FITC-Polysucrose (FITC-Ficoll®)

Fluoresceinisothiocyanate-Polysucrose

Trade name: FITC-Polysucrose

Chemical names: Polysucrose(3',6'dihydroxy-3-oxospiro(isobenzofuran-1(3H),9'- [9H]xanthen]-5(or 6)-yl)carbamothioate
Fluorescein isothiocyanate-Fluoresceinyl thiocarbamoyl-Polysucrose
FITC-Ficoll®

Structure:

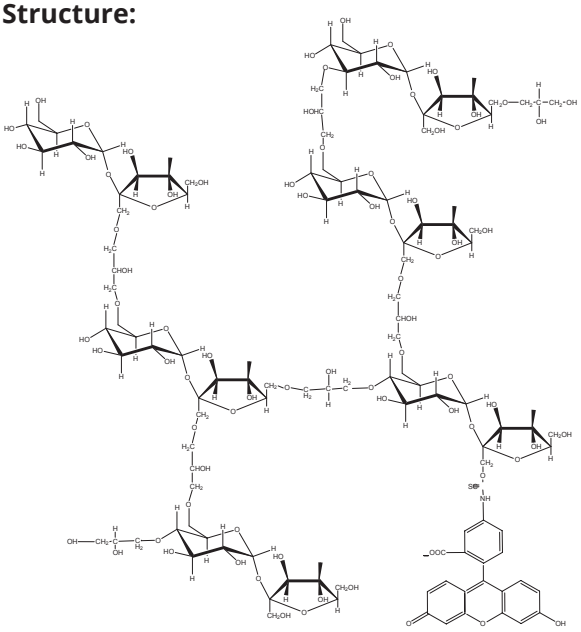

Fig. 1 Structural representation of fragment of FITC-Polysucrose molecule.

Synthesis

Polysucrose fractions are 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 Polysucrose. The FITC-Polysucroses carry from 0.001-0.008 mol. FITC per glucose unit and at these low levels of substitution confer minimal charges to the Polysucrose, which is an essential requirement for permeability studies.
References:

Description:

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

Polysucrose(Ficoll®)is synthesized by polymerizing sucrose with epichlorohydrin. The resulting polymer is highly branched and behaves as a globular molecule in solution (see section Physical chemical properties). Polysucrose contains only primary and secondary hydroxyl groups.

Spectral data:

Excitation is best performed at 496nm and fluorescence measured at 525 nm (see Fig.2). The dependence of fluorescence from a FITC-Polysucrose solution in the range pH 3-9 is shown in Fig.2. Measurements in biological media may significantly affect the fluorescence intensity which may be enhanced or depressed.

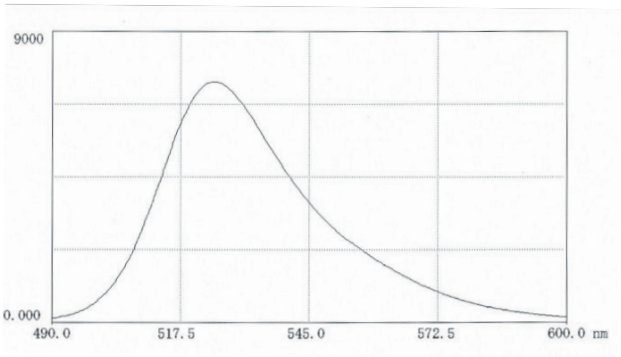

Fig. 2 Fluorescence scan of FITC-Polysucrose 70 in 0.025M borate pH 9.0 (9.9 mg in 50 ml buffer) Excitation 493nm; Emission 523nm

Physical chemical properties of FITC-Polysucroses(FITC-Ficoll®)

The Polysucrose molecule behaves as a globular molecule in solution as is to be expected from its structure. In Table 1 (below) a comparison of the Stokes radius of dextran and Polysucrose fractions reflects these differences in molecular flexibility. The molecule is best regarded as an intermediate between a hard solid sphere and a flexible coil. Thus when comparing Polysucrose and dextran fractions of similar molecular weights, the molecular dimensions of the Polysucrose will always be smaller. It is unsuitable to use dextran calibration for gpc determinations of the molecular weight of Polysucrose products.

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Mw x 10-3	Dextran Stokes radius	Polysucrose Stokes radius	Albumin Stokes radius
		-	
500	147	106	
70	58	49,5	35
40	44,5	40	

Table 1. Molecular dimensions of Polysucrose and dextran expressed as Stokes Radii (Å)

Polysucrose solutions have very low osmotic pressures compared to sucrose solution of equivalent concentrations. Thus a 10% solution of Polysucrose 70 has an osmolality of 3 mOs/kg compared to 150 for a 10% sucrose.

Storage and stability:

FITC-Polysucrose powder when stored in air-tight containers at ambient temperatures is stable for at least 6 years in solution. The stability of FITC-Polysucroses has not been investigated in detail. However, the stability of the thiocarbamoyl linkage between the fluorescein moiety and Polysucrose will be similar to that with dextran (see data-file for information on stability of FITC-dextran). Only at elevated pH (>9) and elevated temperatures is there a risk for hydrolysis of the thiocarbamoyl linkage.
FITC-dextran was found to be stable at pH4 for up to 1 month at temperatures up to 35°C but this is not to be recommended for Polysucrose based products owing to the lability of the glycosidic linkages in sucrose. Polysucrose itself can be autoclaved at neutral and slightly alkaline pH.

