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Affinity chromatography on novel perfluorocarbon supports

Immobilisation of C.I. Reactive Blue 2 on a polyvinyl alcohol-coated perfluoropolymer support and its application in affinity chromatography

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ABSTRACT

The triazine dye C.I. Reactive Blue 2 has been immobilised on a particulate perfluorocarbon support by preparation of a hydrophilic polymeric coating comprising polyvinyl alcohol (average molecular weight, M_r 14 000) esterified with perfluorooctanoyl chloride and securely adsorbed on the perfluorocarbon support by multiple Van der Waals interactions. This polyvinyl alcohol-based coating wets the perfluorocarbon support and provides a neutral barrier to non-specific adsorption of proteins. Reaction with the triazine dye C.I. Reactive Blue 2 allows secure immobilisation of this versatile pseudo-affinity ligand and yields a remarkably stable adsorbent. The performance and capacity of the perfluorocarbon-based adsorbent was assessed: an 8-fold purification of lactate dehydrogenase was achieved from a crude rabbit muscle extract in 100% yield. The albumin-binding capacity of a dyed perfluorocarbon support containing 2.2 ± 0.2 μmol dye per g wet support was determined to be 15.7 mg/ml by frontal analysis. These novel supports compare favourably with conventional polysaccharidic affinity supports.

INTRODUCTION

The reactive textile dyes are known to participate in complex hydrogen bond, electrostatic, hydrophobic and steric interactions at the active sites of biological macromolecules and are becoming popular as versatile group-specific ligands for application in affinity chromatography[1]. The reactive chlorotriazine ring enables facile coupling to hydroxyl-containing chromatographic matrices, thereby allowing the preparation of affinity adsorbents. However, the stability of the triazine dyes to a wide range of stringent chemical conditions highlights the inherent limitations of the base matrix to which they are invariably attached. In particular, there is still a requirement for the development of novel chromatographic matrices that are able to

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withstand not only high pressures for application in high-performance affinity chromatography, but also extremes of acid and alkali to allow *in situ* column clean-up, chemical sanitisation and depyrogenation[2].

Perfluorocarbons are chemically and biologically inert, high-density (1.8–2.1 g/ml), thermally stable synthetic materials that are totally insoluble in aqueous and organic solvents. Aggregates of powdered polytetrafluoroethylene have been used as adsorbents for gas chromatography[3] and as hydrophobic adsorbents for the fractionation of small organo-fluorine-containing compounds[4] and the separation of proteins and nucleic acids[5]. However, perfluorocarbon surfaces display extremely low surface tensions and are wetted by water only in the presence of fluorosurfactants that adsorb to the surface[6]. Consequently, enzmes may be immobilised to fluorocarbon surfaces by cross-linking with the hydrophilic moieties of the adsorbed fluorosurfactant layer[7]. In an alternative approach, urease may be immobilised on perfluorocarbon surfaces by prior perfluoroalkylation of the protein with perfluorooctyl isothiocyanate[8]; the macromolecule is adsorbed on the perfluorocarbon surface by strong fluorocarbon–fluorocarbon Van der Waals interactions between the perfluoroalkyl chains of the covalently modified protein and the exposed perfluoromethyl groups of the polymer. Similarly, we have recently described the immobilisation of three dichlorotriazinyl dyes on solid and liquid perfluorocarbon surfaces by prior bis-perfluoroalkylation of the triazine rings of the dyes[9]. Lactate dehydrogenase was successfully purified from a crude rabbit muscle extract on dyes immobilised on a modified perfluoropolymer matrix. The adsorbents exhibited similar capacities and degrees of purification as previously reported for dyed agarose[10] and silica[11]. However, further work clearly showed that these bis-perfluoroalkylated triazine dyes were insufficiently anchored to the perfluorocarbon matrix and leached in the presence of albumin and organic solvents. Secure anchorage could be achieved by the introduction of additional perfluorinated groups to the ligand. It has recently been reported[12] that the strength of interaction between highly fluorinated compounds and perfluorocarbon stationary phases increases exponentially as the number of perfluoroalkyl chains per molecule increases. Furthermore, double-stranded molecules are more strongly retained than the single-stranded compound containing the equivalent overall number of fluorinated carbon atoms, indicating the strong cooperative effect of multiple anchorage sites.

