Affinity Adsorption of Recombinant Human Interferon-α on Monosize Dye-Affinity Beads

Evrim B. Altıntaş, Adil Denizli

Department of Chemistry, Biochemistry Division, Hacettepe University, Ankara, Turkey

Received 10 March 2006; accepted 17 July 2006
DOI 10.1002/app.25273
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Monosize, nonporous poly(glycidyl methacrylate) [poly(GMA)] beads were prepared by dispersion polymerization. Cibacron Blue F3GA was covalently attached onto the poly(GMA) beads for adsorption of recombinant interferon-α (rHuIFN-α). Monosize poly(GMA) beads were characterized by scanning electron microscopy. Dye-carrying beads (1.73 mmol/g) were used in the adsorption–elution studies. The effect of initial concentration of rHuIFN-α, pH, ionic strength, and temperature on the adsorption efficiency was studied in a batch system. Nonspecific adsorption of rHuIFN-α on the beads was 0.78 mg/g. Dye attachment significantly increased the rHuIFN-α adsorption up to 181.7 mg/g. Equilibrium adsorption of rHuIFN-α onto the dye-carrying beads increased with increasing temperature. Negative change in free energy ($\Delta G^0 < 0$) indicated that the adsorption was a thermodynamically favorable process. ΔS and ΔH values were 146.1 J/mol K and −37.39 kJ/mol, respectively. Significant amount of the adsorbed rHuIFN-α (up to 97.2%) was eluted in the elution medium containing 1.0*M* NaCl in 1 h. To determine the effects of adsorption conditions on possible conformational changes of rHuIFN-α structure, fluorescence spectrophotometry was employed. We concluded that dye-affinity beads can be applied for rHuIFN-α adsorption without causing any significant conformational changes. Repeated adsorption–elution processes showed that these beads are suitable for rHuIFN-α adsorption. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 103: 975–981, 2007

Key words: monosize beads; interferon; protein purification; dye affinity adsorbents

INTRODUCTION

Interferons are potent biologically active proteins synthesized and secreted by somatic cells of all mammalian species. Interferon proteins have been classified into three types: α, β, and γ, based on antigenic and structural differences.[1] Human interferon-α (IFN-α) comprises a family of extracellular signaling proteins with antiviral, antiproliferating, and immunomodulatory activities. They are produced by peripheral blood leukocytes and lymphoblastoid and myeloblastoid cell lines on viral activation.[2] The interest in this protein is connected with its therapeutic value against certain types of tumors such as brain tumors and malignant melanomas.[3] Recombinant IFN-α is also used for the treatment of AIDS-related Kaposi's sarcoma, hairy cell leukemia, and chronic hepatitis B and C. The isolation and purification of human interferons from various sources has been attempted by a variety of methods, including metal chelation, precipitation, ion-exchange chromatography, gel-filtration chromatography, hydrophobic chromatography, dye-affinity chromatography, and immunoaffinity chromatography over many years and some protocols have been proposed that yield homogeneous protein.[4–9]

Recently, the preparation and applications of micron-sized particles have attracted considerable attention because of their unusual physical and chemical properties owing to small size and large specific surface area.[10,11] A major advantage of the nonporous microparticles is that significant intraparticle diffusion resistances are absent; this is particularly useful for the rapid analysis of proteins with high efficiency and resolution.[12] The rapid separation makes it very useful for quality control, on-line monitoring, and purity check of biomolecules such as peptide mapping of recombinant products. However, silica is unstable in extreme pH, and polystyrene-based particles are hydrophobic, which makes them exhibit pronounced nonspecific protein adsorption[13] Therefore, the development of other polymeric adsorbents with low nonspecific protein adsorption is desirable. Poly(glycidyl methacrylate) [poly(GMA)] has attracted much attention for its hydrophilic characteristics.[14] Recently, there has been increasing interest in the use of monosize beads in medical, bioengineering, and biotechnology applications such as in nuclear medicine for diagnostic imaging and in studying the phagocytic process,[15] biopolymer separation,[16] enzyme immobilization,[17] protein purification,[18] and sequence-specific separation of polynucleotides and nucleic acids.[19]

Classical protein ligands are not an attractive choice because of their high cost, orientation of ligand, low binding capacity on immobilization, instability, and leakage.[20] In industrial applications, the use of affinity

Correspondence to: A. Denizli (denizli@hacettepe.edu.tr).

