BIOC2060: Purification of alkaline phosphatase

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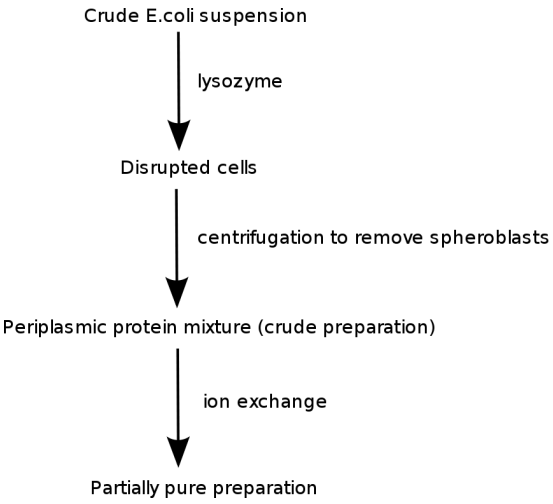
Contents

1 Introduction

A crude mixture of periplasmic proteins was obtained by treating E. coli suspension with lysozyme. Alkaline phosphatase was purified by DEAE-cellulose ion exchange using a NaCl gradient. Samples before ("crude preparation") and after ("partially pure preparation") were assayed for alkaline phosphatase activity (reaction with 4-nitrophenyl phosphate) and protein activity (Bradford assay).



2 Procedure

2.1 Lysozyme treatment

The original cell suspension was diluted until it had a turbidity between 6 and 9 (as measured by absorbance at 550nm). The turbidity of a x100 dilution was 0.587, indicating that the turbidity of the original suspension was 58.7, and that a x8 dilution should result in a turbidity of $\frac{58.7}{8} = 7.1$. It was found to be 7.69. The volume of the suspension was not recorded.

The progress of the reaction with lysozyme was observed by measuring the Abs_{550} of a x16 dilution at roughly 3-minute intervals.

Table 1: turbidity measurements of varying dilutions of the original suspension

Sample	Dilution	Abs_{550}
Undiluted	x1	>2
	x10	>2
	x100	0.587
Diluted x8	x1	>2
	x10	0.769

2.2 Partial purification

Fractions showing high alkaline phosphatase activity were pooled to give a partially pure preparation. 6 adjacent fractions were used for a total of 27ml.

2.3 Protein determination (Bradford assay)

The most accurate protein concentration values are those towards the centre of the calibration curve, i.e. where the absorbance is between 0.3 and 0.6. The

Figure 1: Progress of reaction with lysozyme, as measured by Abs_{550} of x16 dilutions.

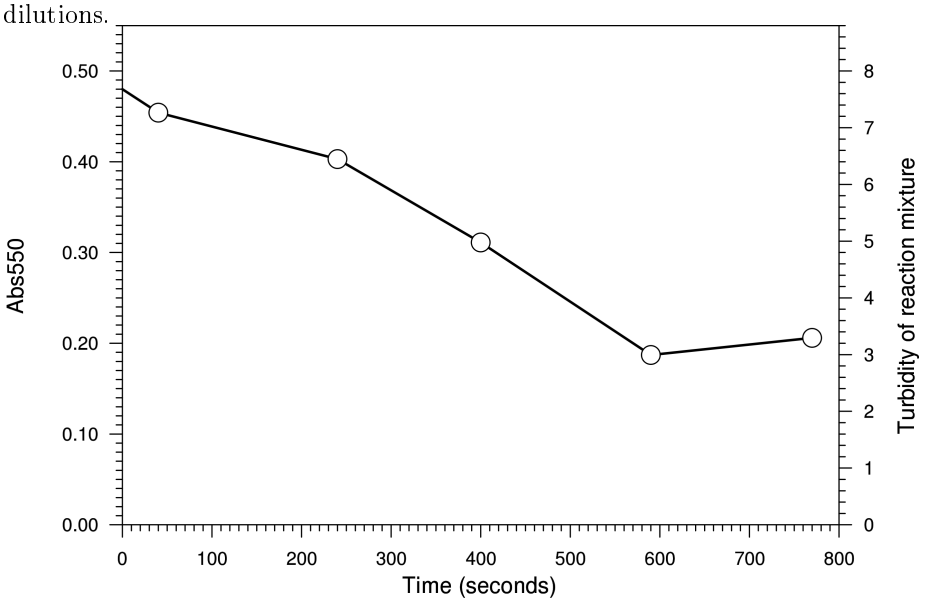


Figure 2: Amount of protein per fraction, as determined by Abs_{630} after treatment with Coomassie.