Adsorbed-coating technology has been developed by Alpert and Regnier[13] to prepare alternative ion-exchange and reversed-phase silica and organic packings for use in high-performance liquid chromatography (HPLC). The technology involves the polysulphonation of the matrix followed by electrostatic adsorption of polyethyleneimine. In an analogous fashion, perfluorocarbon matrices may be coated with polymers by hydrophobic interactions, provided that the polymers contain highly fluorinated regions that are able to interact with the perfluorocarbon surface. However, in order to be effective in affinity chromatography, the polymeric coating should be neutral, stable, easily derivatised and exhibit low non-specific adsorption of proteins. Polyvinyl alcohol is a common neutral polymer derived from the hydrolysis of polyvinyl acetate and used extensively in the paper, food and printing industries. This paper describes the application of a perfluorooctanoyl-polyvinyl alcohol coating to a particulate perfluorocarbon matrix, its subsequent derivatisation with the triazine dye C.I. Reactive Blue 2 and its stability and performance as a chromatographic

adsorbent in the purification of lactate dehydrogenase from a crude rabbit muscle extract and albumin from human plasma.

EXPERIMENTAL

Materials

The triazine dye C.I. Reactive Blue 2 was obtained from BDH (Poole, U.K.). Polyvinyl alcohol [average molecular weight (M_r) 14000, 100% hydrolyzed] was purchased from Sigma (Poole, U.K.), together with the biochemicals tris(hydroxymethyl)methylamine, NADH (grade 2), sodium pyruvate and the diagnostic reagent kit for albumin determination. Lactate dehydrogenase [L-lactate:NAD^+ oxidoreductase, EC1.1.1.27; rabbit muscle (500 U/mg)] and pure human serum albumin were also purchased from Sigma, while human plasma was obtained from a known donor at the National Blood Transfusion Centre (Nottingham, U.K.) and tested negative for HIV III, HBS antigen and syphilis. Perfluorooctanoyl chloride was obtained from Fluorochem (Old Glossop, U.K.) while pre-packed Sephadex G-25 (PD-10 columns) was purchased from Pharmacia Biotechnology (Milton Keynes, U.K.). With the exception of acetone (SLR), all solvents were of analytical grade. The particulate perfluoropolymer matrix (6–8 m2/g, mean particle size 35 μm) was kindly provided by E.I. Du Pont de Nemours (Wilmington, DE, U.S.A.).

Modification of the perfluorocarbon matrix

Polyvinyl alcohol (1 g, 72 μmol) was dissolved in 100 ml water to which was added perfluorooctanoyl chloride (400 μl, 15 mmol). The mixture was shaken for 10 min, after which perfluorocarbon matrix (100 g) was added, and the suspension stirred overnight at room temperature. The modified support was sequentially washed on a sintered glas funnel with distilled water (1 l), acetone–water (50:50, v/v) (1 l), acetone (500 ml), acetone–water (50:50, v/v) (500 ml), acetone–water (30:70, v/v) (500 ml) and finally distilled water (1 l). Care was taken to avoid the exposure of the perfluorocarbon support to air between washes.

Immobilisation of C.I. Reactive Blue 2

Wet polyvinyl alcohol-coated-perfluorocarbon matrix aliquots (4 g) were stirred with distilled water (20 ml) to which were added various weights of commercial C.I. Reactive Blue 2 (0, 50, 100, 150, 200 and 250 mg). The suspension was stirred at 50°C for 30 min, after which sodium hydroxide (5*M*, 6 ml) was added and stirring continued at 70°C for a further 30 min. The suspension was allowed to cool and washed on a sintered-glass funnel with water (200 ml), acetone–water (50:50, v/v) (100 ml), acetone (100 ml), acetone–water (50:50, v/v) (100 ml), acetone–water (30:70, v/v) (100 ml) and finally distilled water (200 ml), once again avoiding exposure of the support to air. Higher degrees of dye loading on the support were achieved by repeating both dye application and washing stages.

