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New Insight into the Effects of Various Parameters on the Crystallization of Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase (RuBisCO) from *Alcaligenes eutrophus*

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1. Purification of RuBisCO from *Alcaligenes eutrophus*

Figure S1 represents the chromatogram of DEAE-sepharose Cl-6B anion exchange column. The left side vertical axis indicates the absorbancy scala (optical density) at 280 nm. The right side vertical axis represents the concentration of KCl. The diagonal straight line indicates the gradient of KCl. One can read the KCl concentration of every peak eluated through DEAE-sepharose Cl-6B colum which were bound to the column materials. The horizontal axis indicates the fraction numbers eluated from DE-AE-sepharose Cl-6B column. To identify the RuBisCO, 50 ul were taken out from the fractions responded to the indicated highest peaks (I, II, III, IV, and V) and determined the activity of RuBisCO as described in section 2.3 of the main manuscript. RuBisCO was eluated from the fraction number 62 to 71.

For checking the purity of RuBisCO, 10-20 ul samples of each purification steps were collected and loaded on the SDS-PAGE. This result is represented in Figure S2. The detailed information was described in the legend of Figure S2.

Citation: Choe, H.-W.; Kim, Y.J. New Insight into the Effects of Various Parameters on the Crystallization of Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase (RuBisCO) from *Alcaligenes eutrophus*. *Crystals* **2022**, *11*, 196. https://doi.org/10.3390/cryst12020196

