FITC-inulin

Chemical name: Inulin(3',6'dihydroxy-3-oxospiro(isobenzofuran-1(3H) ,9'-[9H] xanthen]-5(or 6)yl)carbamothioate
Fluorescein isothiocyanate- Inulin
Fluoresceinyl thiocarbamoyl- Inulin

CAS nr: N/A

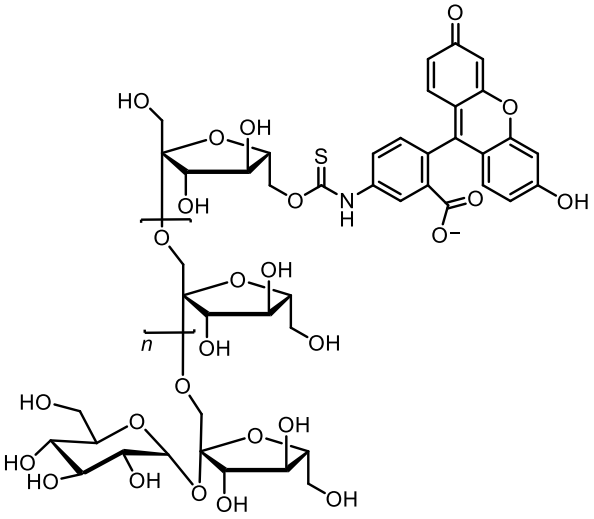

Fig. 1 Structural representation of fragment of FITC-Inulin molecule.

Introduction

Inulin is a low molecular weight polyfructosan and is well tolerated when given parenterally to humans and animals. It is completely filtered by the glomerulus and is not secreted or reabsorbed by the tubules and as such has been recognised as a gold standard for measuring glomerular filtration rate (GFR).

Purified fractions of inulin from various sources are available commercially and the molecular weights are around 3000 to 5000.

Description

FITC-inulin is supplied as a yellow powder which dissolves in water or salt solutions giving a yellow solution. Dilute solutions (1-2%) may remain clear on standing but more concentrated (>10%) form precipitates on standing, since inulin tends to form crystalline aggregates. These precipitates will re-dissolve on heating. Temperatures up to 80°C may be employed providing the solution is around neutral pH. The product also dissolves in DMSO, formamide and certain other polar organic solvents but is insoluble in lower aliphatic alcohols, acetone, chloroform, dimethylformamide.

Synthesis

An inulin fraction obtained from dahlia tubers is labelled with fluorescein by a procedure similar to that described by de Belder and Granath (1). The fluorescein moiety is attached by a stable thiocarbamoyl linkage and the labelling procedure does not lead to any depolymerisation of the inulin. FITC-inulin possesses from 0.001-0.008 mol. FITC per fructose unit and at these low levels of substitution the effect of the charges is minimal.

Spectral data

Excitation is best performed at 490nm and fluorescence measured at 520 nm (see Fig.2). The dependence of fluorescence from a FITC-inulin solution in the range pH 3-9 is shown in Fig. 3. Measurements in biological media may significantly affect the fluorescence intensity which may be enhanced or depressed.

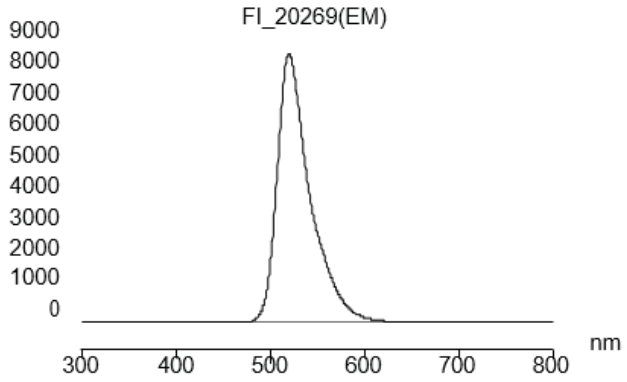

Fig. 2 *Fluorescence scan of FITC-inulin in 0.025M borate pH 9.0 (13.5 mg in 50ml buffer). Excitation 493nm; Emission 520nm.*