Determination of immobilised dye concentration

The immobilised dye concentration of a dyed support was estimated by sucking the adsorbent dry on sintered glass and suspending a weighed aliquot (50 mg) in methanol–water (50:50, v/v) (0.5 ml) to which was added boiling agarose (2%, w/v)

(0.5 ml). After continuous inversion of the cuvette until the gel had set, the difference spectrum of the support between 700 and 500 nm could be prepared against an equivalent sample of undyed support. The system was calibrated as follows: various dilutions of free dyed perfluorooctanoyl polyvinyl alcohol conjugate were prepared in a methanol–water mixture (50:50, v/v) (5 ml) and then incubated with aliquots of wetted perfluorocarbon support (50 mg wet weight) by rotary tumbling for 12 h. The decrease in absorbance of the methanol–water solutions at 620 nm after incubation was directly attributed to the adsorption of the dyed polymer on the support. After extensive washing, this yielded a series of dyed supports with known immobilised dye concentrations. These supports were centrifuged at 4500 *g* for 5 min and resuspended in plastic cuvettes in the methanol–water mixture (0.5 ml), set in agarose as previously described. Comparison of the measured difference spectra of the dyed versus undyed supports with the known dye concentrations showed a difference by a factor of 1.25 which was thereafter incorporated into the estimation of immobilised dye concentrations on all supports.

Determination of adsorbent stability

A wet perfluorocarbon adsorbent containing 2.2 μmol/ml C.I. Reactive Blue 2 was sucked dry, divided into 100-mg aliquots and suspended in 2 ml volumes of various solutions and solvent by rotary tumbling at room temperature for 24 h. The adsorbent was allowed to settle, the solution/solvent removed and, where necessary, adjusted to neutral pH with a known volume of acid or base. The absorption at 620 nm (λ_{max}) of the solution was then measured against appropriate solution/solvent blanks, tand the dye concentration estimated assuming a molar extinction coefficient of 12 750 M^1 cm^{-1}. The percentage dye lost from the support was estimated assuming that the leached dye remained intact.

Protein preparation, determination and assay

Protein preparations were desalted where necessary by passage on disposable Sephadex G-25 pre-packed PD-10 columns pre-equilibrated in the appropriate buffer. Protein determination was routinely carried out using the Bradford assay[14], the standard reagent (Coomassie brilliant blue G-250, 0.01%, w/v; ethanol, 4.7%,w/v; phosphoric acid, 8.7%, w/v) being diluted one fifth in water prior to use. Protein concentrations in stock solutions were initially determined by absorbance at 280 nm assuming extinction coefficients for crude albumin and crude lactate dehydrogenase as 0.53 (ref. 15) and 1.14 (ref. 16) ml mg^{-1} cm^{-1}, respectively. Serial dilutions (20 μl) of stock protein solutions were incubated with fresh assay mixture (980 μl) for 10 min before measuring the absorbance at 595 nm against a buffer blank, to prepare standard curves from which the protein concentration in unknown samples could be estimated.

The bromocresol green assay[17] for serum albumin was used to determine the albumin content in plasma samples. Diluted plasma samples were compared to a known standard by incubating aliquots (200 μl) with the standard assay mixture (Sigma) (800 μl) for 10 min, followed by measuring the absorbance at 628 nm against the appropriate buffer blank. The activity of lactate dehydrogenase was monitored spectrophotometrically at 340 nm and 25°C by measuring the oxidation of NADH in disposable plastic cuvettes arranged in a Perkin Elmer Lambda 5UV–VIS spectrophotometer. Small volumes of enzyme (10 μl), diluted if necessary, were added to

a prepared solution (1 ml) containing Tris–HCl (200 m*M*), sodium pyruvate (1 m*M*) and NADH (200 μM) at pH 7.3. One unit is defined as that amount of enzyme needed to convert 1 μmol of substrate to product in 1 min at 25°C, assuming a molar extinction coefficient of 6230 l mol^{-1} cm^{-1} for NADH at 340 nm.

Chromatographic procedure

Adsorbents were washed in the appropriate running buffer, then packed in Pharmacia HR 5/10 columns and used in conjunction with the Pharmacia FPLC system (P500 pumps, LCC 500+ controller and UV1 single-path monitor). The flow-rate used throughout was 2 ml/min. Typically, about 10 mg crude protein were loaded (1 ml) and purified protein was eluted as follows: albumin was recovered using potassium chloride (1 *M*), while lactate dehydrogenase was eluted with NADH (5 m*M*). Recovered protein fractions were analysed for activity and protein content and were stored at 4°C for sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).

Dynamic load capacity

Dynamic load capacities of the adsorbent were determined by frontal analysis[18]. For example, human serum albumin-binding capacity was measured by pumping 10 mg/ml pure albumin until the absorbance at 280 nm of the output and input streams were identical. Albumin was subsequently eluted with potassium chloride (2 *M*), and the recovery of bound protein determined. Bound lactate dehydrogenase was eluted with the specific eluent, NADH (5 m*M*).

