TRITCDextran

(Tetramethyl-rhodamine isothiocyanate dextran)

TdB CONSULTANCY

Trade name: TRITC-dextran

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

CAS nr. NA

Structure:

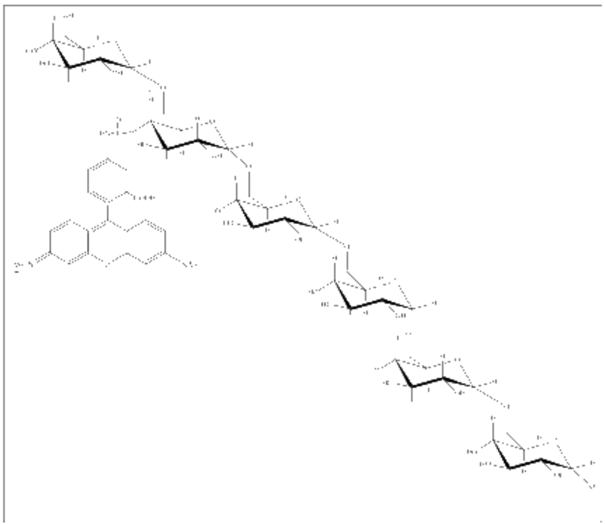

Fig. 1 Structural representation of fragment of TRITC-dextran molecule.

Synthesis

Selected dextran fractions prepard from nati- ve Dextran B512F are labelled with tetramet- hyl-rhodamine B by a procedure similar to that described by de Belder and Granath (1). The rhodamine moiety is attached by a stable thio- carbamoyl 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 char- ges to the dextran, which is an essential require- ment 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 or- ganic solvents but is insoluble in lower aliphatic alcohols, acetone, chloroform, dimethylforma- mide.

Spectral data:

Excitation is best performed at 550nm 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. Mea- surements in biological media may significantly affect the fluorescence intensity which may be enhanced or depressed.

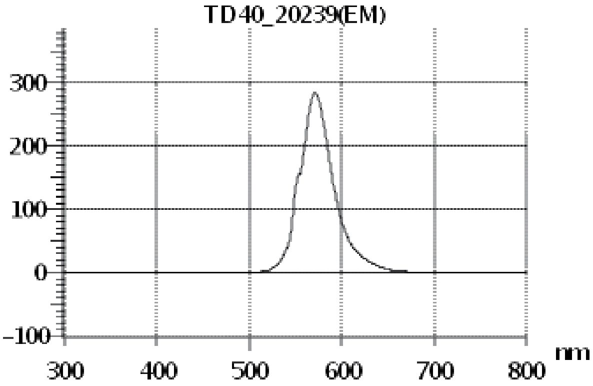

Fig. 2 Fluorescence scan of TRITC-dextran 40 in 0.025M borate pH 9.0 (9.9 mg in 50 ml buffer) Exitation 550nm; Emission 571.5nm

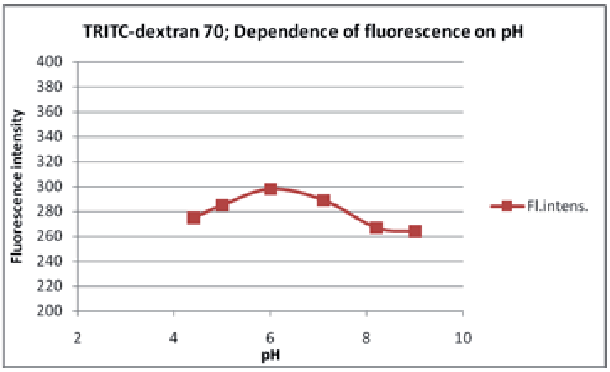

Fig. 3 Dependence of fluorescence of TRITC-dextran with pH in the range 4-9.

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Physical chemical properties of TRITC-dext- rans
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)	Stoke's radius
2 million	270
500 000	147
100 000	69
70	58
40	44.5
10	23.6
Albumin	35

Table 1. Molecular dimensions of dextran ex- pressed as Stokes Radii (Å)

Dextrans andTRITC-dextrans will exhibit New- tonian flow characteristics i.e. the viscosity is independent of shear rate. Studies in the range pH 4 - 10 establish that the viscosity is indepen- dent of pH. The viscosities of dextran fractions at various concentrations is shown in Fig. 4.

