Sepharose-Avidin Column for the Binding of Biotin or Biotin-Containing Peptides

The method of attaching a biologically active protein, e.g., an enzyme to an activated polysaccharide matrix[1] has become widely used in recent years, since on the inert surface the enzyme retains most of its activity and is easy to handle. Selective purification methods for proteins based on such affinity chromatography[2] were devised like the single step isolation-purification of avidin from egg white[3].

We found that avidin can be coupled to Sepharose 4B activated with cyanogen bromide. A column prepared of this Sepharose-avidin showed considerable biotin or dye[4] binding capacity. For binding of biotin or biotin containing peptides such columns are quite efficient, recovery of the biotin containing material may be less practical.

In a typical experiment 20 ml agarose gel (Sepharose 4B from Pharmacia) was suspended in 20 ml water and a solution of 2 g CNBr in 20 ml water was added. The pH was raised and maintained at about 11 by the dropwise addition of 4 N NaOH. When the pH did not fall any more (about 10 ml 4 N NaOH was needed) the suspension was filtered on Buchner funnel and washed with 0.1 M $NaHCO_3$. To this activated gel were added 20 mg of avidin (Worthington) in 2 ml 0.1 M $NaHCO_3$ and the mixture was stirred at 4 °C for 20 h. The Sepharose-avidin was then poured into a chromatography tube, washed with 0.1 M $NaHCO_3$, and finally with 0.2 M phosphate buffer of pH 6.8. The UV-absorption of the effluents was measured at 280 nm to find unbound avidin. At least 80% of the avidin was bound by the gel.

A sample of this Sepharose-avidin (1 ml after low speed centrifugation) turned pink when treated with a 100 μM solution of 4-hydroxyazobenzene-2′-carboxylic acid. It was washed with 3 ml of 0.2 M phosphate buffer, pH 6.8, and then titrated with a biotin solution (0.034 mg/ml) until the red color disappeared (0.45 ml biotin solution). Thus, the Sepharose-avidin has a biotin binding capacity of 0.06 μmol/ml or 15 γ/ml.

The Sepharose-avidin was used to bind biotin containing fragments from a tryptic digest of methylmalonyl-oxaloacetic transcarboxylase[5] containing tritiated biotin. The solution, 336,000 counts/min in 4.5 ml of 0.2 M phosphate buffer pH 6.8 was poured over a column prepared from 4 ml Sepharose-avidin and was washed with 0.2 M phosphate buffer of pH 6.8. The column was dyed red with 5 ml 100 μM 4-hydroxyazobenzene-2′-carboxylate in 0.2 M phosphate buffer of pH 6.8, and washed with 6 ml of the same buffer. According to previous titration this column can bind 0.24 μmol of biotin. When the transcarboxylase digest was poured over the red column, its top one-third was discolored. The column was then washed with 0.2 M phosphate buffer and fractions of 8–10 ml were collected and counted. A total of 900 counts/min was found in 4 fractions. Elution with a 6 M guanidine-hydrochloride-hydrochloric acid solution of pH 1.5[3] removed only a total of 80,000 counts/min, or about 24% of the biotin containing fragments.

Zusammenfassung. Avidin konnte an das Polysaccharid Sepharose 4B, das zuvor mit Bromcyan aktiviert wurde, gebunden werden. Um Biotin oder Biotin-haltige Peptide zu binden, erwies sich eine Sepharose-Avidin-Säule als besonders günstig.

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[1] R. AZEN, J. PORATH and S. ERNBACK, Nature *214*, 1302 (1967).
[2] P. CUATRECASAS, M. WILCHECK and C. B. ANFINSEN, Biochemistry *61*, 636 (1968).
[3] P. CUATRECASAS and M. WILCHEK, Biochem. biophys. Res. Commun. *33*, 235 (1968).
[4] N. M. GREEN, Biochem. J. *94*, 23c (1965).
[5] H. G. WOOD, S. H. G. ALLEN, R. STJERNHOLM and B. JACOBSON, J. biol. Chem. *238*, 547 (1963).

Fluorometric Detection of Serotonin Using o-Phthaldialdehyde: an Improvement

Reaction of *o*-phthaldialdehyde (OPT) with serotonin standards increases amine detection sensitivity[1] over the direct BOGDANSKI procedure[2]; however, no detailed information is available concerning the blank which is a consistent problem when low concentrations of serotonin are being analyzed. The blank arises primarily from reagents and glassware and is variable and not easily controllable[3]. In the BOGDANSKI procedure, if blank fluorescence is large relative to serotonin fluorescence, the peak of maximal serotonin fluorescence (545 nm) may not be present but appears in the spectrum at a shorter wavelength. This apparent shift (atypicallity) is due to overlap of blank (ca. 400 nm wavelength) and serotonin (545 nm wavelength) emissions which are detected additively. Readings from the atypical peak or at 545 nm wavelength are inaccurate. We have reported[3] that the blank in the serotonin-OPT method constitutes a greater problem than in the BOGDANSKI procedure since reagent OPT produces exaggerated primary scattering, the increased volume of HCl required to achieve optimal acidity[4] produces greater blank fluorescence, and the peak of maximal serotonin-OPT fluorescence occurs at 473 nm wavelength in comparison to the maximal emission of serotonin at 545 nm. Reported here are the advantages of a chloroform wash in the serotonin-OPT method; chloroform removes major blank emissions and reduces primary scattering.

[1] R. P. MAICKEL and F. P. MILLER, Analyt. Chem. *38*, 1937 (1966).
[2] D. F. BOGDANSKI, A. PLETSCHER, B. B. BRODIE and S. UDENFRIEND, J. Pharmac. *117*, 82 (1956).
[3] J. H. THOMPSON, C. A. SPEZIA and M. ANGULO, Biochem. Pharmac., submitted.
[4] J. H. THOMPSON, C. A. SPEZIA and M. ANGULO, Experientia *25*, 927 (1969).