SDS-PAGE

SDS-PAGE was carried out essentially as described by Laemmli[19] using an LKB Multiphore II vertical electrophoresis unit, comprising gel lanes (1.5 mm in width) of 10% (w/v) acrylamide resolving gel and 3% (w/v) stacking gel. Protein samples (10–20 μl) were loaded and the gels stained with Electran PAGE Blue G-90 (BDH, Poole, U.K.). Gels were stored in aqueous acetic acid (3.5%, v/v).

RESULTS AND DISCUSSION

Fig. 1 shows two possible reaction routes to achieve the immobilisation of the triazine dye C.I. Reactive Blue 2 on a perfluorocarbon matrix via a highly fluorinated polymeric coating. The preferred strategy involves the preparation of the poly(perfluorooctanoyl)-substituted polyvinyl alcohol in water, coating the perfluorocarbon matrix and, finally, coupling the triazine dye to the adsorbed hydroxyl-containing coating. Alternatively, the dye may first be coupled to the polymer, thereby allowing the spectrophotometric estimation of the dye polymer ratio; the perfluorooctanoyl chloride can then be added separately or in conjunction with the perfluorocarbon matrix. Typical estimates for the extent of coupled dye, determined after reaction in free solution, lie in the range 1–4 mol dye per mol polymer (M_r14 000). With regard to the perfluorooctanoyl-anchoring groups, a least a 200-fold molar excess of perfluorooctanoyl chloride was used to ensure secure anchorage. Alternative highly fluorinated reactive compounds may also be used, of which pentafluorobenzoyl chloride proved the most viable alternative. This compound has been used by Keese