Journal of Applied Polymer Science, Vol. 103, 975–981 (2007)
© 2006 Wiley Periodicals, Inc.

WILEY InterScience® DISCOVER SOMETHING GREAT

beads carrying antibody or protein ligands is hampered by the cost of biomolecules and the need to validate the process, where the problems come from the biological sources of biomolecules and by their leakage into purified products. Therefore, affinity beads with synthetic ligands which exhibit selectivity and a wide range of applications are desirable. Textile dyes have been considered as one of the important alternatives to the natural biological ligands to circumvent many of their aforementioned drawbacks.[21–24] For example, the antraquinone dye Cibacron Blue F3GA is widely used in affinity chromatography for purifying nucleotide-binding enzymes, such as dehydrogenases and kinases, and human cholesteryl ester transfer protein. Cibacron Blue F3GA is also able to bind to several proteins, including human serum albumin, lysozyme, and immunotoxins such as ricin A.[25] Dye ligands are biologically inert, chemically and physically stable, inexpensive, and can be easily immobilized to matrices at high loading, resulting in high-capacity adsorbents.

The aim of this study was to prepare monosize dye-affinity beads for efficient separation of recombinant human interferon-α (rHuIFN-α). Nonporous poly (GMA) monosize beads were obtained by dispersion polymerization of GMA. A major advantage of nonporous beads is the absence of significant intraparticle diffusion resistance, making it particularly useful for rapid separation of proteins. The poly(GMA) beads carrying Cibacron Blue F3GA are shown to be very effective for the adsorption of rHuIFN-α.

EXPERIMENTAL

Materials

Recombinant human interferon α (rHuIFN-α) (sterile filtered white lyophilized (freeze-dried) powder; 99% purity as determined by reversed phase-high performance liquid chromatography (RP-HPLC)) was purchased from ProSpec-Tany TechnoGene (USA). The specific activity as determined in a viral resistance assay using bovine kidney MDBK cells was 3.5×10^8. Cibacron Blue F3GA was obtained from Polyscience (Warrington, USA) and used without further purification. Glycidyl methacrylate (GMA, Fluka A.G., Buchs, Switzerland) was purified by vacuum distillation and stored in a refrigerator until use. Azobisisobutyronitrile (AIBN) and poly(vinyl pyrrolidone) (MW: 30,000; BDH Chemicals, Poole, England) were selected as the initiator and the steric stabilizer, respectively. AIBN was recrystallized from methanol. Ethanol (Merck, Germany) was used as the diluent without further purification. All other chemicals were the guaranteed or analytical-grade reagents commercially available and used without further purification. Laboratory glassware was kept overnight in a 5% nitric acid solution. Before use the glassware was rinsed with deionized water and dried in a dust-free environment. All water used in the experiments was purified using a Barnstead (Dubuque, IA) ROpure LP® reverse osmosis unit with a high-flow cellulose acetate membrane (Barnstead D2731) followed by a Barnstead D3804 NANOpure® organic/colloid removal and ion-exchange packed bed system.

