TRITC-dextran

(Tetramethyl-rhodamine isothiocyanate dextran)

Trade name: TRITC-dextran

Chemical names: Dextran (3´6´tetramet-hylamino dihydroxy-3oxospiro (isobenzofuran-1(3H),(9H) xanthen)-5(or 6)-yl)carbamothioate

CAS nr: N/A

Structure:

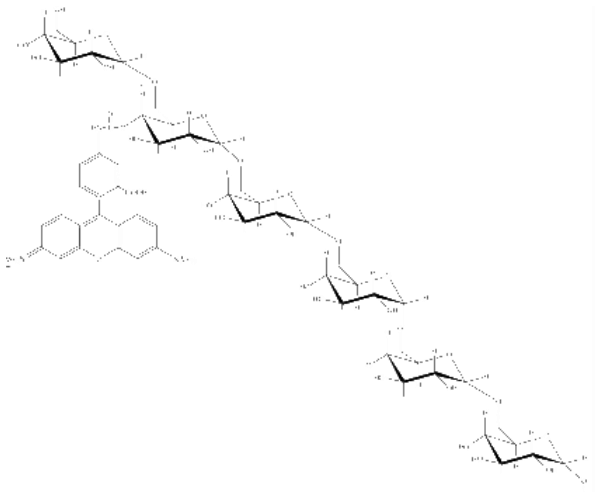

Fig. 1 Structural representation of fragment of TRITC-dextran molecule.

Synthesis

Selected dextran fractions prepared from native Dextran B512F are labelled with tetramethyl rhodamine B by a procedure similar to that described by de Belder and Granath (1). The rhodamine moiety is attached by a stable thiocarbamoyl linkage and the labeling procedure does not lead to any depolymerization of the dextran. TRITC-dextrans contain from 0.001-0.008 mol. TRITC per glucose unit and at these low levels of substitution confer minimal charges to the dextran, which is an essential requirement for permeability studies.

Properties

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

Spectral data

Excitation is best performed at 550 nm and fluorescence measured at 572 nm (see Fig.2). Studies in our laboratories have shown that the fluorescence from a TRITC-dextran solution shows only a slight increase with decreasing pH over the range pH 3-9 (see Fig.3). Similar results have been reported earlier (2.). This is of interest when making quantitative measurements. Measurements in biological media may significantly affect the fluorescence intensity which may be enhanced or depressed.

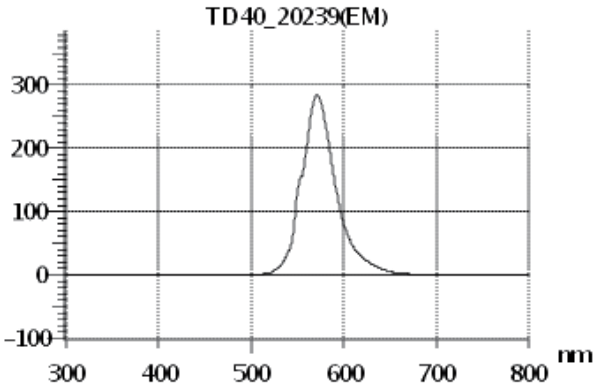

Fig. 2 Fluorescence scan of TRITC-dextran 40 kDa in 0.025M borate pH 9.0 (9.9 mg in 50 ml buffer). Excitation 550 nm; emission 571.5 nm.

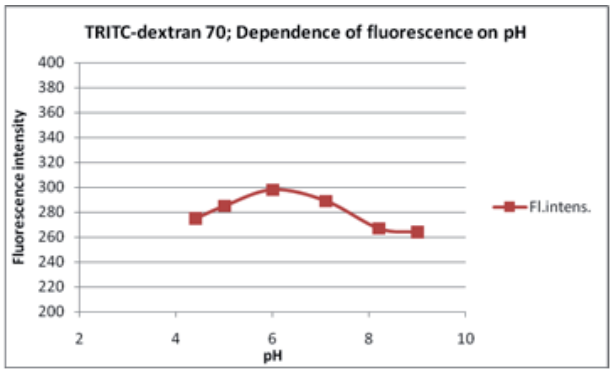

Fig. 3 Dependence of fluorescence of TRITC-dextran with pH in the range 4-9.

Physical chemical properties of TRITC-dextrans

The dextran molecule at molecular weights greater than 5000 Daltons behaves as a flexible and extended coil in solution. Table 1 (below) shows the molecular dimensions at various molecular weights.

Dextran (Mw, Da)	Stoke's radius
2 million	270
500 000	147
100 000	69
70 000	58
40 000	44.5
10 000	23.6
Albumin	35

Table 1. Molecular dimensions of dextran expressed as Stokes Radius (Å).

Dextrans and TRITC-dextrans will exhibit Newtonian flow characteristics i.e. the viscosity is independent of shear rate. Studies in the range pH 4 - 10 establish that the viscosity is independent of pH. The viscosities of dextran fractions at various concentrations are shown in Fig. 4.

