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Note

Hydrophobic chromatography of N-acetyl-β-D-hexosaminidase

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The hydrophobic chromatography of different natural biopolymers, including enzymes, is currently being developed with some success. This technique has been used successfully to purify chymotrypsin and chymotrypsinogen[1–3], ovalbumin and bovine serum albumin[1,4], β-galactosidase[5,6] and many other enzymes[7–17].

We have synthesized various alkylated derivatives of agarose, differing in the alkyl chain length and the presence of different functional groups, with a view to applying hydrophobic chromatography to the isolation and purification of N-acetyl-β-D-hexosaminidase (E.C. 3.2.1.30). Ethanolamine, ethylenediamine, tetramethylenediamine and heptamethylenediamine were added to Sepharose 4B activated by trichlorotriazine. The N-acyl derivatives of tetramethylenediaminoagarose and heptamethylenediaminoagarose were obtained by acylating the two compounds with benzoic and acetic acids (Table I).

EXPERIMENTAL

General methods

The activity of N-acetyl-β-D-hexosaminidase was determined in 0.1 *M* phosphate buffer (pH 3.5) using *p*-nitrophenyl-2-acetamido-2-deoxy-β-D-glucopyranoside as a substrate. The reaction was terminated by adding 1 ml of 1 *M* sodium carbonate solution, and the amount of *p*-nitrophenol obtained was determined spectrophotometrically at 440 nm on a Spekol instrument (Carl Zeiss, Jena, D.D.R.). The protein concentration was determined according to Lowry *et al.*[18].

The activation of Sepharose 4B and addition of alkyldiamines was performed as described earlier[19]. In this instance, every twentieth sugar residue in agarose gel was modified (analysis of trichlorotriazine of activated Sepharose: N = 1.76%, Cl = 2.08%).

Acylation of aminoalkylsepharoses

Aminoalkyl-Sepharose (20 ml) in dioxan (20 ml) and 4 m*M* dicyclohexylcarbodiimide (0.8 g) were added to a 2 m*M* solution of benzoic acid (0.25 g) in methanol (2 ml). The mixture was agitated for 12 h at 18°, then the sorbents were filtered off and washed with aqueous methanol, aqueous acetone and water (200 ml of each). The corresponding N-acetyl sorbents were obtained by an anhydride method.

TABLE I
SORPTION OF N-ACETYL-β-D-HEXOSAMINIDASE ON AMINOALKYLSEPHAROSES

Sorbent No.	*Hydrophobic sorbent*	*Enzyme bonding (%) at pH 5.0 in 0.05 M phosphate buffer*	*Elution (%) of enzyme with 1 M NaCl in 0.05 M phosphate buffer (pH 5.0)*	*pK_a and pK_b values of ionizing groups*
I	R = –OH	100	100	$pK_{a_1} = 3.95$; $pK_{b_1} = 7.0$
II	R = –$NHCH_2CH_2OH$	100	100	$pK_{a_1} = 4.05$; $pK_{b_1} = 6.7$; $pK_{a_2} = 9.1$
III	R = –$NHCH_2CH_2NH_2$	28*	28	
IV	R = –$NHCH_2CH_2CH_2CH_2NH_2$	4.5*	4.5	
V	R = –$NHCH_2CH_2CH_2CH_2NHCOCH_3$	50*	50	$pK_{a_1} = 4.0$; $pK_{b_1} = 6.8$; $pK_{a_2} = 9.2$
VI	R = –$NHCH_2CH_2CH_2CH_2CH_2CH_2CH_2NH_2$	49*	49	
VII	R = –$NH(CH_2)_4NHCOC_6H_5$	95*	95	
VIII	R = –$NH(CH_2)_7NHCOCH_3$	100	33**	$pK_{a_1} = 3.9$; $pK_{b_1} = 6.7$; $pK_{a_2} = 9.1$

* Remaining N-acetyl-β-D-hexosaminidase was not bonded by sorbent when spotting sample.
** Remaining 67% of N-acetyl-β-D-hexosaminidase was eluted from sorbent with 50% ethylene glycol in 0.05 *M* phosphate buffer, pH 5.0.