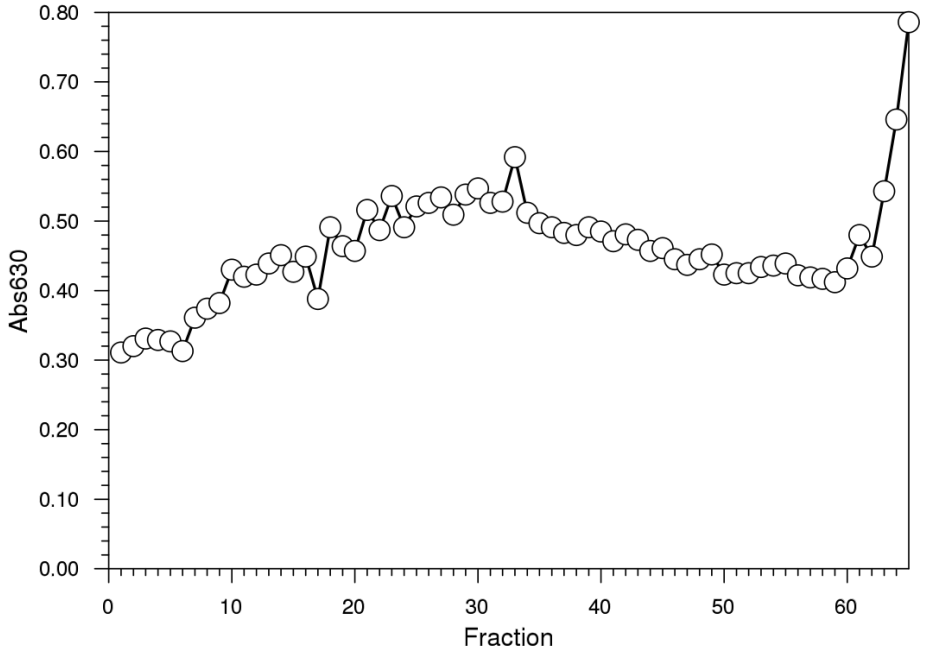


Figure 3: Alkaline phosphatase activity per fraction (Abs_{405} after addition of 4-nitrophenyl phosphate). Fractions with high activity were pooled as "partially pure".

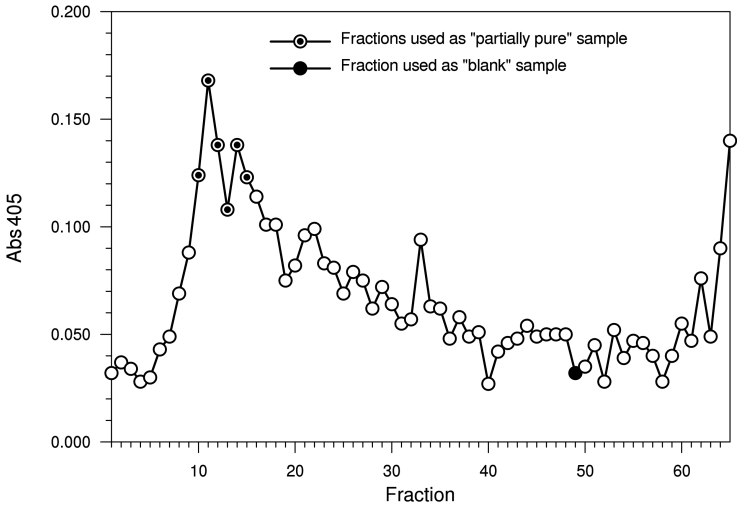


Figure 4: Calibration curve using BSA standard. Linear regression was used to find a relationship between absorbance and protein concentration.

