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Application note

Oligomerization of Interleukin-2

A commercially available human interleukin-2 is assessed by microfluidic diffusional sizing across a dilution series. The hydrodynamic radius is observed to increase with increasing concentration, in a way which suggests a monomer-trimer equilibrium with positive cooperativity is established.

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Introduction

Interleukin-2 (IL-2) is a lymphokine with a range of crucial roles in immune activation and homeostasis (1). It is produced by a subset of activated T-lymphocytes and acts as a growth factor for T cells and NK cells (2), and promotes proliferation, activation-induced cell death, antibody secretion, and cytokine production in various cell types (3). Intravenous treatment with high dose IL-2 has been used in patients with certain cancers (4) (5).

IL-2 has a molecular weight of 15.5 kDa and is globular, which corresponds to a size of 2.04 nm for a monomeric form (6).

Here we use Microfluidic Diffusional Sizing (MDS) on a Fluidity One instrument to assess the hydrodynamic radius (R_h) over a dilution series. The tests use a commercially available lyophilized form with no additives. We observe that the R_h increases with increasing concentration, in a manner which indicates a monomer-trimer equilibrium with positive cooperativity.

This result challenges much of the modern literature which assumes a monomeric form (7) (8) (9), or in some instances a dimer (10). One study by Siddarth et al uses analytical ultracentrifugation and produced data which cannot rule out a monomer-trimer equilibrium, though a monomer-dimer model is eventually concluded (11). It should be noted that a different IL-2 source and buffer is used in this study.

Methods

50 µg of lyophilized human IL-2 recombinantly expressed in E. coli (Sigma-Aldrich code SRP3085) was dissolved in 10 mM acetic acid at 1 mg/mL.

A 10 µL aliquot of this stock was diluted with 23.3 µL of 138.6 mM acetic acid to produce a solution of nominally 300 µg/mL in 100 mM acetic acid. (The subsequent measured concentrations of the dilution series being consistently higher than expected suggests that this initial stock was > 300 µg/mL, likely due to the purchased 50 µg containing more than expected.)

This 300 µg/mL solution was diluted in 2-fold dilution series using 100 mM acetic acid to give 7 varying concentrations, with a lower limit of 4.7 µg/mL.

Each concentration was analysed using a Fluidity One prototype instrument to measure the R_h. Tests were performed in triplicate, with the values reported below being an average of the repeats.

The observed R_h change over changing concentration was then assessed against different models to infer the nature of the oligomerisation.

Results

The concentrations of the dilution series along with the measured R_h and concentration are shown in Table 1.

Given the molecular weight of IL-2, the theoretical Rh for a monomer is 2.04 nm, dimer is 2.62 nm and trimer is 3.03 nm. As such the sizes observed at higher concentrations are consistent with trimer formation.

Figure 1 shows fitting of the data points to a monomer-dimer equilibrium model (see appendix 1), which does not accurately represent the data.

Table 1: Results of MDS analysis on the IL-2 dilution series

Nominal Concentration (µg/mL)	Average Measured R_h (nm)	Average Measured Concentration (µg/mL)	Notes
4.7	1.87	4.32	-
9.4	2.00	10.75	Average of 2 runs
18.8	2.29	22.53	-
37.5	2.85	40.47	-
75	2.98	88.90	-
150	2.91	190	Average of 2 runs
300	3.05	411	-

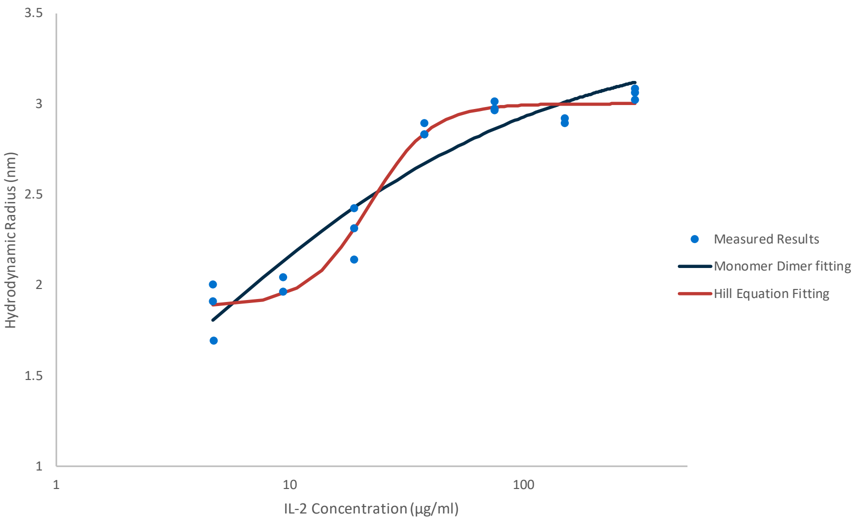

Figure 1: Results of dilution series analysis fitted to both a monomer-dimer equilibrium model, and a Hill Equation

It is clear that the monomer-dimer fitting fails at capturing the steepness of the curve, indicating that there may be cooperative behaviour.

Fitting the data instead to a Hill equation provides a more representative fit, as shown in Figure 1. Akaike's Information Criterion Test also shows that the Hill equation is ~3000-fold more likely than the monomer-dimer equilibrium model.

The steepness of the slope observed in this fitting indicates there is positive cooperativity in the oligomerisation, as indicated in Table 2.

Table 2: Hill equation fitting of the dilution series data

No cooperativity; n = 1

Positive cooperativity; n >1

$y = MR_h + (DR_h - MR_h)*x^n/(K_d^n + x^n)$	Result
MR_h (nm)	1.88 ±0.05
DR_h (nm)	3.00 ±0.04
K_d (µg/mL)	22 ±2
K_d (µM)	1.42 ±0.13
n	3.3 ±0.7
R^2	0.961

Conclusion

A change in the R_h of IL-2 on dilution has been observed using microfluidic diffusional sizing on a Fluidity One instrument. The R_h changed from 3.1 nm at 300 µg/mL concentration, to 1.9 nm at 4.7 µg/mL.

The change in size indicates that oligomerization occurs at concentrations above 20 µg/mL. The size observed at 300 µg/mL is consistent with a trimer.

When the data is analysed, a Hill equation provides a better fit compared to a monomer-dimer equilibrium model. The steepness of the curve on the Hill equation fit indicates positive cooperativity in the assembly of the trimers.

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Appendix 1 - Data fitting equations

Fitting equation for Monomer-Dimer equilibrium: $K_d = [M]^2 / [D]$

Total concentration of IL-2: x

Concentration of IL-2 monomer: $[M] = x - 2z$

Concentration of IL-2 dimer: $[D] = z$

Quadratic equation:

$$z = \frac{(4x + K_d) - \sqrt{(4x + K_d)^2 - 16 \times x^2}}{8}$$

Equation for Average R_h:

$$AveR_h = \left(\frac{(4x + K_d) - \sqrt{(4x + K_d)^2 - 16 \times x^2}}{8}\right)\frac{2}{x} \times DR_h + \left(1 - \left(\frac{(4x + K_d) - \sqrt{(4x + K_d)^2 - 16 \times x^2}}{8}\right)\frac{2}{x}\right) \times MR_h$$

Average R_h: $AveR_h$

Monomer R_h: MR_h

Dimer R_h: DR_h

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