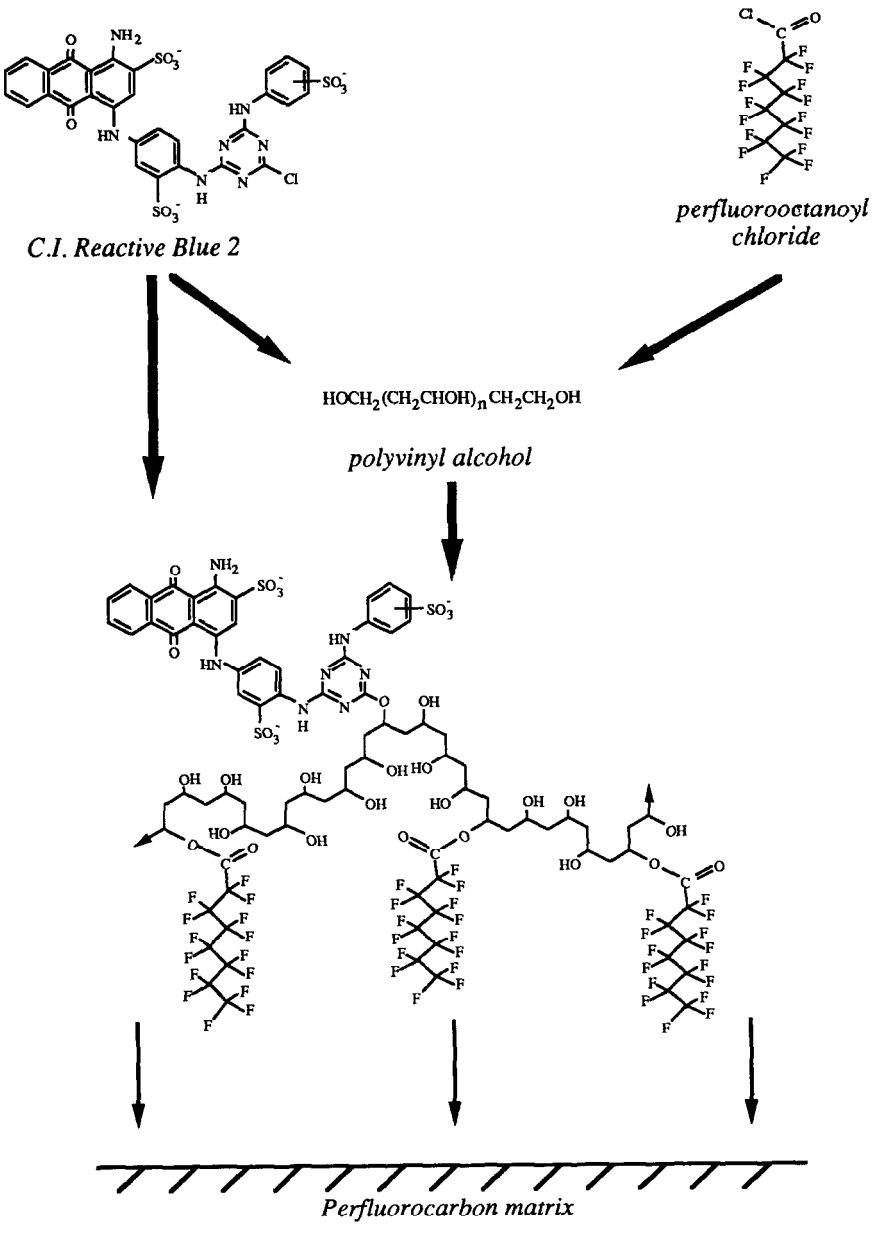

Fig. 1. Strategies for the preparation of C.I. Reactive Blue 2 securely adsorbed on a perfluorocarbon surface (mean n = 324).

and Giaever[20] to anchor polylysine on liquid perfluorocarbon droplets as a substrate for cell growth in culture media.

Estimation of the concentration of dye immobilised on the perfluorocarbon matrix is complicated by the inert nature of the base material, which precludes its

solubilisation. Solid perfluorocarbons are extremely unreactive at room temperature and cannot be dissolved, hydrolysed, oxidised or otherwise degraded. Measurement of the visible spectrum of the support suspended in water is complicated by rapid sedimentation of the particles; the use of a viscous medium such as glycerol only marginally improves this situation. By preparing mixed suspensionsin hot agarose, the perfluorocarbon are held in place upon gelling such that the difference spectra between dyed and undyed adsorbents can be determined. However, because of light loss froms scattering, a correction factor was estimated using lightly dyed supports prepared with known concentrations of immobilised dye (<0.5 μmol/g) (only lightly dyed supports can be prepared by this method). By assuming that this correction factor applies across the entire range of dye concentrations it is possible to estimate the concentration of immobilised dye on more heavily dyed supports.

The difficulties experienced in estimating the immobilised ligand concentration reflect the durability of the perfluorocarbon support. Table I assesses the stability of the polyvinyl alcohol-coated perfluorocarbon matrix derivatised with the triazine dye C.I. Reative Blue 2, by measuring the extent of dye leakage after exposure to a variety of conditions for 24 h. In strong acid and basic conditions (5 *M* hydrochloric acid, 6 *M* sodium hydroxide) the perfluorocarbon-based support showed some leakage of dye, while in long-term studies lasting more than nine months, no leakage of the triazine dye from the perfluorocarbon matrix was apparent, within the spectrophotometric limits of detection, in water, sodium hydroxide (1 *M*), hydrochloric acid

TABLE I

STABILITY OF PERFLUOROCARBON-IMMOBILISED C.I. REACTIVE BLUE 2 UNDER VARIOUS CONDITIONS

Suction-dried wet dyed perfluorocarbon support containing 2.2 μmol/ml C.I. Reactive Blue 2 was divided into 100 mg aliquots, suspended in 2 ml of solution/solvent and incubated at 18°C by rotary tumbling for 24 h. The absorption at 620 nm of the supernatants were measured after appropriate adjustment to neutrality with a known volume of acid or base. Dye concentrations were estimated assuming a molar extinction coefficient of 12 750 M^{-1} cm^{-1}. Dye concentrations below the spectrophotometric limits of detection (<0.005 A.U. at 620 nm; <0.4 μM) are indicated by a dash.

Solution/solvent	*Concentration of C.I. Reactive Blue 2 released (μM)*
Distilled water	–
Sodium hydroxide (1 *M*)	–
Sodium hydroxide (6 *M*)	7.7
Hydrochloric acid (1 *M*)	–
Hydrochloric acid (5 *M*)	7.1
Urea (8 *M*)	–
Aqueous glutaraldehyde (1%, w/v)	–
Aqueous triethylene glycol (25%, v/v)	–
Aqueous glycerol (25%, v/v)	–
Acetone	–
Acetonitrile	–
Dimethyl formamide	–
Dimethyl sulphoxide	–
Methanol	–

(1 *M*), urea (1 *M*) or acetone. Thus, the perfluorocarbon-based adsorbent appears to offer superior stability to conventional polysaccharide-based absorbents, particularly under the harsh conditions necessary for column cleaning and depyrogenation[2]. We are at present comparing dye leakage from a number of supports using both radioisotopic and immunological (competitive enzyme-linked immunosorbent assay) methods.