Preparation of poly(GMA) beads

Poly(GMA) beads were prepared as described elsewhere.[26] The dispersion polymerization was performed in a sealed polymerization reactor (volume: 500 mL) equipped with a temperature control system. A typical procedure applied for the dispersion polymerization of GMA is given below. The monomer phase was comprised of 40 mL GMA. AIBN (250 mg) was dissolved into the monomer phase. The resulting medium was sonicated for about 5 min at 200 W within an ultrasonic water bath (Bransonic 2200, England) for the complete dissolution of AIBN in the polymerization medium. Poly(vinyl pyrrolidone) (4.0 g) was dissolved in a homogeneous solution of ethanol (100 mL) and water (100 mL) and placed in a polymerization reactor. The reactor content was stirred at 500 rpm during the monomer addition, completed within about 5 min, and the heating was started. Then, the mixture was degassed by purging with nitrogen for about 20 min. Then, the sealed reactor was placed in a shaking water bath at room temperature. The initial-polymerization time was defined when the reactor temperature was raised to 65°C. The polymerization was carried out at 65°C for 4 h with continuous stirring. After completion of the polymerization period, the reactor content was cooled down to room temperature and centrifuged at 5000 rpm for 10 min for the removal of dispersion medium. This polymerization reaction led to the formation of white beads. Poly(GMA) beads were redispersed in 10 mL of ethanol and centrifuged again under similar conditions. The ethanol washing was repeated three times for complete removal of unconverted monomers and other components. Finally, poly(GMA) beads were redispersed in 10 mL of water (0.10% by weight) and stored at room temperature.

Dye attachment to poly(GMA) beads

Cibacron Blue F3GA was covalently attached to the poly(GMA) monosize beads. First, Cibacron Blue F3GA was dissolved in 100 mL of water (dye concentration: 5 mg/mL). This aqueous dye solution was transferred to poly(GMA) beads (total mass: 1.0 g) in 100 mL distilled water, and then 4.0 g of NaOH were added. The medium was heated at 80°C in a sealed reactor and was stirred magnetically for 4 h. Under

these experimental conditions, a chemical reaction took place between the chlorine containing group of the Cibacron Blue F3GA and the epoxy groups of the GMA monomer. Afterward, the remaining epoxy groups were blocked with 2*M* ethylene diamine at pH 10 for 16 h under gentle stirring. To remove the nonspecifically attached dye molecules, an extensive cleaning procedure was applied, which was as follows: The beads were first washed with deionized water. The monosize beads were dispersed in methanol, and the dispersion was sonicated for 2 h in an ultrasonic bath. At the last stage, beads were washed again in deionized water. Cibacron Blue F3GA-attached poly(GMA) beads were stored at 4°C with 0.02% sodium azide to prohibit microbial contamination.

The release of the Cibacron Blue F3GA from the dye-attached monosize beads was investigated at different pH values in the range of 4.0–8.0. It should be noted that these media were the same which were used in the rHuIFN-α adsorption experiments given below. Cibacron Blue F3GA release was also determined in the medium at 0.05*M* Tris/HCl buffer containing 0.5*M* NaSCN which was the medium used for the rHuIFN-α elution experiments. The medium with the Cibacron Blue F3GA-attached beads was incubated for 24 h at room temperature. Then, beads were removed from the medium, and the Cibacron Blue F3GA concentration in the supernatant was measured by spectrophotometry at 630 nm.

Characterization of monosize beads

The amount of attached Cibacron Blue F3GA was determined using an elemental analysis instrument (Leco, CHNS-932, USA). The amount of Cibacron Blue F3GA attachment on the monosize beads was calculated by considering the sulfur stoichiometry.

Poly(GMA) beads were gold coated (about 100 Å thickness) under a high vacuum, 0.1 Torr, high voltage, 1.2 kV and 50 mA. Coated beads were examined using a scanning electron microscope (JEOL, JEM 1200 EX, Tokyo, Japan) to characterize the morphology and size of beads.