Academic Editors: Borislav Angelov, Kyeong Kyu Kim and T. Doohun Kim

Received: 24 December 2021
Accepted: 25 January 2022
Published: 28 January 2022

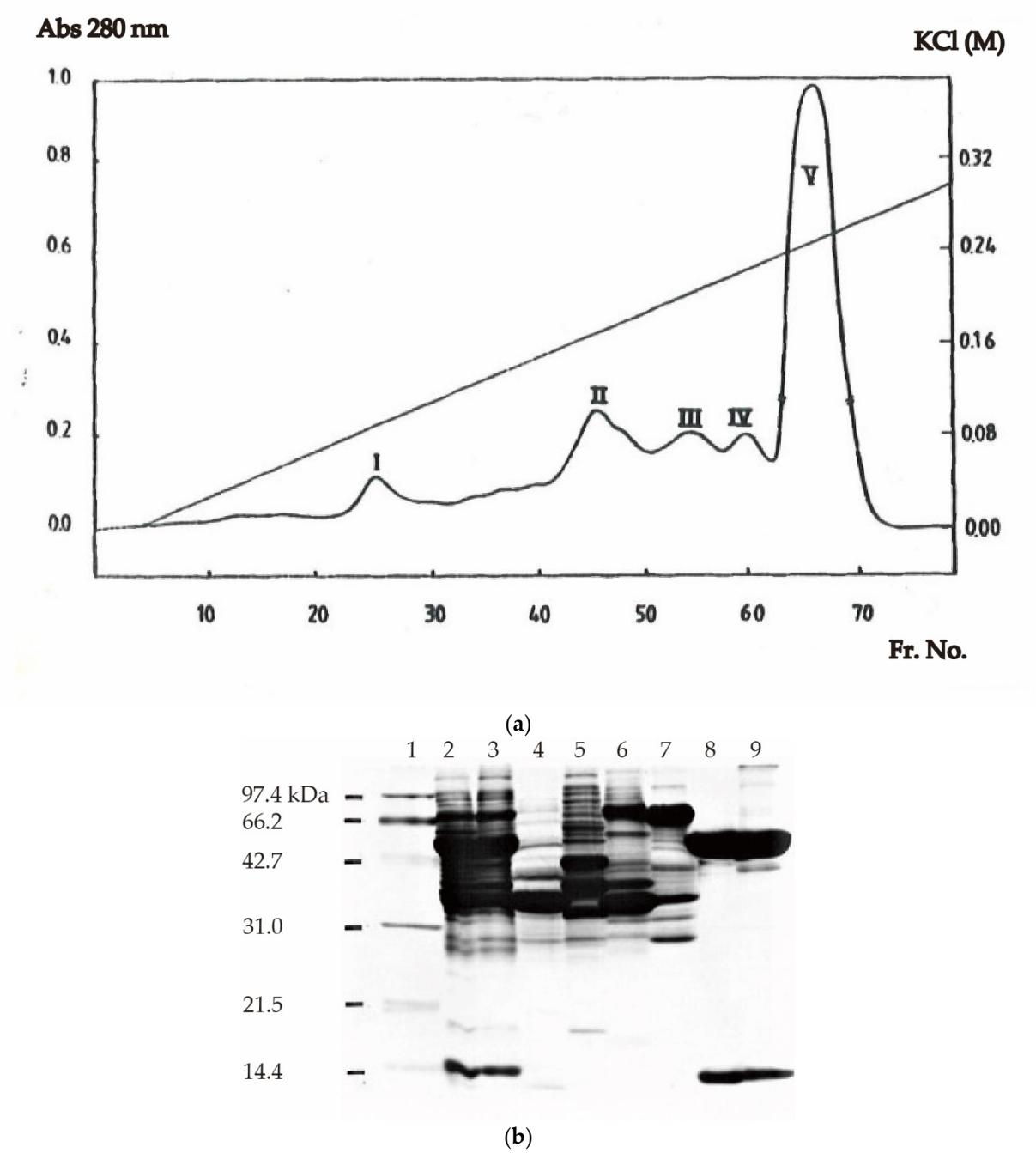

Figure S1. (**a**) Chromatogram of RuBisCO using by DEAE-Sepharose Cl -6B column. The diagonal line indicates the KCl concentration gradient. The peaks from I to V were loaded on the SDS-PAGE. Peak V indicates the RuBisCO fractions (Fraction number 62-71). (**b**) SDS-PAGE. Samples were taken out from the main fractions after the purification by DEAE- sepharose Cl-6B column. The lanes indicate as follows. (1) Marker, (2) Crude extract, (3) Ammonium sulfate fractionation (40%), (4) Peak I, (5) Peak II, (6) Peak III, (7) Peak IV, (8) Peak V, and (9) Side cuts of Peak V from the profile of the chromatogram shown as Figure S1a respectively.

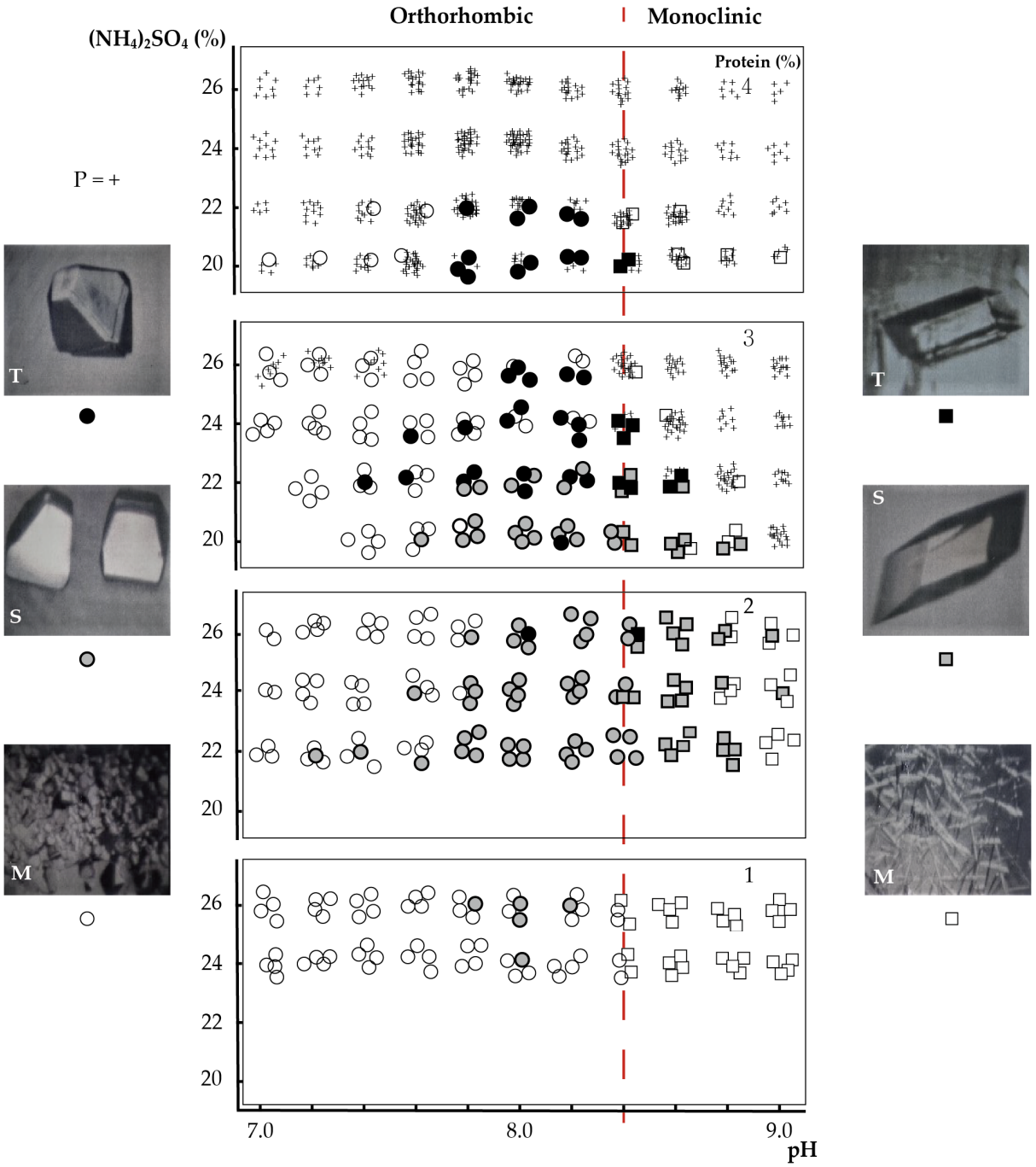

Figure S2. Influences of RuBisCO concentrations versus pH changes by the crystallization The numbers of crystallization droplets which were observed orthorhombic twin crystals (T) are represented as ●, those of single crystals (S) marked as ◍, and those of microcrystals (M) represented as ○, while the numbers of crystallization droplets observed monoclinic twin crystals (T) are marked as ■, those of monoclinic single crystals (S) are shown as ◪, and those of microcrystals (M) are marked as □, at 20 ℃. P represents + and stands for the area of precipitates. The concentrations of RuBisCO lower than 0.5% are blank in this diagram. Blank areas represent the unsaturated zones which could be observed neither crystals nor precipitates. The vertical red colored dotted line denotes the boundary of pH between the orthorhombic- or monoclinic morphology of RuBisCO crystals.