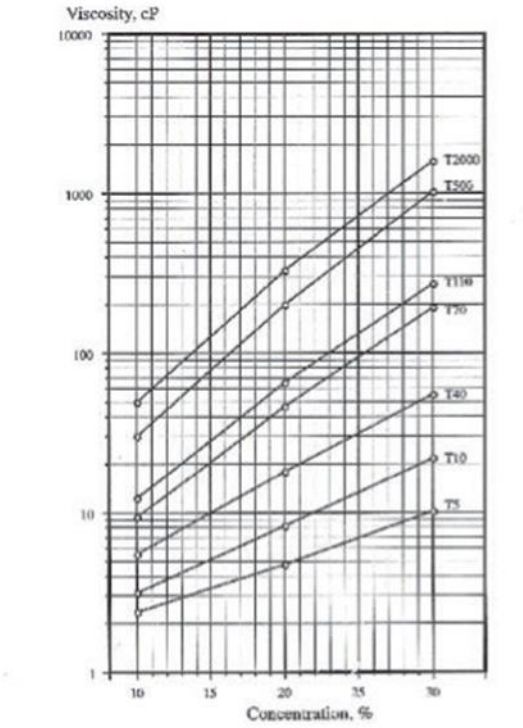

Fig.4. The dependence of viscosity on concentration for various dextran fractions.

Storage and stability

The stability of TRITC-dextrans has not been investigated in detail but it is presumed to be similar to that of FITC-dextrans (see data-file). Only at elevated pH (<9) and elevated temperatures is there a risk for hydrolysis of the thiocarbamoyl linkage. For further details, the reader is referred to the data-file for FITC-dextran.

Toxicity

The toxicity patterns follow those of the parent dextrans. Clinical dextran 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). The reactions vary from mild skin reactions to severe shock states. The incidence of severe reactions is about 1 in 2000. TRITC-dextrans are also likely to display this type of behavior but few reports of problems with experimental animal have appeared.

Applications

TRITC-dextrans are primarily used for studying permeability and transport in cells and tissues (5). An added benefit is that 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 1µg/ml can be detected in tissue fluids. Unlike FITC-dextrans, the fluorescence of TRITC-dextrans is not dependent on pH in the range 4 to 9. A further important property is that TRITC-dextran does not bind to artery walls (6,7).

General Procedures

The microvasculature of the hamster cheek pouch has proved to be a useful model for studying plasma leakage, e.g. following ischemia/re-perfusion, exposure to inflammatory mediators (8-10). With this technique, vascular permeability 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 and images are captured with a digital camera (see Fig.5). A 5% TRITC-dextran 150 solution in normal saline is administered i.e. (approx. 100mg/kg bodyweight).

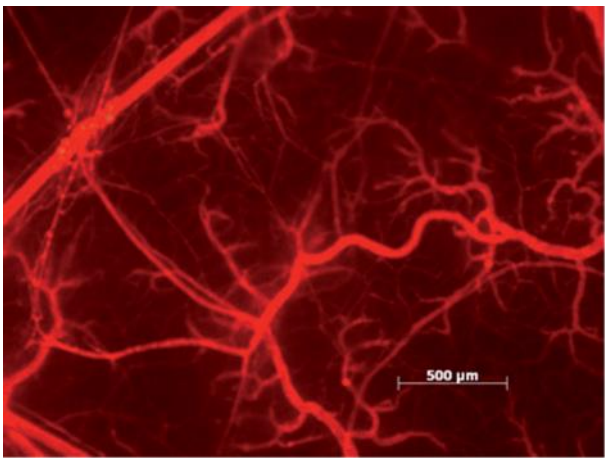

Fig.5 *TRITC-dextran 150 injected in a hamster cheek pouch 15 min. after histamine challenge (image by kind permission of E. Svensjö).*

An alternative procedure using rabbit ear chambers has been described. The regenerative titanium ear-chambers (rabbits) were used to study the blood/lymph systems in the microcirculation with fluorescent dextrans. Lymph ingrowth is seen after 4-8 weeks of implantation (11).

Permeability studies using combined florescence stereomicroscopy were reported by Thorball (5). Extensive studies of issue fixation techniques in the presence of FITC-dextrans are described, which are relevant also for TRITC-dextrans. Details of the microscopy setup (filters, illumination) may be found in this article. Mullick and coworkers have described a new method for sequential quantitation of endothelial layer permeability using TRITC-dextran 4 kDa (TD4). The perfusate contained 42 μ g/ml TD4 (12).

Permeability studies in cells

The subcellular fluorescence of FITC- and- TRITC-dextrans was studied in mouse macrophage lysosomes. A comparison of the values enabled estimates of the intracellular pH. i.e. turning a FITC- and TRITC-dextran combines use into a fluorescent probe. Cultures were incubated with 1 mg/ml dextran end concentrations (13).

Other studies describe proton accumulation in living cells using these fluorescent probes (14). Uptake of TRITC-dextran 10 kDa in response to osmotic cell swelling by intestine 407 cells has been described (15). TRITC-dextran 10 kDa (5mg/ml) has also been used as a fluid phase marker (16). TRITC-dextran 4 kDa was used in studies of barrier functioning prostate cancer cell cultures (17).

Mairhofer and colleagues have studied the late endosomal localization of SLP-1 in perinuclear bodies with TRITC-dextran 4 kDa (18). For studies of transcellular protein delivery, a TRITC-dextran 10 kDa (0.1mg/ml) was used to examine the kinetics of internalization of trans-activator fusions (19).

Permeability studies in arteries and micro- vasculature

TRITC-dextran 4 kDa and 70 kDa were used to study permeability in carotid arteries in folate deplete rats-concentrations of 42 μ g/ml were injected (21). TRITC-dextrans have also proved useful in studies of potential therapeutic treatments (22-24).

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