The epoxy group content in the synthesized poly (GMA) samples was determined by the perchloric acid titration method. The poly(GMA) beads were dispersed in 0.1 mol/L tetraethylammonium bromide in acetic acid solution and titrated with 0.1 mol/L perchloric acid solution until the crystal violet indicator changed to blue–green.

rHuIFN-α adsorption studies

rHuIFN-α was selected as a model protein for adsorption studies. rHuIFN-α adsorption of the normal and the Cibacron Blue F3GA-attached beads were studied at various pH. The pH of the adsorption medium was changed between 4.0 and 8.0 by using different buffer systems (0.1*M* acetate for pH 4.0–6.0, 0.1*M* phosphate for pH 7.0–8.0). All pH measurements were made with a digital pH/mV meter. The initial concentration of rHuIFN-α was changed between 0.25 and 2.0 mg/mL. In an adsorption experiment, rHuIFN-α was dissolved in 5 mL of buffer solution and beads were added. The time to reach equilibrium adsorption with continuous stirring was found to be 60 min and in the rest of the study a 60-min adsorption duration was therefore employed. At the end of the equilibrium period, the beads were separated from the adsorption solution. The rHuIFN-α adsorption capacity of beads was determined by measuring the remaining concentration of rHuIFN-α in the adsorption medium spectrophotometrically at 280 nm. A UV–vis diode array spectrophotometer with 10-mm cuvettes was used for absorbance measurements. It should be noted that all adsorption curves are averages of five experiments. The amount of adsorbed rHuIFN-α per unit mass of the beads was calculated by using the mass balance.

rHuIFN-α elution studies

The rHuIFN-α elution experiments were performed in a buffer solution containing 1.0*M* NaCl. The rHuIFN-α adsorbed beads were placed in the elution medium and magnetically stirred for 1 h at 25°C, at a stirring rate of 100 rpm. The final rHuIFN-α concentration within the elution medium was determined by spectrophotometry. The elution ratio was calculated from the amount of rHuIFN-α adsorbed on the beads and the amount of rHuIFN-α desorbed into the medium. To obtain the reusability of the dye-attached poly(GMA) beads, rHuIFN-α adsorption–elution procedure was repeated 10 times by using the same polymeric adsorbent. It should be also noted that, during the elution step of rHuIFN-α, dye release was also monitored continuously.

Antiviral bioassay for rHuIFN-α

The biological activity of the eluted rHuIFN-α was determined by its ability to show cytopathic effect on MDBK cells. Specific activity was determined with reference to standard rHuIFN-α obtained from ProSpec TechnoGene (USA). One unit of activity is defined as the amount of rHuIFN-α required to produce antiviral activity equivalent to that expressed by 1 international unit (IU) of the ProSpec TechnoGene reference standard.

RESULTS AND DISCUSSION

Figure 1 shows the scanning electron micrograph of the prepared poly(GMA) beads, illustrating that the

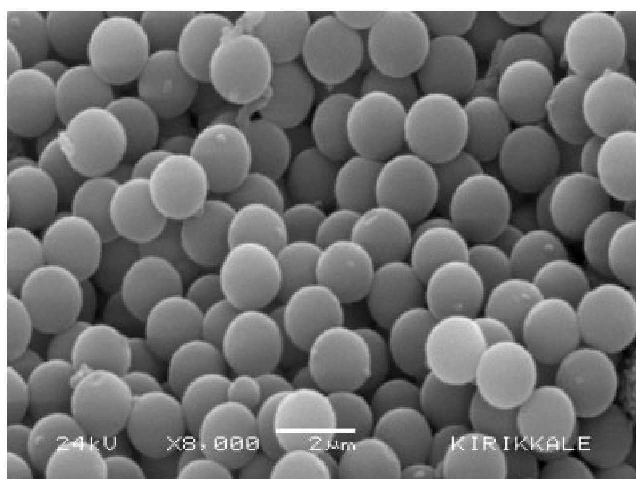

Figure 1 SEM photograph of monosize poly(GMA) beads.

