A Light Scattering Study of the Interaction of Fibroblast Growth Factor (FGF) with its Receptor

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ABSTRACT Light scattering technique has been used to study the interaction between fibroblast growth factor (FGF) and its receptor. In this study, a general mathematical model has been developed where the concentration of product formed by the interaction of two proteins and its dependence on the initial concentration of interacting proteins have been determined using laser light scattering. Calculated hydrodynamic diameters reveal that both human fibroblast growth factor (hFGF-1) and its receptor domain (D2 domain) exist as monomers in solution. Titration of hFGF-1 and the D2 domain of FGFR show that they interact in a 1:1 stoichiometry in solution. The binding stoichiometry does not depend on the concentrations of the interacting proteins. The results of this study, for the first time to our knowledge, provide an unambiguous evidence that the 2:2 binary complex of FGF and FGFR observed in the crystal structures of the FGF-FGFR complex (in the absence of heparin) is possibly a crystallization artifact.

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Fibroblast growth factors (FGFs) are a family of growth factors that participate in key cellular processes such as cell proliferation, cell differentiation, wound healing, and tumor growth (1). FGFs exert their biological activity by binding to their cell surface tyrosine kinase receptors (FGFRs). FGFRs contain an extracellular domain, a short transmembrane, and a cytoplasmic tyrosine kinase domain. The extracellular domain consists of three structural subdomains called D1, D2, and D3. Mutational studies have shown that the D2 subdomain contributes to the ligand (FGF) binding (2). X-ray crystallographic studies consistently show that the FGF-receptor complex is a symmetric dimer both in the absence and the presence of heparin (2,3). The stoichiometry of interaction between FGF and FGFR (in the absence of heparin), derived from the crystal structures, has recently come under intense scrutiny. Previous studies based on size-exclusion chromatography (SEC) have consistently revealed the formation of a 1:1 complex of FGF and FGFR (4). However, the debate on the stoichiometry remains largely unresolved owing to the resolution of the SEC technique, and the problems of stability of the FGF-FGFR complex under the conditions used in the SEC experiments. Therefore, there is an increasing need to develop new approaches to reliably resolve the existing controversy on the stoichiometry of the FGF-FGFR complex.

Light-scattering techniques are versatile optical methods for characterizing physico-chemical properties of macromolecular solutions (5). They can provide information about the size, diffusion coefficient, conformation, and aggregation of biomolecules. Although the sensitivity of dynamic light scattering (DLS) technique usually precludes the determination of affinities and association or dissociation rate constants, we show that DLS can be quite useful in characterizing the stoichiometry of protein complexes at high concentrations in solution. In many cases, biochemical methods provide information about the interaction of protein A with another protein B, but the stoichiometry of this interaction is often elusive. In this study, we describe a new mathematical model that can help in the determination of the stoichiometry, binding constant, and the concentration of protein-protein complexes formed in a pool of two freely interacting protein partners. We have used our model to examine the binding stoichiometry and binding affinity between FGF and its receptor.

Bovine pancreatic ribonuclease (RNase A) was obtained from Sigma Aldrich (St. Louis, MO). All other reagents used were of the highest purity available. Cloning, overexpression, and purification of the D2 domain of FGFR2 and human fibroblast growth factor (hFGF-1) have been reported previously (1,6). All light scattering measurements were performed using Brookhaven Instruments (Holtsville, NY) BI-200SM goniometer and BI-9000AT digital autocorrelator. Solutions were prepared and were filtered (0.2 μm) to remove dust. All of the measurements were carried out at 25°C. The light source was a He-Ne ion laser (Spectra Physics Lasers, Mountain View, CA; $\lambda = 632.8$ nm). A photomultiplier tube mounted on the goniometer-collected photons scattered by the sample at an angle of 90°. Static light scattering intensity is related to the molecular mass of the protein, its concentration, scattering angle, and the wavelength, which in principle are known. Dynamic light scattering measurements utilize the temporal correlations of the scattering intensity fluctuations, which are related to the Brownian motion of the solute. For dilute solutions, the diffusion coefficient D_i is related to the hydrodynamic diameter d_i of the scatterers and the solvent viscosity η through the Stokes-Einstein relation

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$$D_i = k_B T/3\pi n d_i, \quad (1)$$

where k_B is the Boltzmann constant. The nonnegative least-squares algorithm was used in the Laplace inversion of the autocorrelation function to obtain the size distribution. Dynamic light scattering and static light scattering studies revealed the diffusion coefficient, diameter, and molecular mass of hFGF-1 to be 1.173×10^{-6} cm^2/s, 3.8 nm, and ~16 kDa, respectively (see Supplementary Material, Table S1). Both RNase A and D2 domain have nearly the same diffusion coefficient, diameter, and molecular mass of $\sim 1.278 \times 10^{-6}$ cm^2/s, 3.5 nm, and ~13.7 kDa, respectively (Table S1). Dynamic light scattering experiments show that a homodimer is not formed and all the three proteins remain as monomers in the concentration range of 10–650 μM (Fig. S1).