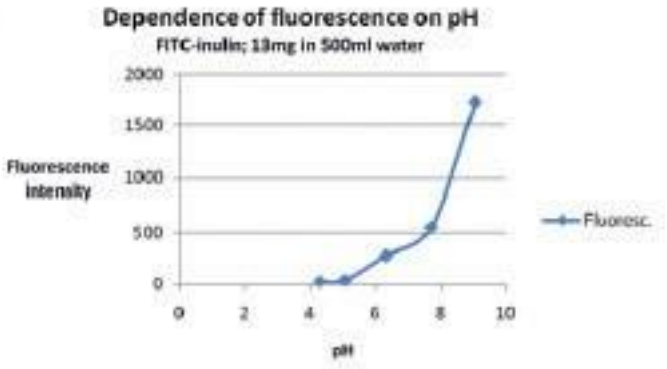

Fig.3*. Dependence of fluorescence of FITC-inulin*

Physical chemical properties of FITC-inulin

The weight average molecular weight (Mw) of FITC-inulin as determined by SEC (Superose 6+ 12; dextran calibration) is approx. 5000. The results for three batches were as follows; 5440, 5165, 5640. Inulin has also been fractionated on Dionex resins and fractions with DP up to 30 were observed, However, the method is not quantitative (2). Phelps determined the Mw from osmotic pressure data and obtained a value of 5640 (3). When determined by light-scattering, a Mw of 7250 was obtained. However, it should be noted that at these low molecular weights, the values derived by light-scattering become uncertain.

The structure of crystalline samples of inulin has been examined by Marchessault and co-workers who proposed a five-fold helix (4). Later studies by André and co-workers using electron diffraction techniques on single crystals of inulin indicated two antiparallel six-fold helices (5).

Storage and stability

FITC-inulin powder when stored in air-tight containers at ambient temperatures is stable for at least 6 years. The stability of FITC-inulin will be similar to that for FITC-dextran (see data-file).

Only at elevated pH (>9) and elevated temperatures is there a risk for hydrolysis of the thiocarbamoyl linkage. Like polysucrose however, the stability at acidic pH is poor owing to the susceptibility of the fructofuranose units to acid hydrolysis.

Toxicity

Inulin fractions exhibit no toxic symptoms when tested orally or intravenously.

Biological aspects and applications

FITC-inulin has primarily been used for studying glomerular filtration rate (GFR) in experimental animals but has also been applied to investigate factors affecting permeability of other tissues.

Measurements of the fluorescence provide quantitative data on transport and permeability of healthy and diseased tissues. Such studies can be performed in real time by intravital fluorescence microscopy. The technique offers high sensitivity and concentrations down to 1 µg/ml can be detected in tissue fluids.

Permeability studies on the glomerular membrane

The tubular fluid to plasma concentration ratio was determined in rats following a bolus injection of FITC-inulin in the femoral vein (6). The validity of the method as a measure of glomerular filtration was established by comparisons with 51Cr-EDTA and [H3]-inulin. A thorough investigation of the use of FITC-inulin for GFR studies was presented by Fleck in 1999(7). The rats were given 4 mg/mL at a rate of 4 ml/100 g bodyweight per hour via the tail vein or jugular vein.

Dunn and co-workers found excellent correlation between creatinine clearance (by 2 methods) and FITC-inulin clearance in mice (8). Two procedures for using FITC-inulin for determining GFR in mice have been described (9). Lorenz and Gruenstein described a simple, non-radioactive method for evaluation single nephron filtration rate using FITC-inulin (10). Fluid absorption was determined using FITC-inulin in studies on kidney tubules in mice (11). Renal function and glucose transport in male and female mice with diet induced type II diabetes mellitus was studied using FITC-inulin (12). Single proximal tubules and kidney opossum (KO) cells using a FITC-inulin perfusate have been studied (13). Several studies

have used FITC-inulin as a fluid phase endocytosis marker together with FITC-albumin as a receptor mediated endocytosis marker in KO cells (14-15). The transport rate of FITC-inulin was measured before and after Ca + switch in kidney epithelial cells (16). More recently, FITC-inulin has been used to assess the barrier function in a bioartificial kidney device (17,18).

