FITC-dextran

(Fluorescein isothiocyanate dextran)

Chemical names:

- Dextran(3',6'-dihyroxy-3-Oxospiro (isobenzofuran-1- (3H), 9'-[9Hxanthen]-5 (or 6)-ylcarbamothiate.
- Fluoresceinisothiocyanate- dextran
- Fluoresceinyl thiocarbamoyl- dextran

CAS number: 60842-46-8

Structure:

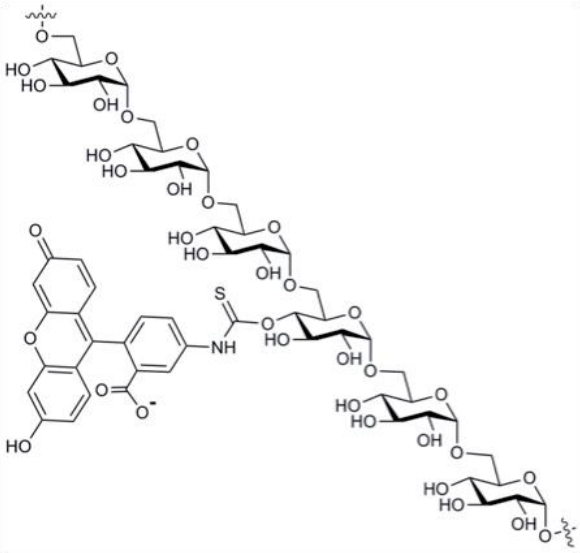

Fig.1. *Structural representation of fragment of FITC-dextran molecule.*

Properties

FITC-dextran is supplied as a yellow/orange powder which dissolves freely in water or salt solutions giving a yellow solution. The product also dissolves in DMSO, formamide and other polar organic solvents but is insoluble in lower aliphatic alcohols, acetone, chloroform and dimethylformamide.

Spectral data

Excitation is best performed at 493 nm and fluorescence measured at 518 nm (Fig. 2). Since the charge status of the fluorescein moiety is dependent on the pH and ionic strength of the medium, the fluorescence intensity will also vary with these parameters. The maximal intensity is observed at pH > 8. Measurements in biological media will significantly affect the fluorescence intensity which may be enhanced or depressed.

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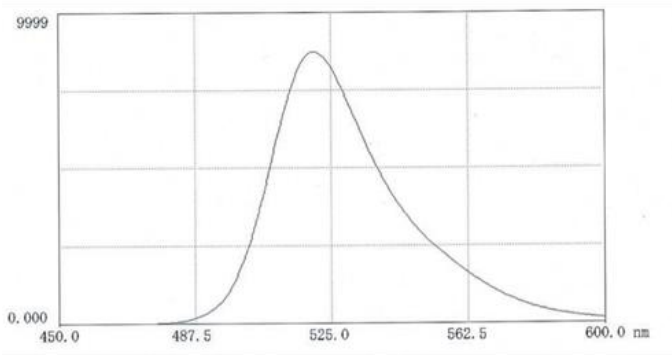

Fig. 2. Fluorescence scan of FITC-dextran 70 in phosphate buffered saline pH 9.

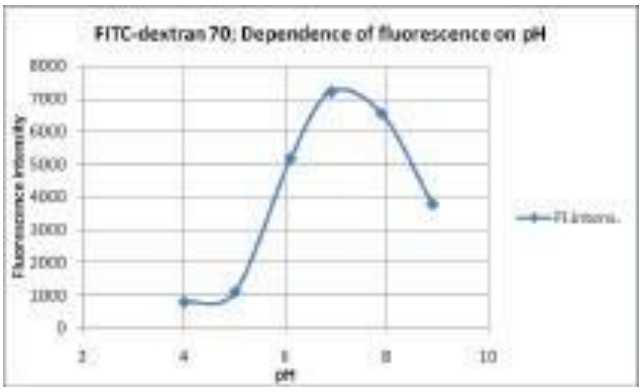

Fig. 3. Fluorescence (Emission 520nm) of FITC-dextran in the range pH 4-9.

Storage and stability

The stability of FITC-dextran has been investigated in various media and at various temperatures. From these studies, it is concluded that the stability of FITC-dextrans in vitro and in vivo is excellent. Only at elevated pH (>9) and elevated temperatures is there a risk for hydrolysis of the fluorescein label. Studies at 37°C in rabbit plasma, muscle homogenate, liver homogenate and urine established that FITC-dextrans are stable for at least 3 days. No changes in the mol. wt and no release of fluorescein moieties was noted. FITC-dextran was stable in 6 % trichloracetic acid at room temperature for 3 days. The hydrolysis shows specific catalysis by hydroxide ions in the pH range 10-10.75 (1).

