WYATT TECHNOLOGY

APPLICATION NOTE

AN4004: Conformation of an oligomeric protein by DLS

Summary

Various structural studies on Migration Inhibitory Factor (MIF) have reported monomer, dimer, and tetramer forms of this 12.3 kDa protein. The subunit arrangement of MIF is important because it may reveal information on the stoichiometry of its interaction with cell surface receptors and the mechanism of signal transduction through cell membranes. Dynamic light scattering (DLS) reveals key structural aspects of MIF including oligomeric form and shape.

Introduction

Migration inhibitory factor (MIF) was first discovered in the 1960s as a T-cell product that inhibits the random migration of macrophages, and is implicated in delayed-type hypersensitivity reactions. It has recently been rediscovered as a pituitary product that modulates systematic inflammatory responses and may play a role in mediating septic shock. Crystallographic studies of MIF indicate a form containing a non-crystallographic 3-fold axis of symmetry. The structure of this trimeric form of MIF is shown in Figure 1.

Dynamic light scattering is a versatile technique for determining protein structure, providing both hydrodynamic radius (R_h) and, through static light scattering (SLS) functionality, molecular weight. It was used to confirm the existence of the MIF as a trimer in solution, as well as the conformation of this form.

Materials and Methods

A 200 µL sample of MIF at 8 mg/mL in 20 mM Tris buffer (pH 8.0) and 20 mM NaCl was filtered through a 0.02 mm Anotop filter into a microcuvette and placed in the DynaPro® dynamic/static light scattering instrument. This instrument utilizes separate, dedicated SLS and DLS detection modules, and is capable of simultaneously determining molecular weight and hydrodynamic size with as little as 2 µL of solution.

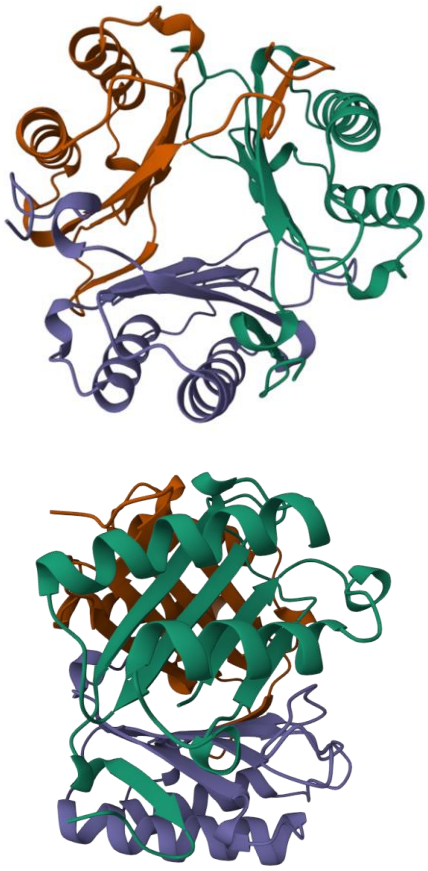

Figure 1. Crystallographic structure of the trimeric form of Migration Inhibitory Factor, top and side views. (RCSB PDB, http://doi.org/10.2210/pdb1MIF/pdb).

Analysis of the autocorrelation function by cumulants led to the results shown in Table 1, where D_{25} is the translational diffusion coefficient at 25° C given in units of

10^{-7} cm^2/s, R_h is the hydrodynamic radius in nm, and SOS is the sum of squares fitting error.

Table 1. DLS results by Method of Cumulants.

#	Amp	D_{25} (10^{-7} cm/s^2)	R_H (nm)	Baseline	SOS
1	0.835	8.91	2.7	0.999	0.464
2	0.846	8.87	2.7	1.001	0.545
3	0.839	8.93	2.7	1.001	0.579
4	0.836	8.92	2.7	1.001	0.519

The molecular weight of the sample was estimated both from SLS and by comparison to a MW vs. R_H calibration curve, prepared from globular protein standards. For a hydrodynamic radius of 2.7 nm, the equivalent molecular weight is 34.1 kDa, consistent with the SLS result of 36.4 kDa and the value expected for an MIF trimer, 36.9 kDa.

Results and Discussion

From the 2-dimensional top view of the protein in Figure 1, MIF appears to be spherical. The side view, however, indicates a disk shape. In the absence of the structure, information regarding particle shape can be obtained by comparison of the measured hydrodynamic radius to that of a hypothetical sphere of the same mass and density of the protein. In the DYNAMICS® software package, the calculations are incorporated into the Axial Ratio Calculator. Figure 2 shows the results for the axial ratio calculations for MIF and indicates a frictional ratio of 1.235 ($F = f/f_o = R_{sph}/R_H$). For spherical particles, a frictional ratio of 1 would be expected. The observed value of 1.235 is consistent with the disk shape of the MIF trimer.

Conclusion

By combining DLS and SLS, DynaPro instruments reveal key structural aspects of proteins and their oligomers while using very little sample..

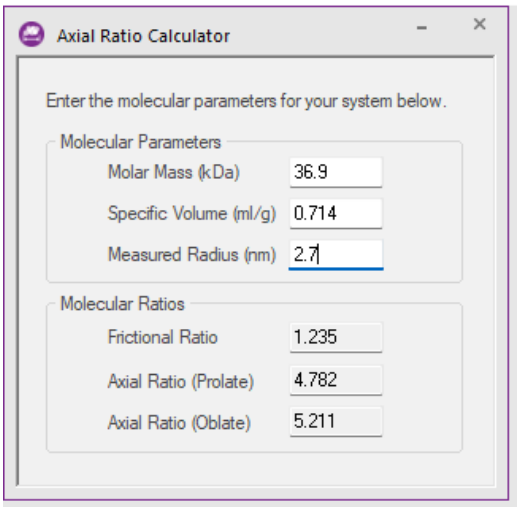

Figure 2. DYNAMICS Axial Ratio Calculator for estimating the frictional ratio of proteins.

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