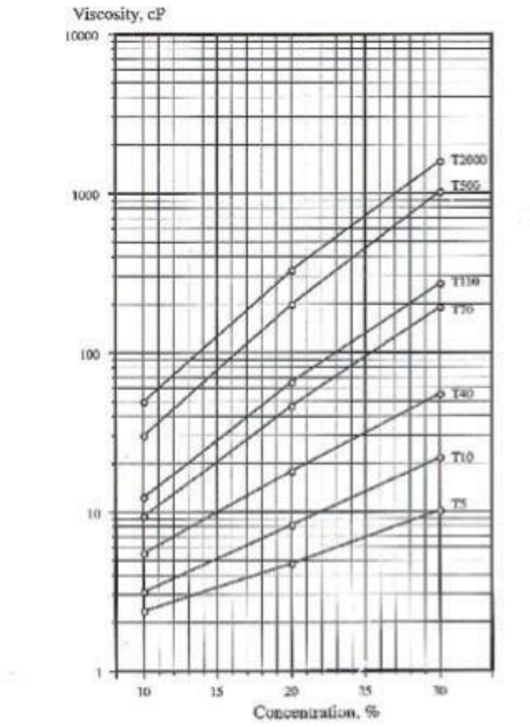

Fig.4. The dependence of viscosity on concentration for various dextran fractions.

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 tempera- tures is there a risk for hydrolysis of the thiocar- bamoyl linkage. For further details, the reader is referred to the data-file for FITC-dextran.

Toxicity
Their toxicity patterns follow those of the 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). 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-detrans are primarily used for studying permeability and transport in cells and tiss- ues(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 micros- copy. The technique offers high sensitivity and concentrations down to 1µg/ml can be detected in tissue fluids. Unlike FITC-dextrans, the flu- orescence 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 stu- dying 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-dext- ran 150 solution in normal saline is administe- red i.v.(approx. 100mg/kg bodyweight).

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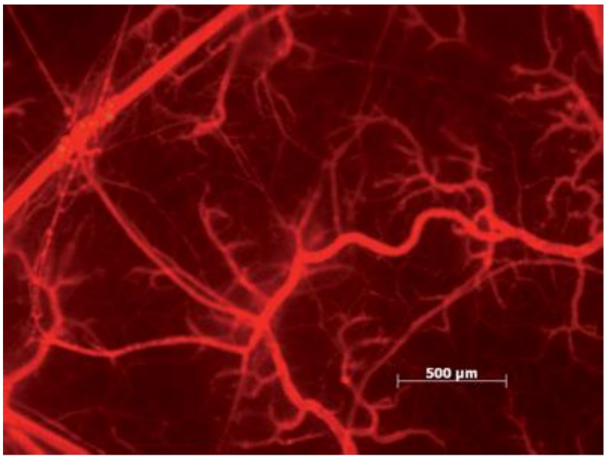

Fig.5 TRITC-dextran 150 injected in a hamster cheek pouch 15 min. after histamine challenge(by kind permission of E.Svensjö).

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

Permeability studies using combined flu- orescence stereo-microscopy were reported by Thorball(5). Extensive studies of issue fixa- tion techniques in the presence of FITC-dext- rans are described which are relevant also for TRITC-dextrans. Details of the microscopy set-up(filters, illumination) may be found in this article. Mullick and coworkers have ddescribed a new method for sequential quantitation of en- dothelial layer permeability using TRITC-dextran
4. The perfusate contained 42µg/ml TD4(12).

Permeability studies in cells
The subcellular fluorescence of FITC- and- TRITC-dextrans was studies in mouse macrop- hage lysosomes. A comparison of the values enabled estimates of the intracellular pH. Cultures were incubated with 1 mg/ml dextran concentrations(13).

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

Mairhofer and colleagues have studied the late endosomal localization of SLP-1 in perinuclear bodies with TRITC-dextran 4(18). For studies of trans-cellular protein delivery, a TRITC-dextran 10(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 and 70 were used to study per- meability in carotid arteries in folate deplete rats
-concentrations of 42µg/ml were injected(21). TRITC-dextrans have also proved useful in stu- dies of potential therapeutic treatments(22-24).

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