normal and pathological growth control. EGFR is a 170 kDa membrane glycoprotein consisting of three functional domains: an EGF-binding cell-surface domain, which is heavily glycosylated, a short transmembrane region, and a cytoplasmic domain with tyrosine kinase activity. Since overexpression of EGF receptors has been observed in many types of human tumors, structure-based drug design for therapeutic modulation of receptor functions would be of high medical interest. In order to overcome the known problems of membrane protein crystallization, only the hydrophilic external domain of the EGF receptor was used for this study. This protein, the EGF receptor ectodomain, is secreted by A431 human tumor cells as a truncated 100 kDa protein with functionally intact ligand binding site (Weber *et al.*, 1984; Günther *et al.*, 1990; Lax & Schlessinger, 1991; Brown & O'Connor-Mccourt, 1994). The results presented in this communication suggest that using this approach it should be possible to produce crystals of the hydrophilic external domain that will support a low-resolution X-ray analysis.

2. Methods

The secreted form of the EGF receptor (sEGFR) was purified as described by Weber *et al.* (1984) with a modification according to Lengyel *et al.* (1996). An extensive crystallization screen yielded two crystal morphologies growing at identical conditions (Figs. 1*a* and 1*b*), but suggests that crystallization of sEGFR can be accomplished only in the presence of equimolar amounts of mouse EGF. Mouse EGF, isolated and purified according to Savage & Cohens (1979), is 68% homologous to human EGF and the binding affinity to the receptor is comparable. EGF from mouse was purchased from IC Chemikalien GmbH, Ismaning, Germany. Best results were obtained by the hanging-drop vapour-diffusion technique with 12–15 mg ml^{-1} receptor complexed with equimolar amounts of EGF, mixed with 2.0 *M* sodium/potassium phosphate pH 7.5 and exposed to 0.5 ml reservoir solution (same as precipitant). At a controlled temperature of 290 K these crystals typically grew to terminal size in about 6 weeks. Small-angle X-ray scattering and dynamic light-scattering techniques were

© 1998 International Union of Crystallography
Printed in Great Britain – all rights reserved

applied to obtain preliminary information about the shape, conformation and a potential dimerization of the receptor alone, or in complex with the ligand (Greenfield *et al.*, 1989; Lemmon & Schlessinger, 1994), as well as to analyse the crystallization process in more detail.

A new approach was recently initiated to calculate *ab initio* the solvent envelope from native data at low resolution (Harris, 1997). A series of small-angle scattering experiments were started at the EMBL beamline X13 (Rapp, 1992). For the initial experiments at room temperature an experimental set up with a sample-to-detector distance of 350 cm in combination with a gas-filled one-dimensional detector (Gabriel & Dauvergne, 1982), a beam diameter of 1.5 × 0.8 mm at the sample and exposure times of 60 s were utilized. Several preparations of the complex in a molar ratio of 1:1 and a concentration of 20 mg ml^{-1} were analysed. The radius of gyration (R_g) measurements fall in a range 3.8–4.2 nm. Additional measurements comparing the receptor alone and in the complex indicate dimerization consistently, which is shown for one experiment (Fig. 2).

Finally, a newly developed dynamic light-scattering (DLS) system [described in Schulze (1996), provided by Dierks & Partner, Systemtechnologie (Hamburg)] was applied both to analyze the protein material prior to crystallization and also to follow the preliminary aggregation process. Typical sample volumes of 30 µl and concentrations of 12–15 mg ml^{-1} were used. Hydrodynamic radii (R_h) from several preparations are in the range 8.0 ± 0.5 nm. Most preparations, however, were revealed to be polydisperse. In addition to a low molecular diameter fraction, reflecting monomers of the sEGFR and EGF complex, these analyses indicate higher aggregates are present in various sizes and amounts (Fig. 3).

3. Results and concluding remarks

After optimizing cryo-conditions, numerous crystals have been tested and analyzed with synchrotron radiation. Probably due to the extent of glycosylation (30% of the molecular mass), to structural flexibility, and to the high crystal solvent content, the maximum resolution observed for the better crystal form has so far been limited to about 4.5 Å. The unit-cell parameters are a = 207.4, b = 113.3 and c = 120.4 Å with the space group assigned to be $P2_12_12$. Assuming two molecules of sEGFR and two ligands in the asymmetric unit, the packing parameter V_M can be as high as 3.4 Å^3 Da^{-1} (Matthews, 1968), which is equivalent to approx. 65% solvent. After repeated attempts with synchrotron radiation at DESY and ELETTRA, we were recently able to collect a complete data set from one flash-frozen crystal (at 103 K). This data set was collected on a MAR Research image-plate detector at the X-ray diffraction beamline at storage ring ELETTRA, Trieste, Italy. The wavelength was 1 Å and the exposure time was set to 15 min for images of 1.5° rotation. The images were processed using the program *DENZO* (Otwinowski, 1991). The data set contains 3840 unique reflections in the range of 25–7.5 Å with R_{merge} = 10.2%. The completeness is 99.9%. A systematic screen of heavy-atom derivatives has been started. At this point, we have identified one potential candidate (Thimerosal). Attempts to attach more electron-dense heavy-atom clusters to the EGF receptor complex are presently in preparation.