Model for calculating concentration of product formed by the interaction of two proteins

Since all species contribute to scattering, correlation time (Γ) for the scattered-intensity-measure weighed diffusion constant is given by

$$\frac{1}{D} = \frac{\sum_i c_i m_i^2}{\sum_i c_i m_i^2 D_i}, \quad (2)$$

where c_i, m_i, and D_i are, respectively, the molar concentration, molecular mass, and diffusion coefficient of protein i. Using the Einstein-Stokes relation, we find that effective diameter d_{eff} determined by light scattering is related to the diameter of each species by

$$d_{eff} = \left[\frac{(x-z)\,m_1^2/d_1 + (y-z)m_2^2/d_2 + z\,m_3^2/d_3}{(x-z)\,m_1^2 + (y-z)\,m_2^2 + z\,m_3^2}\right]^{-1}, \quad (3)$$

where x, y denote the initial concentrations of the interacting proteins, and z that of the product complex. With the help of this formula, we can extract the value of z, the concentration of the protein complex, from the measured d_{eff} because all the other parameters are either known or can be calculated using the standard diameter versus molecular mass relation $d_i = 1.235\,M_i^{0.404}$ where d_i is the diameter and M_i is the molecular mass in kiloDaltons. Effective diameter and molecular mass values of two proteins, hFGF-1 (effective diameter 3.8 nm and molecular mass 16 kDa) and RNase A as well as D2 domain (effective diameter 3.5 nm and molecular mass 13.7 kDa) obtained by dynamic light scattering, fit well to the standard curve (Fig. S2).

When two proteins interact, the effective diameter will depend on the concentration, molar mass, and effective diameter of each of the proteins as well as those of the product formed by the interacting proteins. The change in hydrodynamic diameter was measured by titrating a fixed concentration of hFGF-1 (100 μM) with increasing concentrations of the D2 domain (in the concentration range of 50–600 μM; Fig. 1).

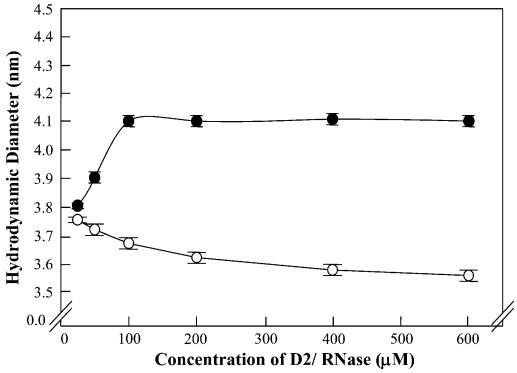

FIGURE 1 Dynamic light scattering of hFGF-1 (100 μM), in the presence of increasing concentrations (50–600 μM) of the D2 domain of FGFR (*solid circles*), and RNase A (*open circles*). Experiment was performed at 25°C in 10 mM phosphate buffer (pH 6.5) containing 50 mM NaCl.

The diameter steeply increases from 3.8 nm to 4.1 nm until the concentration of the D2 domain reaches 100 μM (Fig. 1). Increase in the D2 domain concentration beyond 100 μM does not result in a significant change in the hydrodynamic diameter. Control experiments involving the titration of 100 μM hFGF-1 with RNase A, which is not known to interact with hFGF-1, caused a marginal decrease in the hydrodynamic diameter (Fig. 1). Similarly, the maximum hydrodynamic diameter value attained, upon varying the concentrations (in the range of 50–750 μM) of both hFGF-1 and the D2 domain, is 5.1 nM (Fig. 2 *A*).

Hydrodynamic diameter 5.1 nm, when extrapolated to the standard plot (of molecular mass versus hydrodynamic diameter), corresponds to a molecular mass of ~31 kDa; this is approximately equal to combined molecular mass of hFGF-1 and the D2 domain. These results are consistent with 1:1 stoichiometry of binding between hFGF-1 and the D2 domain. Control experiments involving the variation in the concentra-