Toxicity:

Polysucrose fractions exhibit no toxic symptoms when tested orally or intravenously. Intravenously Polysucrose fractions (100 000 to 500 000) when administered at doses up to 12 g/kg in experimental animals showed no toxic symptoms. Polysucrose is not however degraded in the blood and accumulates in the liver, spleen and kidneys. Polysucrose shows excellent biocompatibility with cells, virus or microorganisms and has been used for many decades in separation technology.

Biological Aspects and Applications

Polysucrose(Ficoll®) and derivatives thereof offer many interesting characteristics for studying the physiology of various organs. Many articles on the glomerular membrane have appeared over the years. Polysucrose unlike dextran has a more compact globular structure but nevertheless appears to possess some flexibility and is best regarded as an intermediate between a hard globular protein and the loose flexible coil of dextran. Polysucroses exhibit excellent biocompatibility and are not secreted or reabsorbed by the renal tubules.
The diffusion coefficients of narrow fractions of inter alia dextran and Polysucrose has been studied in membranes(2). It was shown that the diffusion properties of Polysucrose differed significantly from the linear polymers which diffused faster through the membranes. The authors concluded that Polysucrose behaves more like a solid sphere. A close examination of the size and conformation of Polysucrose using gpc in combination with light-scattering and viscosity detectors indicated that the Polysucrose molecule is best regarded as intermediate between a solid sphere and a well-solvated linear random coil(3). Venturoli and Rippe(4) have reviewed the available data on the two polysaccharides Ficoll and dextran for assessing the glomerular permselectivity as compared to glomerular proteins. The authors elaborate on the various properties which may influence the results such as molecular size, shape, charge and flexibility and assess their results in various pore models.

FITC-Polysucroses(FITC-Ficoll®) are primarily used for studying permeability and transport in cells and vessels and tissues. 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.

General procedures

The microvasculature of the hamster cheek pouch has proved to be a useful model for studying plasma leakage in different experimental conditions, e.g. following ischemia/reperfusion, 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 (490/520nm) and images are captured with a digital camera. A suitable concentration of FITC-Polysucrose for infusion in

experimental animals is 5 % (approx. 100mg/kg bodyweight)(5-7). 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(8).

1. Permeability studies in cells

The effect of LPS on different splenic non-lymphoid cells was monitored by selective uptake of FITC-Polysucrose(9). Marginal zone macrophages could be distinguished by the lack of uptake of FITC-Polysucrose and antibody staining(10). Other studies on liposomes and non-lymphoid cells using FITC-Polysucroses have been reported(11,12).

2. Permeability studies on the glomerular membrane

The available data on the two polysaccharides Polysucrose(Ficoll®) and dextran for assessing the glomerular permselectivity as compared to glomerular proteins has been reviewed(13). Polydisperse polysaccharides are excellent probes for measuring glomerular permselectivity and are reproducible, reliable and elegant. The authors elaborate on the various properties which may influence the results such as molecular size, shape, charge and flexibility and assess their results in various pore models.

Studies of the glomerular permeability of Polysucrose when infused intravenously showed that it has a cut-off at about 50Å whereas dextran is excreted up to 60-70Å – this is explained by the greater flexibility of dextran . The clearance of FITC-Polysucrose in mice lacking endothelial caveolae was studied in order to elucidate macromolecular transport pathways(14). The glomerular filter was studied at different glomerular filtration rates using FITC-Polysucrose 70 and 400 (also FITC-inulin)(15).
Studies on the glomerular filtration of dextran and Polysucrose showed that the glomerular membrane presented a much more restrictive barrier to Polysucrose than to dextran(16). Interestingly, the values of the sieving coefficient θ for Polysucrose approximated to those reported for uncharged globular proteins. Glomerular sieving in rats following surgery and muscle trauma monitored using FITC-Polysucrose 70/400(17). The rats were dosed with a mixture of FITC-Polysucrose 400 (960µg), FITC-Polysucrose 70 (40µg) and FITC-inulin (500µg) as a priming bolus. Glomerular sieving measured in caveolin-1 knockout mice monitored using FITC-Polysucrose 70/400 was used to investigate the factors affecting glomerular permeability(18).

FITC-Polysucrose 70 and albumin were used to estimate the fractional clearances in mice before and after treatment with enzymes degrading various glycosaminoglycans in the glycocalyx(19). FITC-Polysucrose 70 and albumin were infused in rats to explore the effects of temperature and ammonium chloride on the fractional clearance. The sieving coefficients between 8 and 37°C were not significantly different. Polysucroses perform differently to dextrans and theta was lower over the range 20 -70 Å than the corresponding dextrans.- solute shape and may outweigh size and charge(20,21).
To further explain the transport of protein across the capillary walls, mice lacking endothelial caveolae were studied with various permeability probes including FITC-Polysucrose(22). Fractional clearance of FITC-Polysucrose was determined at low ionic strength in perfusion fixed isolated kidney(23). Perfusates contained approx. 70mg FITC-Polysucrose 70/L were used to evaluate whether the increase in clearance of native albumin after 9 weeks of diabetes was due to reduced charge selectivity or to an alteration in the proportion of large pores(24).
More recent studies have explored the role of nitric oxide(25), reactive oxygen species(26) and scavengers(27) on glomerular permeability.

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