The chromatographic behaviour of the polyvinyl alcohol-coated perfluorocarbon support in the absence of immobilised dye was examined by repeated applications at room temperature of small aliquots (100 μg, 0.1 ml) of pure rabbit muscle lactate dehydrogenase (in 50 m*M* Tris–HCl, pH 7.3) or human serum albumin (in 20 m*M* phosphate, pH 5.0). No non-specific adsorption of either protein was observed. The polyvinyl alcohol coating effectively wets the strongly hydrophobic perfluorocarbon surface and provides an effective barrier to the non-specific adsorption of proteins on the underlying perfluorocarbon matrix.

Fig. 2a shows the purification of lactate dehydrogenase from a crude rabbit muscle extract achieved by chromatography on C.I. Reactive Blue 2 immobilised on the polyvinyl alcohol-coated perfluorocarbon support. Elution with the coenzyme NADH (5 m*M*) promoted an overall 8-fold increase in specific activity in 100% yield, the main eluted fraction showing a 12.7-fold purification (Fig. 2b, lane B); the purity of the eluted protein compares favourably with commercial pure rabbit muscle lactate dehydrogenase (lane A). Table II presents the data for the overall purification.

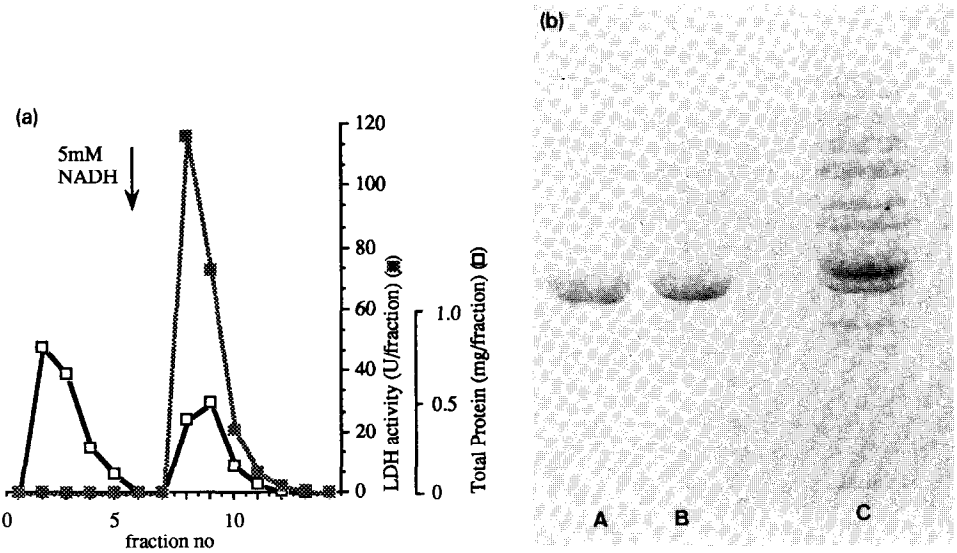

Fig. 2. (a) Purification of lactate dehydrogenase (LDH) from a crude rabbit muscle extract on C.I. Reactive Blue 2 immobilised on polyvinyl alcohol-coated perfluorocarbond Column volume, 1 ml; immobilised dye concentration, 2.2 μmol/ml; running buffer, 50 m*M* Tris–HCl, pH 7.3; eluting buffer, 5 m*M* NADH in running buffer; fraction volume, 1 ml; flow-rate, 2 ml/min. Protein measured by Bradford assay, enzyme activity detected as described in the text. (b) SDS-PAGE of lactate dehydrogenase from a crude rabbit muscle extract. (A) Pure lactate dehydrogenase, Sigma type II; (B) pooled NADH-eluted fractions from C.I. Reactive Blue 2 polyvinyl alcohol-coated perfluorocarbon adsorbent; (C) crude rabbit muscle lactate dehydrogenase extract, Sigma.