beads are uniform in size (R.S.D. < 1%) with a diameter of 1.6 μm. Poly(GMA) beads are hydrophilic polymer networks capable of imbibing large amounts of water yet remain insoluble and preserve their three-dimensional shape. The equilibrium swelling ratio of the poly(GMA) beads is 45%. Epoxy groups in the poly(GMA) structure were reacted with dye ligand Cibacron Blue F3GA in the presence of NaOH. To evaluate the degree of dye attachment, elemental analysis of the beads was performed. The physicochemical properties of poly(GMA) beads are presented in Table I.

rHuIFN-α adsorption–elution studies

Adsorption isotherms

Figure 2 shows the rHuIFN-α adsorption isotherm of the plain and dye-affinity beads. Note that one of the main requirements in dye affinity chromatography is the specificity of the adsorbent. The nonspecific interaction between the support, which is the poly(GMA) beads in the present case, and the molecules to be adsorbed, which are the rHuIFN-α molecules here, should be minimal to consider the interaction as specific. As presented in this figure, with increasing rHuIFN-α concentration in solution, the amount of rHuIFN-α adsorbed by the beads increases almost linearly at low concentrations, below about 0.5 mg/mL, then increases less rapidly and approaches saturation. The steep slope of the initial part of the adsorption isotherm represents a high affinity between rHuIFN-α and Cibacron Blue F3GA. It becomes constant when the rHuIFN-α concentration is greater than 0.5 mg/mL. A negligible amount of rHuIFN-α adsorbed nonspecifically on the plain beads (0.78 mg/g). This nonspecific adsorption value was obtained at 25°C. Dye-immobilization significantly increased the rHuIFN-α coupling capacity of the beads (up to 205 mg/g). This increase in the rHuIFN-α coupling capacity may have resulted from cooperative effect of different interaction mechanisms such as hydrophobic, electrostatic, and hydrogen bonding caused by the acidic groups and aromatic structures on the Cibacron Blue F3GA and by the side chains of amino acids on the rHuIFN-α molecules. It should be mentioned that Cibacron Blue F3GA is not very hydrophobic overall, but it has planar aromatic surfaces that prefer to interact with hydrophobic groups in rHuIFN-α structure.

TABLE I
Some Properties of the Monosize Poly(GMA) Beads

Particle diameter	1.6 ± 0.01 μm
Polydispersity index	1.006
Theoretical epoxy group content	4.2 mmol/g
Experimental epoxy group content	3.8 mmol/g
Swelling ratio	45%
Wet density	1.09 g/mL
Dye content	1.73 mmol/g

Adsorption isotherm

Langmuir adsorption isotherm is expressed by eq (1). The corresponding transformations of the equilibrium data for rHuIFN-α gave rise to a linear plot, indicating that Langmuir model could be applied in these systems and described by the equation:

$$q^* = q_{\max}\, b\, C_{eq}/(1 + b\, C_{eq}) \quad (1)$$

where q is the amount of adsorbed rHuIFN-α in the adsorbent, C_{eq} is the equilibrium rHuIFN-α concentration in solution, b is a coefficient related to the affinity between the sorbent and adsorbate, and $q_{\max}$ is

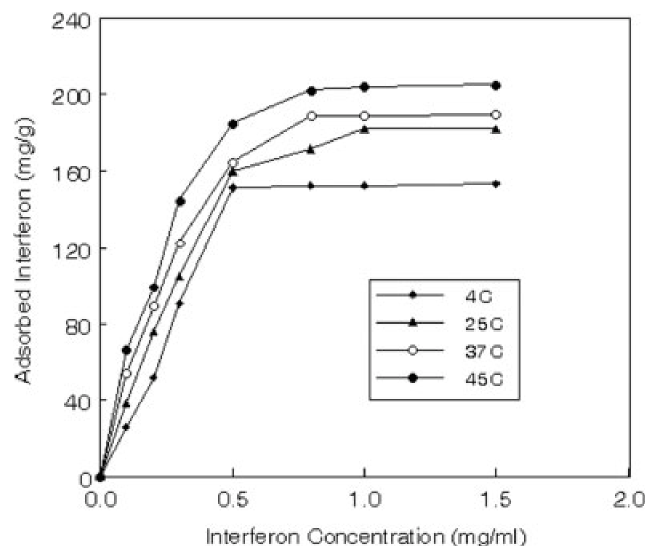

Figure 2 Adsorption isotherms of rHuIFN-α on poly (GMA) beads. Dye loading: 1.73 mmol/g; pH: 6.0.

