ELECTRONIC SUPPLEMENTARY INFORMATION

Protein crystals as a key for deciphering macromolecular crowding effects on biological reactions

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Table of Contents

SUPPLEMENTARY METHODS

Data collection and processing: X-ray diffraction data of lysozyme crystals produced in the absence of crowders were recorded from single cryocooled (100 K) crystals at beamline ID29[1] of the European Synchrotron Radiation Facility (Grenoble, France; two passes (with 90 or 99% beam attenuation) of 120 frames each in 1° oscillation steps and 1 s exposure) or in-house on a Gemini PX Ultra diffractometer (Oxford Diffraction) equipped with an Onyx CCD detector using Cu $K\alpha$ radiation (676 1° oscillation frames with 20 s exposure in 8 ω and 5 ϕ scans). The latter experimental setup was also used to record diffraction data from lysozyme crystals grown in the presence of 300 g/L sucrose (211 0.75° oscillation frames with 17 s exposure in 3 ω scans) or 300 g/L Ficoll-70 (98 0.75° oscillation frames with 60 s exposure in 2 ω scans). The diffraction data were integrated with XDS,[2] scaled with AIMLESS,[3] and reduced with utilities from the CCP4 program suite.[4] The structures were solved by molecular replacement with PHASER,[5] using the coordinates of PDB entry 2W1X as search model.[6] The models were completed in alternating cycles of refinement with PHENIX [7] and manual model building with COOT.[8] The final coordinates and structure factors were deposited at the Protein Data Bank under entries 6RT1 (Native in-house), 6RT3 (Native ESRF), 6RT9 (Sucrose) and 6RTA (Ficoll). The experimental data were deposited at the SBGrid Data Bank,[9] under entries doi:10.15785/SBGRID/654 (6RT1), doi:10.15785/SBGRID/655 and doi:10.15785/SBGRID/656 (6RT3), doi:10.15785/SBGRID/657 (6RT9), and doi:10.15785/SBGRID/658 (6RTA). Data collection and processing and refinement statistics are summarized in Table S1. Software used in this project was curated by SBGrid.[10]

SUPPORTING TABLES

Table S1. Crystallographic data collection, processing and refinement statistics.

	Native in-house	Native ESRF	Sucrose	Ficoll
Source	In-house sealed tube	ESRF ID29	In-house sealed tube	In-house sealed tube
Wavelength (Å)	1.5406	0.9795	1.5406	1.5406
Resolution range (Å)	14.6 – 1.34 (1.36 – 1.34)	39.4 – 1.05 (1.07 – 1.05)	14.8 – 1.55 (1.58 – 1.55)	14.6 – 1.80 (1.84 – 1.80)
Space group	$P4_32_12$	$P4_32_12$	$P2_12_12_1$	$P4_32_12$
Unit cell dimensions	a=78.6 Å; b=78.6 Å; c=37.0 Å	a=78.9 Å; b=78.9 Å; c=36.8 Å	a=30.1 Å; b=56.1 Å; c=72.5 Å	a=78.7 Å; b=78.7 Å; c=37.1 Å
Total reflections	543,281 (1,739)	532,395 (4,316)	61,197 (1,827)	51,828 (1,657)
Unique reflections	26,070 (655)	53,373 (1,955)	18,289 (859)	11,092 (616)
Multiplicity	20.8 (2.7)	10.0 (2.2)	3.3 (2.1)	4.7 (2.7)
Completeness (%)	96.9 (50.7)	97.6 (73.8)	99.1 (95.5)	98.5 (95.5)
Mean I/sigma(I)	30.4 (1.2)	11.4 (0.5)	11.7 (0.8)	15.5 (1.3)
R_{merge}	0.068 (0.735)	0.098 (1.001))	0.071 (0.872)	0.083 (0.720)
$R_{p.i.m.}$	0.014 (0.450)	0.027 (0.810)	0.044 (0.754)	0.042 (0.510)
CC1/2	1.000 (0.526)	0.998 (0.349)	0.991 (0.349)	0.997 (0.570)
R_{work}	0.122	0.124	0.184	0.164
R_{free}	0.162	0.146	0.228	0.191
Non-H atoms	1257	1328	1283	1227
Macromolecules	1064	1111	1057	1042
Ligands	18	37	26	3
Solvent	175	180	200	182
Protein residues	129	129	129	129
RMSD bonds (Å)	0.007	0.006	0.005	0.006
RMSD angles (°)	0.94	0.90	0.89	0.79
Ramachandran favoured (%)	97.6	99.2	98.4	99.2
Ramachandran allowed (%)	2.4	0.8	1.6	0.8
Ramachandran outliers (%)	0.0	0.0	0.0	0.0

Statistics for the highest-resolution shell are shown in parentheses.

