PRO EXPERIMENTIS

A Radioimmunoassay for Dopamine-β-Hydroxylase Using a Glass-Bound Antibody

A solid radioimmunoassay using antibodies to sheep adrenal dopamine-β-hydroxylase (EC 1.14.17.1) (DβH) and ^{125}I-labelled sheep adrenal DβH has been described[1]. The procedure has been modified and applied to the assay of human serum DβH[2]. These methods utilized the adsorption of the antibody to polystyrene tubes as described by CATT and TREGEAR[3]. In this communication we describe a new solid-phase assay for bovine DβH utilizing an antibody covalently bound to glass beads. This assay can be completed much faster and offers other advantages in comparison to the assay reported previously.

Purified bovine DβH, ^{125}I-DβH and rabbit antisera to bovine DβH were prepared as described previously[1,4].

Preparation of tissue for assay. A 10% homogenate of the tissue in 50 m*M Tris* buffer (pH 6.8) containing 0.1% Triton X-100 was centrifuged at 10,000 *g* at 0 °C. The proteins in the supernatant fraction were precipitated in 80% saturated $(NH_4)_2$ SO_4 solution at 0 °C. The precipitate was collected by centrifugation at 10,000 *g* and dissolved in 0.15 *M* NaCl. 2 aliquots of the solution were submitted to the radioimmunoassay.

Preparation of the glass-bound antibody. Controlled-pore arylamine glass (1100 Å pore diameter, 1–4 μ average diameter) was generously supplied by Corning Glass Co., Corning, New York. About 370 mg of the arylamine glass was treated with 200 mg of sodium nitrite in 2 ml of 2 *N* HCl at 0 °C with stirring. The diazotized glass was washed with water. Anti-DβH serum (0.5 ml) was adjusted to pH 8.5 with borate buffer at 0 °C. The diazotized glass was added in small portions with stirring, and the pH was maintained by adding 0.1 *N* NaOH. The slurry was washed with water and borate buffer by alternate centrifugation and decantation. The final precipitate was suspended in sufficient 0.01 *M* phosphate buffer pH 7.5 to produce a mobile slurry on vigorous mixing, and stored at 4 °C.

Radioimmunoassay. The buffer used in these experiments was phosphate buffer (10 m*M*, pH 7.5) containing 0.15 *M* NaCl and 1% bovine serum albumin. In a typical assay a mixture of standard DβH or the sample, the glass bound antibody (final dilution of 1:32,000) and 40,000 cpm of ^{125}I-DβH was shaken vigorously and incubated for 16 h at room temperature. The mixture was centrifuged and the sediment was counted in a gamma counter. The DβH concentration in the tissue extract was calculated by reference to the standard curve. In some studies comparisons were made with the results obtained by a method described previously in which the DβH antibody was coated on polystyrene tubes[1].

Standard curves were constructed by plotting the percent ^{125}I-DβH bound as a function of Dβ H added to the system. The Figure shows results obtained following parallel incubations for 2 h and 16 h, respectively. It is evident that the 16 h incubation with the glass bound antibody (GBA) resulted in a greater percentage of binding and in a greater range of linearity than that obtained following 2 h of incubation.

The method was applied to the assay of DβH in the bovine adrenal gland and in various areas of the bovine brain. Attempts to measure DβH levels in the brain homogenates directly were unsuccessful, presumably because of the presence of interfering substances. The single step of purification by the precipitation with $(NH_4)_2$ SO_4 yields a preparation which was found to be suitable for measurements of DβH levels by the GBA as well as by the antibody coated tube (ACT) procedure.

It is evident from the data in the Table that with the GBA method high DβH levels were found in the adrenal gland and in various regions of the CNS known to be enriched with norepinephrine cell bodies and nerve endings (i.e. locus ceruleus and hypothalamus). DβH levels in the medulla oblongata were much lower. Virtually undetectable levels were found in the striatum. In another experiment 10 ng of purified DβH was added to each tissue extract and the DβH levels were estimated by the GBA method. The increase in DβH level closely reflected the amount added.

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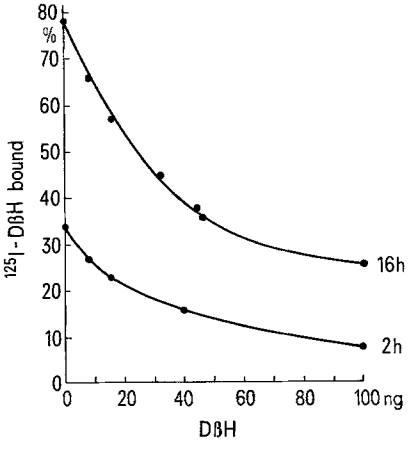

Standard radioimmunoassay curve for bovine DβH Upper curve follows incubation for 16 h; lower curve follows incubation for 2 h.

