Phosphorylation of DegU is essential for activation of *amyE* expression in *Bacillus subtilis*

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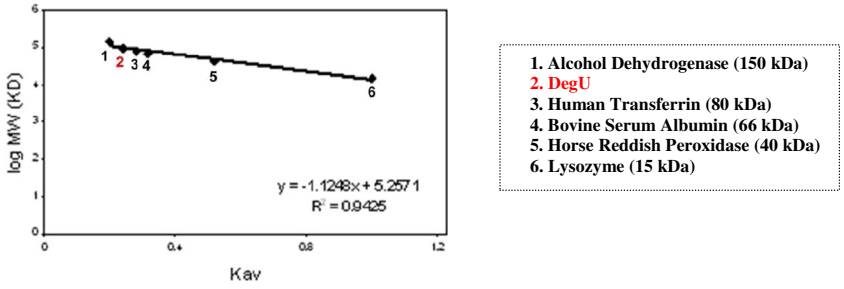
Supplementary material



Supplementary figure 1. Gel filtration analysis of DegU. Plot of K_{av} (Materials and methods) vs log molecular weight of five standards and the test (DegU). Positions of all samples have been marked with numbers (DegU is in red font).

Gel filtration chromatography of DegU on Sephacryl S100 (Methods)

The native molecular weight of DegU was determined on a pre-calibrated Sephacryl S-100 column (Sigma, total bed volume 12.5 mL). The column was equilibrated with Phosphate-NaCl buffer (50 mM Phosophate, 300 mM NaCl, pH 8.0) and was operated at a flow rate of 0.3 ml/min (gravity flow). Blue dextran was used as a marker to determine the void volume of the column. Following marker proteins were analyzed for calibration: Lysozyme (15 kDa), Horsereddish peroxidase (40 kDa), BSA (66 kDa), Human Transferrin (80kDa), Alcohol Dehydrogenase (150 kDa). All the above markers were loaded individually. Purified DegU (~1 mg), in a total volume of 1 mL was loaded onto the equilibrated column. Fractions of 500 µL were collected and analyzed for protein by the Bradford method of estimation. The native molecular weight of DegU was calculated from a plot of K_{av} against logarithmic molecular weight. The K_{av} was calculated using formula (procedures for molecular weight determination were as per Amersham Biosciences Gel Filtration handbook):

$$K_{av} = \frac{V_e - V_o}{V_t - V_o}$$

Where

V_e = elution volume for the protein
V_o = column void volume = elution volume for Blue Dextran 2000
V_t = total bed volume

The calculated values of K_{av} (of all the standards) were plotted against the log molecular weights (Y axis) and the following equation was derived (figure 1):

$$y = 1.1248x + 5.2571$$
$$R^2 = 0.9425$$

Determination of native molecular weight of DegU (supplementary figure 1)

Samples	K_{av} (X axis)	log MW (Y axis)	MW(kDa)
Alcohol Dehydrogenase	0.2	5.2	150
Human Transferrin	0.28	4.9	80
Bovine Serum Albumin	0.32	4.8	66
Horse reddish Peroxidase	0.52	4.6	40
Lysozyme	1.0	4.2	15
DegU	**0.24**	**5. 0**	**100**