Table S2. Thermodynamic analysis by the crystallization, enzyme kinetics, and precipitation methods. The activity coefficients (γ), enthalpic contribution (Δh) and entropic contribution ($-T\Delta s$) are obtained from the experimentally determined values of lysozyme solubility (crystallization and precipitation methods) and Michaelis constant (enzyme kinetics method). The uncertainty intervals correspond to the error propagation of standard deviation.

Method	Additive	Thermodynamic Parameter		
		γ (-)	Δh (kcal/mol)	$-T\Delta s$ (kcal/mol)
Crystallization	Ficoll	1.667 ± 0.030	-0.75 ± 0.02	1.07 ± 0.03
	Sucrose	0.294 ± 0.011	-0.75 ± 0.02	0.00
Enzyme kinetics	Ficoll	2.781 ± 0.726	-0.26 ± 0.12	0.89 ± 0.20
	Sucrose	0.661 ± 0.133	-0.26 ± 0.12	0.00
Precipitation	Ficoll	0.014 ± 0.004	-0.91 ± 0.38	-1.71 ± 0.42
	Sucrose	0.230 ± 0.142	-0.91 ± 0.38	0.00

SUPPORTING FIGURES

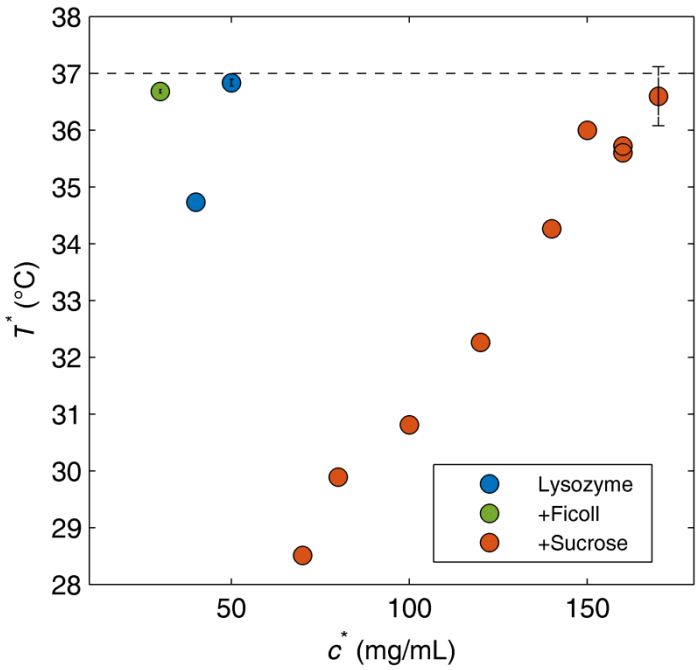

Figure S1. Measurements of lysozyme solubility (symbols) using the crystallization method. These preliminary results were used to determine the crystal solubility (c^*) of lysozyme at a saturation temperature $T^* = 37$ °C in the presence and absence of 300 g/L of crowders. Error bars represent standard deviations of triplicate measurements of T^*.

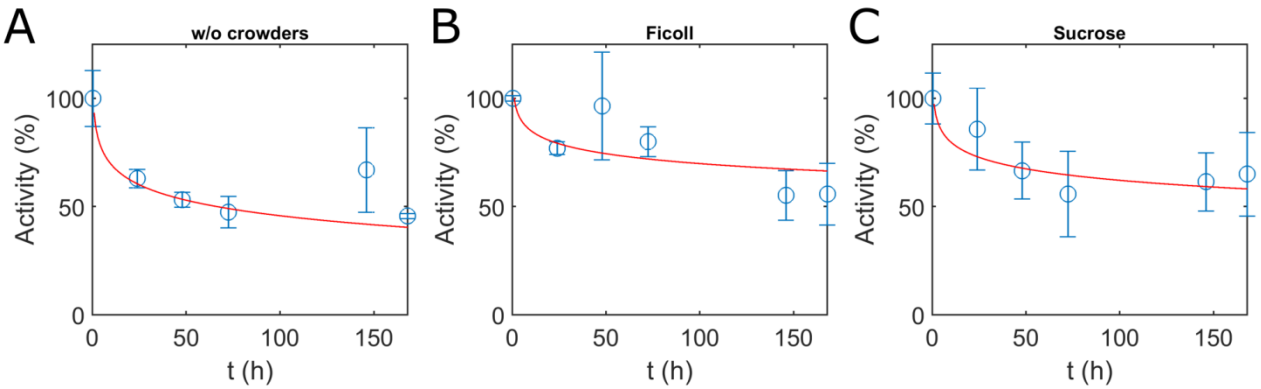

Figure S2. Influence of crowding on the loss of catalytic activity of lysozyme. (A to C) Symbols and error bars: means and standard deviations of the normalized enzymatic activity measured after different periods of incubation and in the presence of (A) no crowders, (B) 300 g/L of Ficoll-70 and (C) 300 g/L sucrose. Solid lines are visual guides. No major changes in the enzymatic activity are observed after long periods of incubation in the presence and absence of crowders.

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