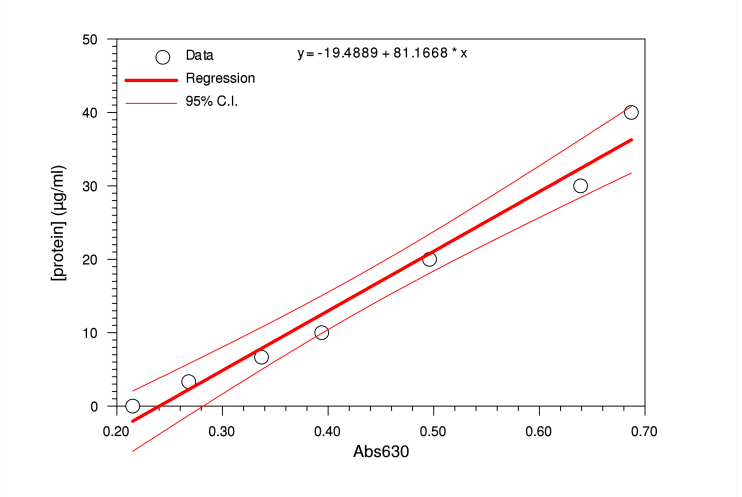


Table 2: protein concentrations in crude and partially pure preparations

	Crude			Partially pure		
		[protein] ($\mu g/ml$)			[protein] ($\mu g/ml$)	
Dilution	Abs_{630}	Diluted	Original	Abs_{630}	Diluted	Original
x1	1.119	N/A		0.409	13.7	13.7
x5	0.697	37.1	185	0.272	2.59	12.9
x10	0.443	16.5	165	0.241	0.07	N/A
x20	0.289	3.97	79.4	0.222	-1.5	N/A
x30	0.272	2.59	77.6	0.247	0.56	N/A
x40	0.251	0.883	35.3	0.233	-0.6	N/A
x50	0.343*	8.35	N/A	0.230	-0.82	N/A

Table 3: Enzyme activity calculation

	Crude		Partially pure	
Dilution	x30	x60	x30	x60
T_{start}	0	10	10	40
T_{end}	60	100	100	180
ΔT	60	90	90	140
ΔA_{400}	0.721	0.448	0.067	0.056
$\Delta A_{400}/min$	0.721	0.299	0.045	0.024
$\Delta A_{400}/min/ml$	7.21	5.98	0.446	0.480

x1 dilution of the crude extract is above this so cannot be used; the absorbance reading of the x50 dilution is anomalous and probably in error. Dilutions below x10 of the partially pure preparation are too close to zero to measure on the curve. Even discounting these, the range of possible values is quite large.

Bearing all this in mind, the x20 dilution of the crude extract and the x1 dilution of the partially pure preparation were used for determination of the protein concentrations, which are $\sim 80\mu g/ml$ for the crude extract and $\sim 13\mu g/ml$ for the partially pure preparation.

2.4 Enzyme activity

The reaction course for the x30 dilution of the partially pure preparation appears sigmoidal rather than hyperbolic. It is unclear why the x60 dilution of the partially pure preparation showed no change in absorbance for the first 30 seconds.

The changes in absorbance per unit time per ml of protein (and hence activity) were reasonably similar for both dilutions (x30 and x60), for both preparations, which provides some confidence in the values obtained.

2.5 Gel electrophoresis

SDS-PAGE was carried out on the crude preparation and the partially pure preparation. Many bands were present in the crude sample but there were only

Figure 5: Reaction courses for crude preparation.

