Streptavidin Properties and Characterization

OVERVIEW

Streptavidin is a biotin-binding protein found in the culture broth of the bacterium *Streptomyces avidinii*. Like its namesake avidin, streptavidin binds 4 moles of biotin per mole of protein with a high affinity virtually unmatched in nature (Kd~10-15). Streptavidin lacks carbohydrate side chains present on avidin and has an isoelectric point nearer to neutralitymwhere most useful biological interactions occur (pI of 5-6 vs 10 for avidin). As a result, streptavidin frequently exhibits lower levels of non-specific binding than does avidin when the proteins are used in applications relying upon the formation of avidin/biotin complexes (Chaiet and Wolf, 1964).

In ELISA-based diagnostic systems, antibodies directed against a particular antigen may be covalently attached to reporter enzymes. Antigens are then quantitated by enzymatic assay after binding to these conjugated molecules. Unfortunately, the precise conditions for accomplishing such covalent attachments must be determined individually for each antibody/reporter combination, and often result in significant loss of either the enzymatic activity of the reporter enzyme or the binding functions of the antibodies. Streptavidin finds utility in these systems because antibody molecules are easily modified by the covalent attachment of derivatives of biotin with little or no loss in the ability of the antibody molecules to bind their antigens. These biotinylated antibodies may be detected by their interaction with conjugates of streptavidin and the reporter enzymes (Guesdon et al, 1979). The same preparation of conjugated streptavidin-reporter enzyme may be used with any number of different biotinylated antibodies making this system a highly flexible one.

The reporter molecule may be bound to streptavidin covalently, or biotinylated and attached to streptavidin via the streptavidin-biotin interaction. Since streptavidin is multivalent (binding 4 molecules of biotin per tetrameric protein molecule) it may be used in combination with biotinylated antibody and biotinylated reporter enzymes to obtain amplified signals. Such amplification in ELISA's is otherwise difficult to obtain and requires the introduction of additional antibody components. ELISA systems employing streptavidin can readily detect sub-nanogram amounts of antigens.

Characteristics

The molecular weight of streptavidin is 55,000 daltons. The protein is composed of 4 essentially identical polypeptide chains (homotetramer). The protein contains no cysteine residues, carbohydrate side chains or associated cofactors. Different preparations of streptavidin show considerable heterogeneity at both the amino- and carboxytermini of each subunit polypeptide due to proteolysis during biosynthesis and secretion. Monomeric subunits of streptavidin are synthesized as 183 amino acid prepeptides. During secretion by Streptomyces sp., a 24 amino acid leader sequence is cleaved from these polypeptides resulting in newly secreted monomers of 159 amino acids (Agaraña et al, 1986). Upon longer incubations in culture these monomers are progressively cleaved to "core" subunits containing 125-127 amino acids (Bayer et al, 1989; Hendrickson et al, 1989). Preparations of streptavidin are relatively stable over a wide pH range and extremely heat stable, requiring up to 20 minutes at 100C in 0.2% SDS to dissociate the subunits (Bayer et al, 1986). Strong chaotropic agents such as 6 M urea have been reported to dissociate the streptavidin tetramer into dimers (Sano and Cantor, 1990). These dimers appear to be stable in urea without appearance of monomers. Unproteolyzed and proteolyzed preparations of streptavidin appear to bind biotin with equal affinity. The most highly proteolyzed tetramers may bind over 16 micrograms of d-biotin per milligram of protein. Bayer (1989) reports that biotinylated enzymes bind most effectively to truncated streptavidin in ELISA-type assays.

The dissociation constant for biotin is approximately 10-15 M (Chaiet et al, 1964). The formation of the streptavidin-biotin complex is stable over wide pH and temperature ranges. The complex is generally disrupted only by conditions which lead to irreversible denaturation of the protein. Analogs of biotin such as 2-imino-biotin bind reversibly to the protein with complex formation at high pH (>9.5) and dissociation at low pH (<4) (Bayer et al, 1986; Hofmann et al, 1980).The extinction coefficient of streptavidin is E(0.1% at 280 nm): 3.17 (Suter et al,1988)

BIOTIN BINDING

The biotin-binding activity of streptavidin is determined using a modification of the dye-binding assay of Green (1970). One unit will bind one microgram of d-biotin at pH 7.0.

Reagents

- M 2-(4'-Hydroxyazobenzene) benzoic acid dissolved in 0.01 M sodium hydroxide (HABA)
- 0.2 M sodium phosphate, pH 7.0
- 0.002 M d-biotin in 0.1 M sodium phosphate, pH 7.0
- Streptavidin dissolved at 5-10 mg/ml in de-ionized water. If the sample has a concentration outside this range, adjust the volume of sample in the assay accordingly.

Procedure

1. Adjust spectrophotometer to read at 500 nm.
2. To two tubes labeled A and B add as follows:

	A	B
Streptavidin sample	0.05 ml	0.05 ml
Phosphate Buffer	0.5 ml	0.5 ml
HABA stock	0.1 ml	0.1 ml
Biotin stock	--	0.25 ml
H_2O	0.35 ml	0.1 ml
Total Volume	1.0 ml	1.0 ml

3. After mixing, zero the spectrophotometer with water and read the absorbances in tubes A and B.
4. Calculations:

$$\text{Units/mg} = \frac{(10^6 \mu g/g)(A-B)MV}{E(Cv)} = \frac{141(A-B)}{C}$$

Where:

M= formula weight of d-biotin (244 g/mole)

V= volume of assay in liters (.001 liters)

v= volume of streptavidin in sample (mg/ml)

C= concentration of streptavidin in sample (mg/ml)

E= net molar extinction coefficient of HABA-streptavidin complex at 500 nm (34,500 M^{-1})

SUGGESTIONS FOR USE

Bayer (1989) reports that streptavidin may form aggregates under certain conditions. Streptavidin is highly soluble under alkaline conditions (pH > 8.5). Streptavidin is often supplied lyophilized. Under these conditions there is a tendency for the material to aggregate if it is redissolved in water or other low ionic strength buffers at neutral or acidic pH. As a convenience to customers, Streptavidin has been lyophilized from a dilute sodium chloride solution at mildly alkaline pH. This material is readily soluble in water. The activity of the material recovered after reconstitution under these conditions is undiminished. We recommend dissolving streptavidin in de-ionized water or, preferably, 1.0 mM sodium bicarbonate buffer (pH 9) at twice the desired final protein concentration. The protein may then be diluted with an equal volume of 2x buffer to produce a stock solution. Upon standing some turbidity may develop in certain buffers. Centrifugation will usually yield a clear solution with negligible loss of streptavidin.

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