TABLE II

PURIFICATION OF LACTATE DEHYDROGENASE FROM CRUDE RABBIT MUSCLE EXTRACT USING PERFLUOROCARBON-IMMOBILISED C.I. REACTIVE BLUE 2

Experimental details as for Fig. 2. Fraction, 8, 9, 10 and 11 pooled as "eluted fraction".

Stage	*Total protein (mg)*	*Total activity (U)*	*Specific activity (U/mg)*	*Yield (%)*	*Purification (fold)*
Crude sample	8.8	213.0	24.2	100	1
Eluted fraction	1.1	214.0	194.5	100	8.04

TABLE III

PURIFICATION OF SERUM ALBUMIN FROM HUMAN PLASMA USING PERFLUOROCARBON-IMMOBILISED C.I. REACTIVE BLUE 2

Albumin was purified from diluted plasma (1:4) on a column containing perfluorocarbon-immobilised C.I. Reactive Blue 2; applied sample, 1 ml; column volume, 1 ml; immobilised dye concentration, 2.2 μmol/ml; running buffer, 20 m*M* phosphate, pH 5.0; eluting buffer, 2 *M* KCl, 20 m*M* phosphate, pH 8.0; fraction volume, 1 ml; flow-rate, 2 ml/min. Protein measured by the Bradford assay; specific albumin detected by the bromocresol green assay.

Stage	*Total protein (mg)*	*Albumin (mg)*	*Albumin content (%)*	*Yield (%)*	*Purification (fold)*
Crude sample	18.5	11.6	62.5	100	1
Eluted fraction	6.6	6.3	93.9	98.5	1.5

Determination of the dynamic load capacity for lactate dehydrogenase by frontal analysis for a matrix containing 2.2 μmol dye per g wet adsorbent yielded 5.3 mg/ml bound enzym per ml perfluorocarbon support.

Table III presents the data for the purification of serum albumin by chromatography of human plasma on the blue perfluorocarbon adsorbent (2.2 μmol dye per g); albumin was recovered in 93.9% purity and 98.5% yield. On repeated passage of human plasma, a 1.5-fold purification of albumin was typically achieved in greater than 90% yield. Fig. 3a shows the SDS-PAGE analysis of the purification and clearly demonstrates that the eluted fraction (lane C) is of comparable purity to commercial pure human serum albumin (Sigma, fraction V) (lane B). Fig. 3b illustrates the frontal analysis chromatogram for the measurement of the dynamic load capacity for human serum albumin of the perfluorocarbon adsorbent. The adsorbent, with an immobilised dye concentration of 2.2 μmol/g, bound 15.7 mg albumin per ml support, corresponding to approximately 11% usage of dye on a molar basis. This capacity compares favourably with the quoted albumin capacities for most commercial adsorbents containing C.I. Reactive Blue 2 (5–18 mg/ml). Fig. 4 examines the capacity as a function of the immobilised dye concentration and suggests that the capacity for both albumin and lactate dehydrogenase might be greater if the immobilisd dye concentration were increased. Furthermore, double reciprocal plots of the capacity data given in Fig. 4 indicate that maximum capacities for albumin and lactate dehydrogenase of 32.3 and 8.5 mg/g could be realised at higher dye concentrations.