Table S1. Preliminary X-ray data of differently crystallized RuBisCOs from *A. eutrophus.*

Paper	Protein preparation	Crystallization	Temp.	Period	Ligand	Diffraction	Resol.
Bowien, B. *et al*	0.5% RuBisCO	20 mM Tris-HCl (pH 7.8)	4 ℃	2 weeks	Ternary complex	Space group	3.5 Å
Eur. J. Biochem.	20 mM Tris-HCl (pH 7.8)	$(NH_4)_2SO_4$			Mg^{2+}, HCO_3^-	Tetragonal, $P4_22_12$	
106: 405-410	10mM $MgCl_2$	$MgSO_4$				Unit cell length	
(1980)	50mM Na-HCO_3	MPD				a=b= 112.7 Å, c= 201.4 Å	
[1]		PEG 6000				α=β=γ= 90 °	
Pal, G.P. *et al*	2% RuBisCO	20 mM Tris-HCl (pH 7.8)	20 ℃	1 week	Quaternary complex	Space group	2.8 Å
J.Biol.Chem.	20 mM Tris-HCl (pH 7.8)	1.5-2.0 M $(NH_4)_2SO_4$			Mg^{2+}, HCO_3^-,CABP	Orthorhombic, $C222_1$	
260:10768-10770	10mM $MgCl_2$	10mM $MgCl_2$				Unit cell length (Å)	
(1985)	50mM Na-HCO_3	50mM $NaHCO_3$				a=b= 159.0 Å, c= 200.0 Å	
[2]						α=β=γ= 90 °	
Hansen, S. *et al*	4% RuBisCO	20 mM HEPES (pH 7.5)	4 ℃	2 weeks	Apo-enzyme	Space group	2.7 Å
Acta Cryst. D.	Isoform I	0.7-0.8 M K_2HPO_4 (pH 9.2)				Tetragonal, $P4_32_12$	
55:310-313	50 mM Tris-HCl (pH 7.8)					Unit cell parameter	
(1999)	$(NH_4)_2SO_4$ precipitation					a=b= 112.0 Å, c= 402.7 Å	
[3]						α=β=γ= 90 °	
Hansen, S. *et al*	4% RuBisCO	20 mM HEPES (7.5)	4 ℃	2 weeks	Apo-enzyme	Space group	3.2 Å
Acta Cryst. D.	Isoform II	8.5% PEG 4000				Tetragonal, $P4_32_12$	
55:310-313	50 mM Tris-HCl (pH 7.8)	1 M NaCl				Unit cell parameter	
(1999)	$(NH_4)_2SO_4$ precipitation					a=b= 111.8 Å, c= 400.0 Å	
[3]						α=β=γ= 90 °	
Current	4% RuBisCO	20 mM Tris-HCl (PH 8.5)	4 ℃	3 weeks	Quaternary complex	Space group	2.2 Å
study	20 mM	100 mM NaOAc	20		Mg^{2+},	Monoclinic, $P2_1$	

	Tris-HCl (pH 7.8)	(PH 8.5)	℃	HCO_3^-,CABP	
(2022)	10mM $MgCl_2$	22-28%saturated $(NH_4)_2SO_4$			Unit cell parameter
	50mM Na-HCO_3				a= 158.6 Å, b= 160.2 Å, c= 202.7 Å α=γ= 90 °, β= 91.8 °

Table S2. X-ray 3D structures of RuBisCOs from *A. eutrophus.*

Paper	Protein preparation	Method	Temp.	Period	Ligand	Diffraction	Resol.
Holzenburg, A.	0.15 M Tris-HCl (pH 7.8)	Electron			Quaternary complex		5 Å
et al	10mM $MgCl_2$	microscopy			Mg^{2+}, HCO_3^-,CABP		
Nature 325: 730–732 (1987) [4]	50mM Na-HCO_3						
Hansen, S. *et al*	4% RuBisCO	20 mM HEPES (pH 7.5)	4 ℃	2 weeks	Apo-structure	Space group	2.7 Å
J. Mol. Biol.	Isoform I	0.7-0.8 M K_2HPO_4 (pH 9.2)			(PDB entry: 1BXN)	Tetragonal, $P4_32_12$	
288: 609-621	50 mM Tris-HCl (pH 7.8)	X-ray crystallrography			inactive	Unit cell parameter	
(1999)	$(NH_4)_2SO_4$ precipitation					a=b= 112.0 Å, c= 402.7 Å	
[5]						α=β=γ= 90 °	

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