Permeability studies of other cells

Studies of permeability of intestinal epithelial cell monolayers with FITC-inulin have been reported (19). Neunlist and co-workers have used FITC-inulin and FITC-dextran to investigate the permeability of intestinal epithelial cell monolayers (20). After 2-24 h post irradiation, the integrity of colonic epithelial tight junctions (TJ), adherent junctions (AJ), and the actin cytoskeleton was assessed by immunofluorescence microscopy using FITC-inulin (21). FITC-inulin has also been valuable in studies of disruption of liver and gut tissues (22).

List of references

1. A.N.de Belder and K.Granath. Preparation and properties of fluorescein-labelled dextrans. Carbohydr Res.1973;30:375-378.
2. C.Fleck. Determination of the glomerular filtration rate (GFR); Methodological problems, age-dependen- ce, consequences of various surgical interventions, and the influence of different drugs and toxic sub- stances. Physiol Res.1999; 48:267-279.
3. C.F.Phelps. The physical properties of inulin solu- tions. Biochem J. 1965;95:41-47.
4. R.H.Marchessault, T.Bleha, Y.Deslandes et al. Con- formation and crystalline structure of (2-1)-α-D-fructo- furanan(inulin). Can J Chem. 1980;58:2415-2421.
5. I.André, K.Mazeau, I.Tvaroska et al. Molecular and crystal structures of inulin from electron diffractional data. Macromolecules. 1996;29:4626-4635.
6. M.Sohtell, B.Karlmark and H.Ulfendahl. FITC-in- ulin as a kidney tubule marker. Acta Physiol Scand. 1983;119:313-6.
7. C.Fleck, Determination of the glomerular filtration rate(GFR); Methological problems, Age-dependence, consequence of various surgical interventions and the influence of different drugs and toxic substances. Physiol Res.1999;48:267-279.
8. S.R.Dunn, Z.Qi, E.P.Bottinger et al., Utility of endo- genous creatinine clearance as a measure of renal function in mice. Kidney Int. 2004;65:1959-67.
9. Z.Qi, I.Whitt, A.Mehta et al. Serial determination of glomerular filtration rate in conscious mice using FITC-inulin clearance. Am J Physiol Renal Physiol. 2004;286:F590-6.
10. J.N.Lorenz and E.Gruenstein. A simple, non radio- active method for evaluating single-nephron filtration rate using FITC-inulin. Am J Physiol. 1999;276:F172-7.
11. J.Ba, D.Brown and Friedman. Calcium- sensing receptor regulation of PTH-inhibitable proximal tubule phosphate transport. Am J Physiol Renal Physiol. 2003;285: F1233-43.
12. W.T.Noonan and R.O.Banks. Renal function and glucose transport in male and female mice with diet induced type II diabetes mellitus. Proc Soc Exp Biol Med. 2000; 225: 221-30.
13. J.S.Schwegler, B.Heppelmann, S.Mildenberger et al. Receptor-mediated endocytosis of albumin in cultured opossum kidney cells: a model for a prox- imal tubular protein reabsorption. Pfluegers Arch. 1991;418:383-92
14. M.Takano, N.Nakaanishi, Y.Kitahara et al. Cispla- tin-induced inhibition of receptor-mediated endocyto- sis protein in the kidney. Kidney int. 2002;62:1707-17.
15. Y.Sasaki, J.Nagai, Y.Kitahara et al. Expression of chloride channel, C1C-5, and its role in receptor mediated endocytosis of albumin in OK cells. Biochem Biophys Res Commun. 2001;282:212-8.