Immunoreactive-dopamine-β-hydroxylase levels in bovine adrenal medulla and in various regions of the bovine brain

Tissue	IR-DβH (μg/g tissue)	
	GBA[a]	ACT[b]
Adrenal medulla	185.0	360.0
Hypothalamus	0.56	1.46
Locus ceruleus	0.46	0.92
Medulla oblongata	0.17	0.80
Striatum	N.D.[a]	0.46

Abbreviations used: GBA, glass bound antibody method; ACT, antibody coated tube method; N.D., not detectable (less than 0.1 μg/g tissue). [a] The values represent averages of 3 assays $\pm$ 0.5–1% SEM. [b] The values represent averages of 3 assays $\pm$ 2–5% SEM.

A comparison was made between the results obtained with the GBA method and those obtained with the ACT method. The DβH levels were considerably lower when assayed by the GBA method (Table). However, when the adrenal extract was diluted further the DβH levels as estimated by the GBA method remained constant, whereas when estimated by the ACT method the level declined. This result suggests that in concentrated extracts some unspecific bindings interfere with the DβH assay by the ACT method.

The low levels of DβH in the medulla oblongata and the virtually undetectable levels in the striatum as shown by the GBA method are consistent with the reported enzyme activity levels in these regions of the brain[5]. These results indicate that the GBA method is more specific than the ACT method.

One of the attractive features of the GBA assay is the relative ease of operation as compared to the ACT method. Once prepared, the glass-bound antibody can be stored and used for many assays, whereas the previously described method requires that each tube be coated with antibody separately. Another advantage is superior precision. Replicate assays by the GBA method yield mean values with less than 1% in the standard error, whereas the corresponding value for the ACT method is about 5%.

The simplicity of the GBA method would render it applicable for routine clinical assays of human serum DβH. Human DβH antibodies have been covalently bound to diazotized arylamine glass and studies on measurements of human serum DβH are in progress[6].

Résumé. Les auteurs décrivent une méthode radioimmunologique de dosage de la DβH utilisant un anticorps lié de façon covalente à des billes de verre. La technique a été appliquée à la mesure de la DβH dans les surrénales et différentes régions du cerveau de bœuf. Les principaux avantages de cette méthode sont sa relative facilité d'emploi, sa précision et sa haute spécificité.

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An Ammoniacal-Silver Stain Technique Specific for Satellite III DNA Regions on Human Chromosomes

Cytogeneticists are currently using in situ hybridization of radioactive complementary RNA fractions to human chromosomes in an attempt to localize the different classes of DNA in specific chromosome regions. Parallel efforts are also being directed toward the development of techniques which differentially stain these chromosome regions. The purpose of this paper is to describe a new procedure, which we call the Ammoniacal-Silver III technique (AS III), that selectively stains those regions of human chromosomes in which satellite III DNA has been localized[1,2]. This method, which is a modification of the Ammoniacal-Silver (A-S) technique[3–7], conspicuously stains the secondary constriction region of human chromosome 9 (Figures 1 and 2). In addition, the centromeric regions of the acrocentric D and G group chromosomes will occasionally differentially stain. Whether the silver binds chemically to the satellite DNA III fraction itself, or to an associated histone or non-histone protein, is not presently known.

When human DNA is fractionated in isopycnic CsCl gradients, it consists of at least 4 satellite fractions[1,2,8,9]. The specific chromosomal locations of satellite I and IV are not known. However, in situ hybridization studies have shown that satellite II DNA is localized in the secondary constriction regions of human chromosome pairs 1 and 16, and possibly 9[10]. More recent radioactive labelling experiments have shown that satellite III DNA is conspicuously concentrated on human chromosome pair 9 and to a lesser extent near the centromeres of all chromosomes of the D and G groups[11,12]. It is these satellite III chromosomal regions that the AS III technique differentially stains.

The AS III stain is prepared by slowly dissolving 8 g of silver nitrate into a solution of 10 cm^3 of distilled water and 10 cm^3 of concentrated ammonium hydroxide. The resulting staining solution is colorless and has a pH of 12.0–12.5. The AS III solution is filtered twice into a vial. 4 drops of the AS III stain are pipetted onto the surface of a microscope slide containing standard air-dried human chromosome preparations from leucocyte culture[13]. 4 drops of 3% formalin (neutralized with sodium acetate) are immediately added to develop the AS III stain. The AS III stain and formalin are quickly

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