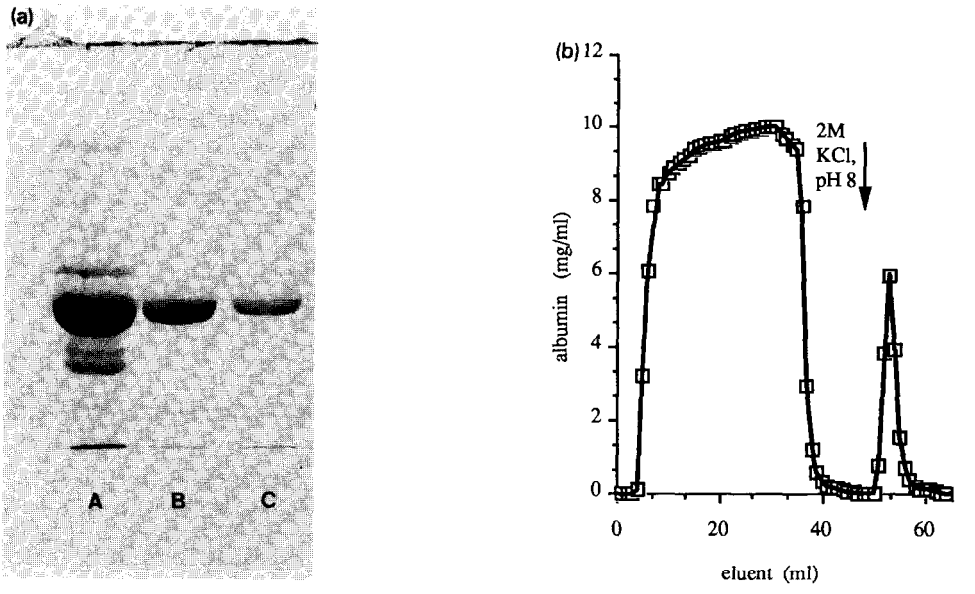

Fig. 3. (a) SDS-PAGE analysis of purification of albumin from human plasma. (A) Crude human plasma; (B) pure human serum albumin, fraction V. Sigma; (C) purified fraction, C.I. Reactive Blue 2 polyvinyl alcohol-coated perfluorocarbon adsorbent. (b) Determination of dynamic loading capacity for albumin by frontal analysis. C.I. Reactive Blue 2 polyvinyl alcohol perfluorocarbon support. Column volume, 1 ml; immobilised dye concentration, 2.2 μmol/ml; running buffer, 20 m*M* phosphate, pH 5.0; eluting buffer, 2 *M* KCl in 20 m*M* phosphate buffer, pH 8.0; flow-rate, 2 ml/min; loading, 10.0 mg/ml pure human serum albumin, fraction V, Sigma; 16.1 mg bound, 15.7 mg eluted with salt. Protein determined by absorbance of fractions at 280 nm (diluted if required).

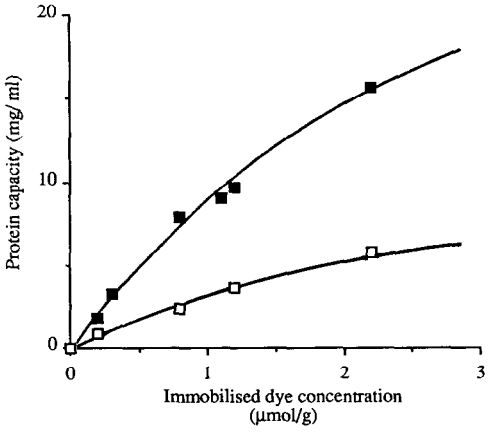

Fig. 4. Comparison of the dynamic loading capacities for human serum albumin (■) and rabbit muscle lactate dehydrogenase (□) of perfluorocarbon supports prepared with varying immobilisd dye concentrations. Immobilised dye concentrations were determined using the difference spectra between dyed perfluorocarbon supports and an equivalent weight of undyed support suspended in a 1% (w/v) agarose gel; observed absorbances at 620 nm were corrected by a factor of 1.25 (see text) to account for losses through light scattering. Dynamic loading capacities were determined by frontal analysis. All column volumes, 1 ml. Chromatographic details for pure albumin —loading concentration, 10 mg/ml; running buffer, 20 m*M* phosphate, pH 5.0; eluting buffer, 2 *M* KCl in 20 m*M* phosphate buffer, pH 8.0. Chromatographic details for lactate dehydrogenase loading concentration, 4.5 mg/ml; running buffer, 50 m*M* Tris–HCl, pH 7.3; eluting buffer, 5 m*M* NADH in running buffer. Flow-rate, 2 ml/min throughout. Protein was determined by absorbance of fractions at 280 nm (diluted if required).

The use of perfluorocarbon supports with smaller particle sizes, and therefore increased total surface areas, may offer still higher capacities while maintaining their overall performance as affinity adsorbents.

CONCLUSIONS

Perfluorocarbon polymers have hitherto found little application in the affinity chromatography of biological macromolecules because of the inherent limitations associated with ligand immobilisation and non-wettability. The preparation of a hydrophilic polymeric coating of polyvinyl alcohol, multiply derivatised with perfluoroalkyl tails with strong affinity for the perfluorocarbon surface, provides a securely adsorbed polyhydroxylic layer on the perfluorocarbon surface to which may be attached a variety of affinity ligands such as the triazine dyes. The chromatographic results suggest that perfluorocarbon supports may offer a viable alternative to many of the current commercially available matrices, while offering superior stability under the harsh conditions required for cleaning *in situ* and depyrogenation.

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