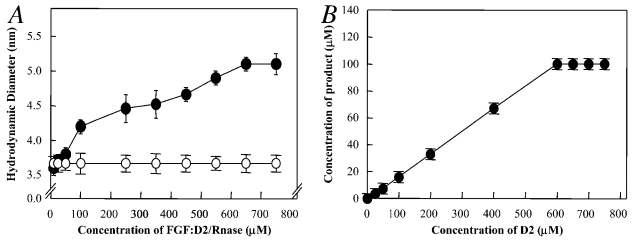

FIGURE 2 Dynamic light scattering obtained upon mixing increasing concentrations of hFGF-1 in equimolar ratio (1:1) with increasing concentrations of the D2 domain of FGFR (*solid circles*), and increasing concentrations of RNase A (*open circles*) (*A*). The range of hFGF-1 concentration used is 25–750 μM. (*B*) Concentration of the hFGF-1-D2 domain binary complex (*z*) formed when a fixed concentration of hFGF-1 (100 μM) was titrated with increasing concentrations of the D2 domain. Experiment was performed at 25°C in 10 mM phosphate buffer (pH 6.5) containing 50 mM NaCl.

tion of both hFGF-1 and RNase A (in the range of 50–750 μM) caused no change in the hydrodynamic diameter (Fig. 2 *A*).

The mathematical model developed in this study allows the calculation of the concentration of the products of interaction (z) based on the observed hydrodynamic diameter. Logically, if the stoichiometry of interaction is 1:1, the value of z is expected to show a linear increase when a fixed concentration (100 μM) of hFGF-1 is titrated with increasing concentrations of the D2 domain. Results presented in Fig. 2 *B* reveal a linear increase in the z-value until the concentration of the receptor domain reached 100 μM. The z-values are observed to reach a plateau when the concentration of the D2 domain is raised beyond 600 μM. The observed plateau is primarily due to the limiting concentration (100 μM) of hFGF-1. These results confirm that hFGF-1 and the D2 domain of FGFR form a 1:1 binary complex in solution. It should be of interest to note that the conclusions drawn from our DLS data are consistent with the biophysical studies reported by Harmer et al. (4).

The binding affinity (K_d) between the interacting proteins can be readily determined based on the calculated z-value. The binding constant (K_d) characterizing the interaction between hFGF-1 and its receptor domain is calculated to be 328 μM. Isothermal titration calorimetry experiments were performed to examine the reliability of the K_d value calculated from the dynamic light scattering experiments. Isothermal titration calorimetry is a useful technique to measure the binding affinity and stoichiometry of protein-protein or protein-ligand interactions. The binding isotherm representing the binding between hFGF-1 and the receptor domain is hyperbolic and proceeds with the evolution of heat. Least-square fitting of the raw isotherm shows the hFGF-1, and the D2 domain interact in a 1:1 stoichiometry (Fig. S3). The K_d value obtained from the isothermal titration calorimetry data is in the same range ($\sim$184 μM) as that calculated from the DLS experiments.

The binding stoichiometry obtained from the DLS experiments is in marked contrast to the 2:2 complex observed in the crystal structures of the FGF-FGFR complex. Crystal structures of the FGF-FGFR complex (in the absence of heparin) reveal that the interactions between residues in the D2 domain of FGFR solely constitute the dimer interface. The results of this study clearly demonstrate that D2 domain exists as a monomer in both its free and FGF-bound forms. Crystal structure of the receptor domain alone is not available, but we believe that the 2:2 binary complex of FGF and FGFR (in the absence of heparin) observed in the crystal structures is possibly a crystallization artifact. The disparity observed between our DLS results and the crystal structure data can possibly be attributed to experimental conditions used in the crystallization process. Crystals of the FGF-receptor complex were grown in reservoir buffers containing high salt (1.6 M ammonium sulfate) plus just 20% glycerol (2), or mixture of 9% sucrose + 2% glucose + 8% glycerol + 8% ethylene glycol (3). High sulfate concentrations (used in the crystallization process) may facilitate the 2:2 dimer formation by promoting intermolecular interactions either through salt-bridging or by screening the repulsive forces that operate between the positively charged residues in the D2 domain. Crystal structure of FGF-receptor complex reveals the presence of a positively charged canyon contributed by residues in the D2 domain (2). In addition, the excluded volume effects of the crowding agents used in the crystallization process may also play a significant role in the dimerization of the receptor. In fact, crowding agent used in the crystallization of G-quadruplex was shown to drive a conformational change (7). It appears that FGF initially interacts with its receptor in a 1:1 stoichiometry, and the dimerization of the receptor, and subsequent autophosphorylation of the cytoplasmic tyrosine kinase domains is triggered only after FGF-FGFR complex is stabilized by binding to the cell surface glycosoamino glycans. We believe that this study will help clear the existing controversy on the structural events involved in FGF signaling. In addition, the mathematical model developed in this study will be generally applicable to calculate the binding stoichiometry and determine the relative concentrations of protein-protein complexes using dynamic light scattering.

SUPPLEMENTARY MATERIAL

To view all of the supplemental files associated with this article, visit www.biophysj.org.

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