Hydrolysis of the thiocarbamoyl linkage gives rise to 4- or 5-aminofluorescein which is readily determined by HPLC. In unpublished studies, the stability of an autoclaved FITC-dextran 70 solution was studied at temperatures from 8 to 50°C over a period of 5 months. Only at 50°C could a slight increase (1%) in free aminofluorescein be noted. Autoclaving alone gives a 2.7 % release of free aminofluorescein. In other unpublished studies, FITC-dextran was found to be stable in solution at pH 4 for up to 1 month at temperatures up to 35°C. At 80°C and pH 4, the thiocarbamoyl linkage was stable for 30 min. However, the dextran may degrade. At pH 9, considerable (24 %) decrease in fluorescence took place at 35 °C over 1 month. Several studies have confirmed the in vivo stability of FITC-dextrans during the duration of the experiments (2).

Toxicity

In studies in mice, FITC-dextrans were found to be tolerated well when injected intravenously or intraperitoneally in doses up to 6 g/kg body- weight. Their toxicity patterns follow those of parent dextrans. Clinical dextrans fractions have been employed for over 50 years as plasma volume expanders. Dextran-induced anaphylactoid reactions (DIARs) have been observed

in humans after injection of clinical dextran solutions (3,4). FITC-dextrans are also likely to display this type of behavior but few reports of problems with experimental animals have appeared.

Synthesis

Selected dextran fractions prepared from native Dextran B512F are labelled with fluorescein (5). The fluorescein moiety is attached by a stable thiocarbamoyl linkage and the labelling pro- cedure does not lead to any depolymerization of the dextran. The FITC-dextrans have from 0.002-0.008 mol FITC per glucose unit and at these low levels of substitution confer minimal charges to the dextran, which is an essential requirement for permeability studies. Physical chemical properties of FITC-dextran. The dextran molecule at molecular weights greater than 5000 Daltons behaves as a flexible and extended coil in solution. Table 1. (below) shows the molecule dimensions at various molecular weights.

Dextran MW	Stokes radius (Å)	Radius of gyration (Å)
2 x 106	270	380
1 x 106	199	275
500 000	147	200
200 000	130	130
100 000	69	95
70 000	58	80
40 000	44.5	62
10 000	23.6	-

Table 1. *Molecular dimensions of dextran*

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Dextrans and FITC-dextrans will exhibit Newtonian flow characteristics i.e. the viscosity is independent of shear rate (Fig. 4). Studies in the range pH 4-10 establish that the viscosity is independent of pH. The isoelectric point of FITC-dextran lies in the range 8-9 and between pH 6.5 and 9.5 they show no migration on electrophoresis (1).

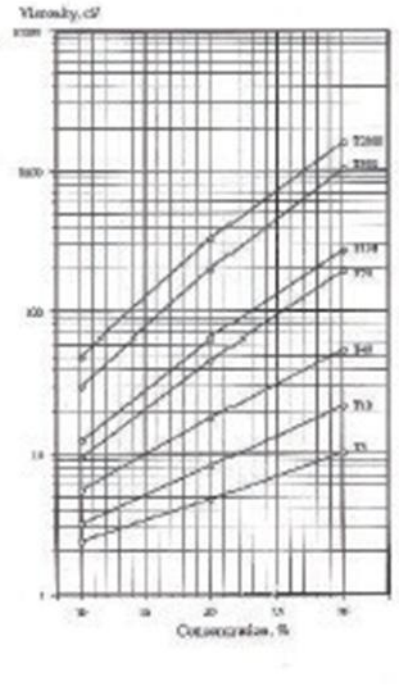

Fig. 4. The viscosities of dextran fractions at various concentrations.

Applications

FITC-dextrans are primarily used for studying permeability and transport in cells and tissues. An added benefit is that measurements of the fluorescence provide quantitative data on the 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 1µg/ml can be detected in tissue fluids. FITC-dextrans have also been used as a pH pro- be in cells (6,7). It may also be noted from polarization experiments that the rotational freedom of fluorescein conjugated to dextran remains high and fluorescent lifetime of the excited state is similar to that before conjugation (6).

1. General procedures

The microvasculature of the hamster cheek pouch has proved to be a useful model for stu- dying plasma leakage in different experimental conditions, e.g. following ischemia/reperfusion, or topical application of a whole range of inflammatory mediators, parasites and bacteria. With this technique, vascular permeability changes can be studied in real time and be related to other microvascular events such as leukocyte adhesion and activation. The cheek pouches are examined by intravital fluorescence microscopy using suitable filters (490/520 nm) and images are captured with a digital camera. A 5 % FITC- dextran 150 solution (approx. 100 mg/kg body- weight in normal saline) is administered i.v. (8-10).

Permeability studies using combined fluorescence stereomicroscopy, fluorescence light microscopy was reported by Thorball (2). This paper also includes tissue fixation techniques in the presence of

FITC-dextran and details of the microscopy set-up (filters, illumination). Regenerative titanium earchambers (rabbits) have been used to study the blood/lymph systems in the microcirculation with FITC-dextrans. Lymph ingrowth is seen after 4-8 weeks of implantation (11).