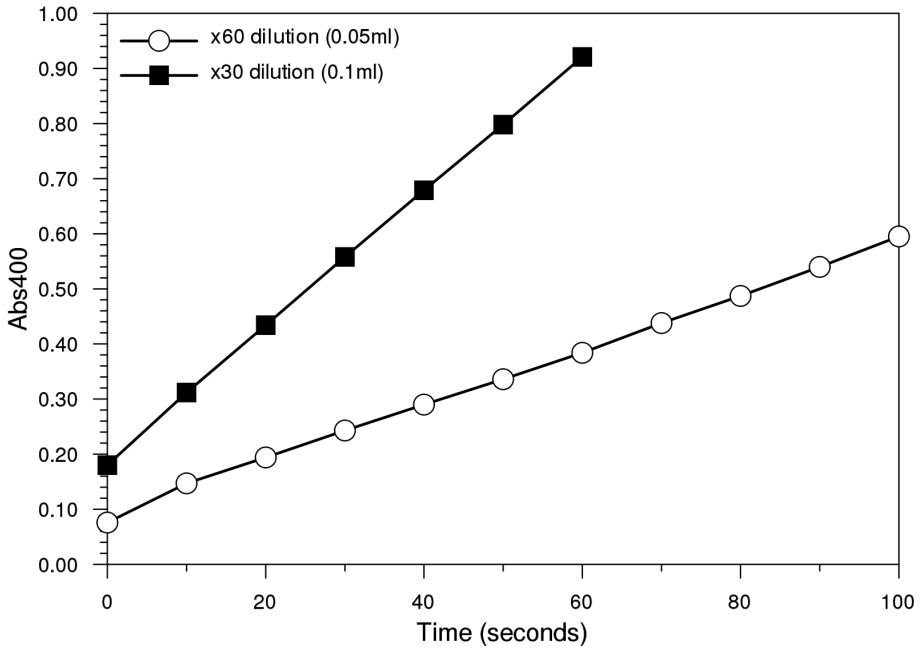


Figure 6: Reaction courses for partially pure preparation

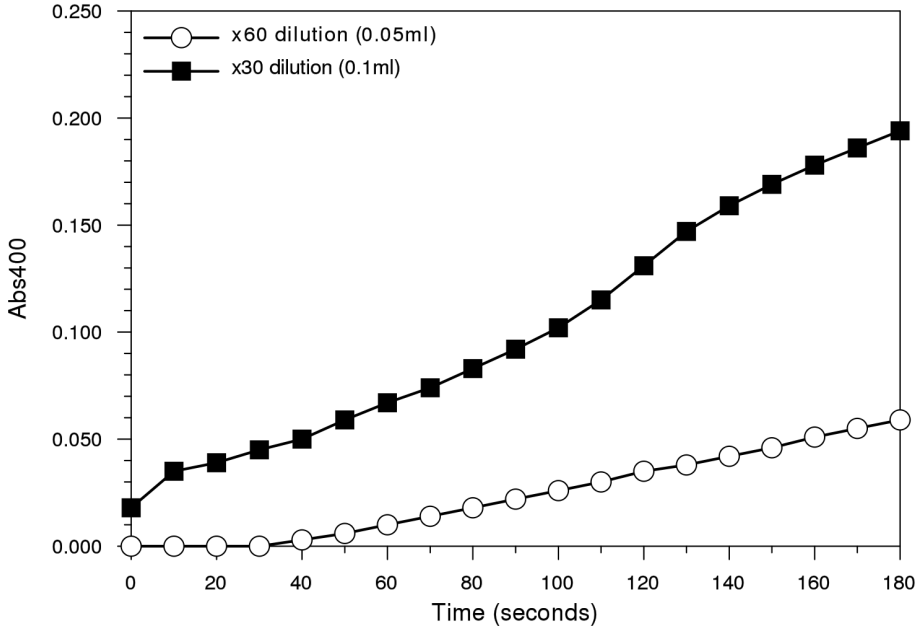


Figure 7: Results of SDS-PAGE. Lanes from left to right: marker, crude preparation, blank, marker, partially pure preparation.

two in the partially pure sample, indicating that some purification had taken place. The size of the molecular markers was not recorded, so it is not possible to deduce the size of the proteins in the preparations.

3 Conclusion

The data is contradictory: the purification factor calculation indicates that the partially pure preparation was only 0.38 times as pure as the initial crude preparation. This value however is sensitive to the values obtained in the Bradford assay, which were imprecise. (It may be worth noting that even using the highest possible value for the amount of protein in the crude preparation, 185 $\mu g/ml$, would give a purification factor of 0.88 which is still less than 1.) However, SDS-PAGE would seem to indicate that fewer proteins are present in the partially pure preparation than were present in the crude preparation.

The total recovery is not high. Most of the alkaline phosphatase was either in

other fractions or was denatured during the purification process.

Opinion: ion exchange seems to work as a purification procedure, although the amount of protein recovered is low; the alkaline phosphatase assay using 4-nitrophenyl phosphate also worked well. The Bradford assay gave very poor results however.

Since ion exchange separates proteins by charge, further purification should exploit another property such as size for maximum efficiency. Therefore size exclusion chromatography (e.g. gel filtration) would seem a promising next step.