Heterologous immunoadsorption in the purification of enzymes: N-acetyl-β-glucosaminidase

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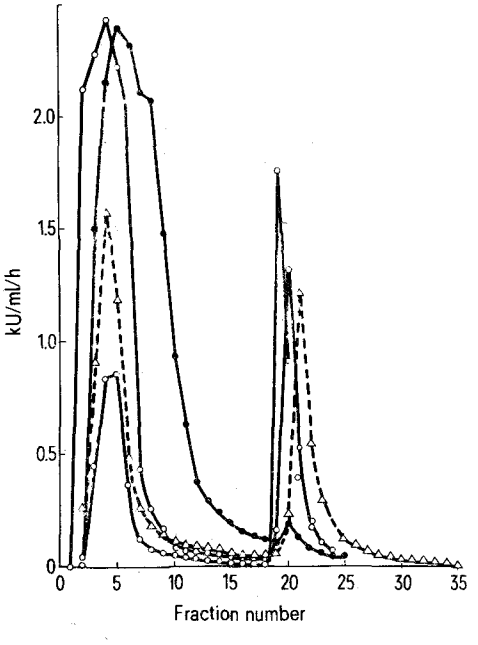
Summary. An immunoadsorbent column was prepared using a specific antibody to *N*-acetyl-β-glucosaminidase of human origin. Although no precipitating activity of the antisera was found with mouse or rat liver extracts, enzyme was easily eluted from the column which provided about 50fold, single-step purifications of these heterologous enzymes.

Immunoadsorption has been frequently used in the purification of enzymes. The technique has usually involved purifying a small amount of enzyme, preparing a specific antibody to the enzyme, and preparing an immunoadsorbent column by coupling antibody to CNBr-activated Sepharose. We previously remarked on some of the advantages of using heterologous immunoadsorption in the purification of enzymes[1]. Antisera to purified enzymes frequently cross-react with the same enzyme in even quite distantly related species and the lower affinity of antibody for the heterologous hapten can be of advantage in eluting the enzyme – the conditions of high salt or dilute acid sometimes necessary in the homologous situation, which usually involves a high avidity situation, frequently leads to denaturation of the enzyme and/or antibody[2]. *Aerobacter cloacae* β-galactosidase was eluted from a column of antibody to *Escherichia coli* β-galactosidase by the buffer in which the enzyme was applied to the column but much after the excluded volume[1]. Such heterologous immunoadsorption was recently used to purify human β-glucuronidase[3] but is not in general use[2]. We report the use of anti-human *N*-acetyl-β-glucosaminidase (this is the enzyme deficient in Tay-Sach's disease) to partially purify the liver enzyme from 2 rodent species.

Elution of liver extracts from column of anti-human *N*-acetyl-β-glucosaminidase

Source of enzyme	Units/ml of immuno-adsorbent retained	Original specific activity KU/mg h	Retained specific activity KU/mg h	Purification factor
Human acid extract $(NH_4)_2SO_4$ ppt	0.8	3.7	113	30.6
Mouse crude extract	1.5	0.23	18.1	79
Rat crude extract	1.16	0.4	17.6	44



Elution of *N*-acetyl-β-glucosaminidase from immunoadsorption column prepared with antibody to human *N*-acetyl-β-glucosaminidase. 4.5 ml fractions were collected from a 10 ml bed volumn column: ○—○, mouse liver homogenate (2 runs); △---△, rat liver homogenate; ●—●, calf liver homogenate.

Human *N*-acetyl-β-glucosaminidase was partially purified from liver by acid extraction, ammonium sulfate precipitation, molecular sieve-gel chromatography, and ion exchange chromatography[4]. We achieved higher specific activities than heretofore described by starting with post-mortem liver of a patient with mucopolysaccharidosis IIIb (San Filippo syndrome, *N*-acetyl-α-glucosaminidase deficiency) which was 10fold enriched in the enzyme. 27 μg of the partially purified *N*-acetyl-β-glucosaminidase was homogenized with Freund's adjuvant, complete for the first injection and incomplete thereafter, and injected subcutaneously 3 times at 1 week intervals into a New Zealand white, male rabbit. After 1 week, 18 μg of antigen in saline was injected intravenously and the rabbit was bled 1 week later. The antibody showed only one precipitation band with extracts of human liver. After extensive washing of the double diffusion gel, the band stained intensely with the histochemical substrate (*N*-acetyl-β-glucosamine-4-methylumbelliferone). The gamma-globulins of the antiserum were partially purified by taking the 33% $(NH_4)_2SO_4$ precipitate (two times, each time the precipitate was taken up in, and dialyzed against, 0.9% NaCl). 50 mg of the precipitated gamma-globulins were coupled to 25 ml of washed, CNBr-activated, Sepharose 4B as previously described[1]. Very small columns of 1 ml volume were prepared in Pasteur pipettes or small columns of about 10 ml volume were prepared in a 0.9 cm dia. glass column.