RESULTS AND DISCUSSION

A solution of purified N-acetyl-β-D-hexosaminidase[20], optical activity of 20.6 unit/mg (2.2 mg of protein), was passed through a column containing a hydrophobic sorbent (5 × 0.5 cm) equilibrated with 0.05 *M* phosphate buffer, pH 5.0. The enzyme was eluted with a 1 *M* solution of sodium chloride in 0.05 *M* phosphate buffer (pH 5.0) to determine the enzyme activity in each eluate fraction. The results obtained are given in Table I, where complete sorption of N-acetyl-β-D-hexosaminidase is considered to be represented by 100% bonding.

The results in Table I show that the sorption of N-acetyl-β-D-hexosaminidase was weak on sorbents I–VII, which contain free amino groups; however, it bonded well with sorbent VIII. The enzyme was eluted from sorbent VIII by 50% ethylene glycol in 0.05 *M* phosphate buffer (pH 5.0). In order to investigate the effect of hydrophobic sorbent charges, we determined the p*K* values of ionizing groups using these polymers (Table I)[21]. By comparing the results in Table I and the p*K* values of ionizing polymer groups, it can be concluded that an increase in alkyl chain length results in stronger bonding of N-acetyl-β-D-hexosaminidase; at the same time, protonated amino groups inhibit hydrophobic bonding of the enzyme.

REFERENCES

1 B. H. J. Hofstee, *Biochem. Biophys. Res. Commun.*, 50 (1973) 75.
2 V. M. Stepanov, G. I. Lavrenova, V. P. Borovikova and G. N. Balandina, *J. Chromatogr.*, 104 (1975) 373.
3 S. K. Sharma and T. R. Hopkins, *J. Chromatogr.*, 110 (1975) 321.
4 S. Hjertén, *J. Chromatogr.*, 87 (1973) 325.
5 O. Raiband, A. Hogberg-Raiband and M. Goldberg, *FEBS Lett.*, 50 (1975) 130.
6 A. H. Nishikawa and P. Bailon, *Arch. Biochem. Biophys.*, 168 (1975) 576.
7 Z. Er-el, J. Zaidenzaig and S. Shaltiel, *Biochem. Biophys. Res. Commun.*, 49 (1972) 383.
8 B. H. J. Hofstee, *Anal. Biochem.*, 52 (1973) 430.
9 J. Porath, L. Sundberg, N. Fornstedt and I. Olsson, *Nature (London)*, 245 (1973) 465.
10 B. H. J. Hofstee, *Biochem. Biophys. Res. Commun.*, 53 (1973) 1137.
11 S. Shaltiel and Z. Er-el, *Proc. Nat. Acad. Sci. U.S.*, 70 (1973) 778.
12 S. Hjertén, J. Rosengren and S. Påhlman, *J. Chromatogr.*, 101 (1974) 281.
13 C. C. Huang and D. Aminoff, *Biochim. Biophys. Acta*, 371 (1974) 462.
14 R. Jost, T. Miron and M. Wilchek, *Biochim. Biophys. Acta*, 362 (1974) 75.
15 P. O'Carra, S. Barry and T. Griffin, *FEBS Lett.*, 43 (1974) 169.
16 J. Visser and M. Strating, *Biochim. Biophys. Acta*, 384 (1975) 69.
17 F. Thanner, D. Palm and S. Shaltiel, *FEBS Lett.*, 55 (1975) 178.
18 O. H. Lowry, N. J. Resebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, 193 (1951) 265.
19 A. A. Artyukov and N. V. Molodtsov, *Bioorg. Khim.*, 1 (1975) 1577.
20 E. V. Sundukova, M. G. Vafina and N. V. Molodtsov, *Biokhimiya*, 39 (1974) 384.
21 A. Yaron, M. C. Otey, H. A. Sober and E. Katchalski, *Biopolymers*, 11 (1972) 607.