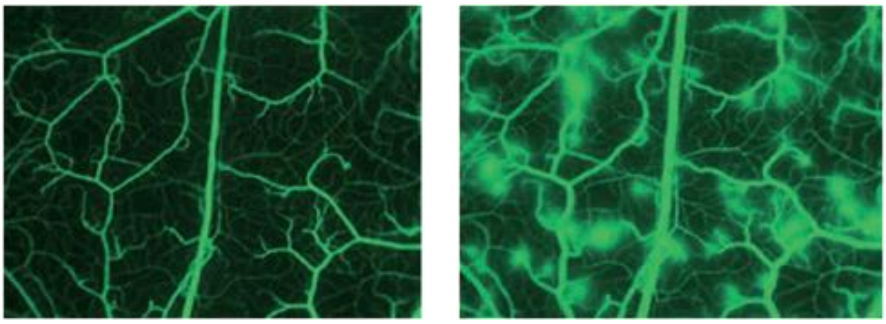

Fig.5. Images taken from cheek pouch after infusion of FITC-dextran 150.The second image shows the leakage of the microvasculature after subjection to histamine. (By kind permission of E. Svensjö).

2. Permeability studies on intestinal tissues

Permeability of intestinal epithelial monolayer during inflammation was studied using a FITC-dextran 150 (12). The action of a protease inhibitor on mucosal erosions and epithelial dysfunction in the GI tract was studied using FITC-dextran 4 (13). Thorball made extensive studies on tissue fixation in connection with FITC-dextrans in the GI tract (2). FITC-dextrans (4000-70000) were used to study permeability changes following cutaneous thermal injury in vitro using modified Ussing chambers (14). See also useful references (15,16).

3. Permeability studies of brain and nervous system.

For studies of FITC-dextrans in the nervous system, techniques are required that immobilize the tracers enabling good optical resolution of features such as neuron, glial cells, sciatic endoneurium, ganglion etc. A method employing freeze-drying of the dissected tissue samples, fixation with formaldehyde vapour at 80 °C and embedding in paraffin wax or plastic proved to give good results (17). The sections from these preparations were dipped in xylene before mounting and examination.

The distribution of intravenously injected FITC-dextran (Mw from 3000 to 150 000) was studied in hamsters and mice. The normal perineural diffusion barrier of the cerebral cortex was not permeable to any FITC-dextran in the above range (19). The hamsters received 5mg FITC-dextran/10g bodyweight in 0.5ml saline (18). Permeability characteristics of the hippocampus were investigated with FITC-dextrans. The blood-brain barrier was subjected to focused ultrasound and the passage of FITC-dextrans was analysed (19).

The time-dependent permeability of the cerebral vessels following ischemia-reperfusion injury was examined using iv. FITC-dextran 150 (20). Brain edema following acute liver failure has been studied by monitoring protein related-permeability changing using FITC-dextrans (21).

3. Permeability studies on neoplastic tissues.

Gerlowski (22) describes the use of transmitted light fluorescence TV microscopy for real time studies of microvascular permeability in neoplastic tissues. The studies were performed in the rabbit ear chamber model using FITC-dextran 150. The authors also examined the quenching of FITC-dextran concentrations from 0.6 mg to 30 mg/100 ml at intervals up to 90 min. Only the very highest

concentrations showed indications of quenching effects. T. Li and co-workers (23) studied membrane permeability of Sarcoma cells following focused ultrasound using FITC-dextran 500. FITC-dextrans were used to study the endocytosis of cells in experiments with ovarian cancer cells (24).

4. Permeability studies within the ocular chamber

E. Mannerma and co-workers (25) studied drug permeation process in the retinal pigment epithelium using FITC-dextran 40 and other probes. FITC-dextrans were used to assess barrier dysfunction in response to TNF-alpha (26). S. Lightman and co-workers (27) were able to study in detail the leakage of the retinal vessels in an inflammation model. Repeated doses of FITC-dextran were well tolerated. Studies on the pathways by which FITC-dextrans and fluoresce- in leave the vitreous body have been described (28). Uveoscleral outflow in normal inflamed eyes has also been examined by C.B.Toris and co-workers (29) using FITC-dextrans. An in vitro model of the outer blood retinal barrier has been described (30).

5. Permeability studies of renal tissues

FITC-dextran 4 is very rapidly excreted into the urine with 70 % of the dose excreted within the first hour (rabbits, rats) (unpublished data). Lencer and coworkers (31) reported studies with FITC-dextran 10 as a probe for endosome function and localization in the kidney. Frozen sections of excised tissue were examined by epifluorescent microscopy.

Diverse applications

FITC-dextrans have been used to explore the permeability of nasal mucosa in vitro (32). In this study charged FITC-dextran derivatives were also employed. Diffusion of drugs vertically into the skin was studied using FITC-dextran 20 loaded dissolving micro-needles (33).

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