Crude homogenates or ammonium sulfate precipitates of liver acid extracts were dissolved in 0.01 *M*, pH 7.0 phosphate buffer for application to the column. After the sample had been applied, the column was washed with the 0.01 *M*, pH 7.0 phosphate buffer until no enzyme activity was eluted, 1 ml fractions being collected from the small column and 4.5 ml fractions from the larger

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column. Elution was then performed with 0.01 *M*, pH 4.6 citrate buffer, 1 *M* in NaCl. Enzymatic activity was assayed with the 4-methyl-umbelliferone substrate as previously described[5].

Double diffusion analyses showed the precipitin line with crude extracts of human liver but not with rabbit (the source of the antibody), sheep, calf, mouse or rat liver. Nonetheless, we suspected that a cross-reaction with some of these species might be present but that the avidity of the antibody would be insufficient for a precipitation reaction (cross-reaction with a single hapten per enzyme molecule seemed less likely). Such low avidity antibodies may allow elution from immunoadsorbent columns at milder conditions. As seen in the figure, rat and mouse liver *N*-acetyl-β-hexosaminidase were retained by the immunoadsorbent column and readily eluted in 1.0 *M* NaCl. However, calf, sheep (not shown), and rabbit (as expected) liver *N*-acetyl-β-glucosaminidase were not retained by the column. Despite large variations in the amount of enzyme applied to the columns, similar amounts were retained by the immunoadsorbent column suggesting that each of the antigenic preparations saturated the available antibody-combining sites about equally well. As seen in the table, the immunoadsorbent column capacity was roughly similar for human, mouse and rat liver *N*-acetyl-β-glucosaminidase. The 79-(mouse) and 44-(rat)fold purifications achieved with the heterologous antigens were actually higher than those achieved with the homologous antigen but this is explained because the human liver starting material was partially purified.

Immunoadsorption purification of enzyme from other species extends the usefulness of an antibody prepared to a purified enzyme of one species. Similar capacities and purification factors are achieved and elution may occur more readily[6].

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Reaction step requiring protein synthesis in DNA synthesis in sea urchin embryos

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Summary. In the acid-soluble fraction extracted from embryos treated with cycloheximide, thymidine triphosphate was largely accumulated and DNA polymerase activity decreased, while both activities of thymidine kinase and thymidylate kinase remained unchanged.

It has been demonstrated that the initiation of in vivo DNA synthesis is dependent on protein synthesis[2–12]. The present study was conducted to investigate which step in the reaction sequence of DNA synthesis from deoxyribonucleosides requires protein synthesis.

Materials and methods. Embryos of the sea urchin, Hemicentrotus pulcherrimus, were cultured to the blastula stage at a population density of 3.5×10^4 per ml in artificial sea water containing 300 units of penicillin and 50 μg of streptomycin per ml for 20 h at 20 °C. Then the suspension was divided into 2 parts. One part was allowed to develop as before (control) and the other was incubated with 10 mM cycloheximide. 60 minutes after adding cycloheximide, samples (10 ml, 3.5×10^5 embryos) of each suspension were exposed to 25 μCi, 2.38 nmoles of [^{3}H]-thymidine for 10 min at 20 °C. After reaction, radioactivities in the medium, the acid-soluble and DNA fractions were measured, and then the acid-soluble fraction was chromatographed and the fractions separated were analyzed as described previously[13]. The activities of DNA polymerase, thymidine kinase and thymidylate kinase were assayed as described previously[13].

Results and discussion. In normal embryos (control), much added [^{3}H]thymidine was taken up into the acid-soluble

Table 1. Effect of cycloheximide on the uptake and incorporation of [^{3}H]thymidine in blastulae and relative proportions of [^{3}H]thymidine and its phosphorylated derivatives in the acid-soluble fraction

	Control (cpm $\times 10^{-5}$)	%	10 mM cycloheximide (cpm $\times 10^{-5}$)	%
Medium after incubation	45.06	23.4	67.82	35.6
Acid-soluble fraction	96.25	50.0	98.25	51.5
dTTP	8.76	(9.11)	45.85	(46.60)
dTDP	2.32	(2.41)	3.55	(3.61)
dTMP	2.52	(2.62)	4.38	(3.45)
TdR	82.50	(85.80)	44.92	(45.65)
DNA fraction	51.02	26.6	24.39	12.9
Total	192.34		190.46	

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