TABLE II
Equilibrium Association Constants and Free Energies

T (K)	q_{max} (mg/g)	K_a	$1/T(10^3)$ (K^{-1})	ln K_a	ΔG^0 (kJ/mol)
277	188.6	4.8	3.61	1.572	−77.9
298	204.0	8.2	3.35	2.106	−80.9
310	200.0	16.7	3.22	2.812	−82.7
318	208.3	48.0	3.14	3.871	−83.8

the maximum adsorption capacity. The maximum adsorption capacity (q_{max}) data for the adsorption of rHuIFN-α was obtained from the experimental data. The correlation coefficients (R^2) were close to 1.0.

Thermodynamic parameters such as free energy (ΔG^0), enthalpy (ΔH^0), and entropy (ΔS^0) changes for the process can be estimated using the following equations:

$$\Delta G^0 = -RT \ln K_a \quad (2)$$

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \quad (3)$$

Here, K_a is the equilibrium association constant ($1/K_d$). The plot of ln K_a versus $1/T$ for the adsorption process must be found linear. Table II shows the corresponding thermodynamic parameters such as maximum adsorption capacity (q_{max}), the equilibrium association constant, and free energy (ΔG^0) calculated from the experimental data at 4°C, 25°C, 35°C, and 45°C, respectively.

The equilibrium adsorption of rHuIFN-α onto the Cibacron Blue F3GA carrying beads significantly increased with increasing temperature. A possible explanation for this behavior is as follows: Chemical interaction between the dye and the rHuIFN-α molecules increased with increasing temperature. The negative change in free energy ($\Delta G^0 < 0$) indicated that the adsorption of rHuIFN-α on the dye-affinity beads was a thermodynamically favorable process. The ΔS value for the adsorption of rHuIFN-α to dye-attached monosize beads was calculated as 146.1 J/mol K. Positive value for the ΔS indicates an increase in the total disorder of the system during adsorption. The calculated ΔH value of the system for the interaction of rHuIFN-α with dye was −37.39 kJ/mol.

Effect of pH

Figure 3 shows the effects of pH. In all the cases investigated, the maximum adsorption of rHuIFN-α was observed at pH 6.0. It should be noted that the isoelectric pH of rHuIFN-α is 5.9. Significantly lower adsorption capacities were obtained in more acidic and in more alkaline pH regions. The decrease in the rHuIFN-α adsorption capacity in more acidic and more alkaline pH regions can be attributed to electrostatic repulsion effects between the opposite charged groups. It has been shown that proteins have no net charge at their isoelectric points, and therefore the maximum adsorption from aqueous solutions is usually observed at their isoelectric points.