Dynamic light-scattering measurements have indicated that crystal growth results from sEGFR preparations are improved when the fraction with smaller R_h is used. Preparations with higher amounts of aggregates tend not to crystallize or yield many small poor quality crystals. Investigations to restore aggregates to monomeric conditions prior to crystallization are continuing. The ratio R_h/R_g obtained from DLS and SAXS experiments indicates a dimerization and a nonglobular, rod-like shape (Murphy, 1997) of the receptor complex. The conformation of sEGFR and the complex will be further analysed in solution by SAXS.

X-ray results demonstrate that is, in principle, possible to analyze the sEGFR structure in complex with its ligand at moderate resolution. This structure should indicate regions of high flexibility that may not be essential for ligand binding. If so, protein engineering techniques will then be applied to stabilize further the three-dimensional structure or to remove disordered regions which may not be of functional interest. The re-designed protein will be the basis for further crystallization and X-ray studies, which we hope will finally result in a structure of the complex at a resolution high enough to reveal the details of ligand binding.

The authors thank the Bundesmisterium für Bildung, Wissenschaft, Forschung und Technologie BMBF under

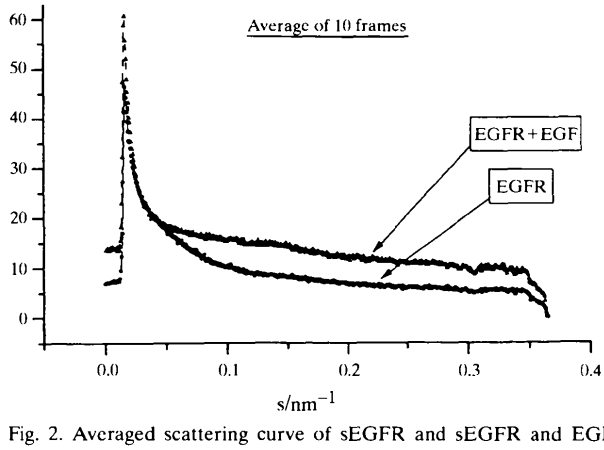


Fig. 2. Averaged scattering curve of sEGFR and sEGFR and EGF indicating dimerization in the presence of EGF.

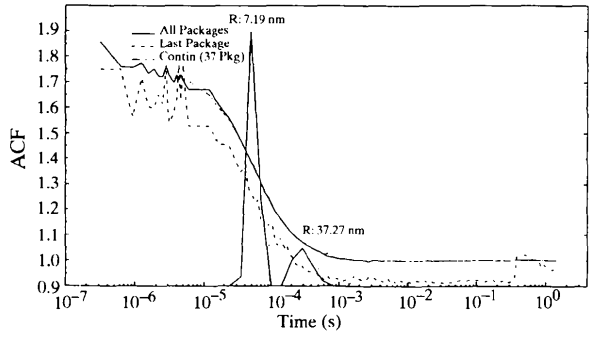


Fig. 3. The results of light scattering analysed by the program *CONTIN* (Provencher, 1982). The material used for crystallization is the dimerized complex, mainly monodisperse with a radius of hydration in the range of 7.2 nm; the second radius indicates aggregation. The vertical axis reflects the normalized auto correlation function (ACF), the horizontal axis the relaxation time.

contract number 641-GKA-6 and DARA (Deutsche Agentur für Raumfahrtangelegenheiten) under contract number 50 WB9544 for financial support.

References

Brown, M. P. & O'Connor-Mccourt, M. D. (1994). *Eur. J. Biochem.* **225**, 223–233.

Greenfield, C., Hiles, I., Waterfield, M. D., Federwisch, M., Wollmer, A., Blundell, T. L. & McDonald, N. (1989). *EMBL J.* **8**, 4115–4123.

Gabriel, A. & Dauvergne, F. (1982). *Nucl. Instrum. Methods*, **201**, 223–224.

Günther, N., Betzel, Ch. & Weber, W. (1990). *J. Biol. Chem.* **256**, 22082–22085.

Harris, G. W. (1997). Abstracts of the Seventeenth European Crystallography Meeting (ECM-17), August 24–28, Lisboa, Portugal, p. 53.

Lax, I. & Schlessinger, J. (1991). *J. Biol. Chem.* **266**, 13828–13833.

Lemmon, M. A. & Schlessinger, J. (1994). *Trends Biochem. Sci.* **19**, 459–463.

Lengyel, H., Günther, N. & Weber, W. (1996). *Eur. J. Cell Biol.* **69** (Suppl. 42), 76–79.

Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.

Murphy, R. M. (1997). *Curr. Opin. Biotechnol.* **8**, 25–30.

Otwinowski, Z. (1991). *DENZO, A Film Processing Program for Macromolecular Crystallography*, Yale University, New Haven, Connecticut, USA.

Provencher, S. W. (1982). *Comput. Phys. Commun.* **27**, 213–227.

Rapp, G. (1992). *Acta Phys. Pol. A*, **82**, 103–120.

Savage, C. R. & Cohens, S. (1979). *J. Biol. Chem.* **247**, 7609–7611.

Schulze, T. (1996). Diploma thesis, University of Hamburg, Germany.

Weber, W., Gill, G. N. & Spiess, J. (1984). *Science*, **224**, 294–297.