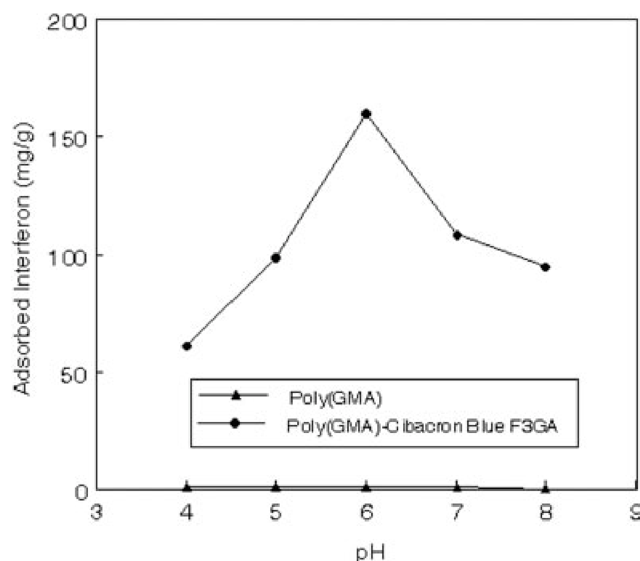

Figure 3 The variation of rHuIFN-α adsorption capacities of the poly(GMA) beads as a function of pH; Dye loading: 1.73 mmol/g; rHuIFN-α concentration: 1.0 mg/mL; T: 25°C.

Effect of ionic strength

The effect of salt concentration on rHuIFN-α adsorption is shown in Figure 4. With an increase of the aqueous phase concentration of sodium chloride, the

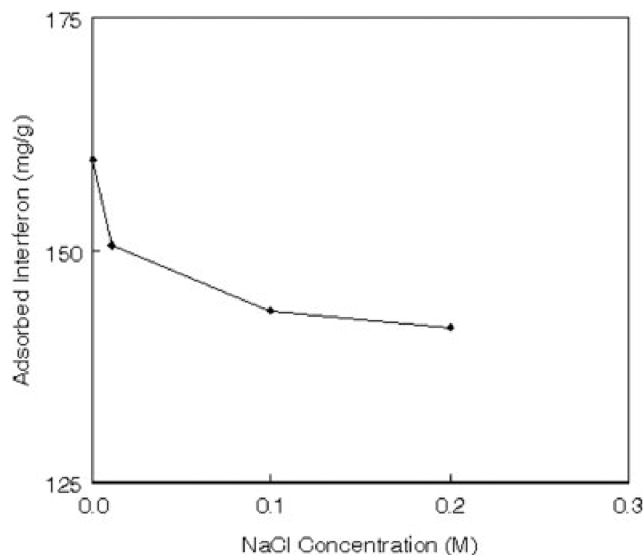

Figure 4 Effect of the ionic strength on rHuIFN-α adsorption; Dye loading: 1.73 mmol/g; rHuIFN-α concentration: 1.0 mg/mL; pH: 6.0, and T: 25°C.

TABLE III
Elution of rHuIFN-α

Concentration (mg/mL)	rHuIFN-α adsorbed (mg/g)	rHuIFN-α desorbed (%)
0.1	25.6	94.1
0.2	52.5	93.6
0.3	90.2	92.9
0.5	151.6	94.8
0.8	152.2	97.2
1.0	152.4	96.3
1.5	153.0	96.0

adsorption capacity decreased drastically. Increasing the ionic strength could promote the adsorption of the dye molecules to the poly(GMA) surface by hydrophobic interaction.[27,28] Moreover, the hydrophobic interactions between the attached dye molecules themselves would also become strong, because it has been observed that the salt addition to a dye solution caused the stacking of the free dye molecules.[29] Thus, the numbers of the attached dye molecules accessible to rHuIFN-α would decrease as the ionic strength increased, and the adsorption of the rHuIFN-α to attached dye became difficult. In addition, the ionic interactions decrease with increasing ionic strength due to the Debye screening effect.[30] The adsorption capacity decreased from 159.7 to 141.6 mg/g polymer with the increase of the NaCl concentration from 0 to 0.2*M*. The adsorption amount of rHuIFN-α decreased by about 11.4%.

Elution

Regeneration of dye-affinity beads is important for their application in affinity separation. The adsorbed rHuIFN-α was desorbed from the beads by the addition of NaCl. Dye-affinity beads carrying different amounts of rHuIFN-α were placed in an elution medium containing 1.0*M* NaCl and the amount of rHuIFN-α released in 60 min was determined. Table III gives the elution data. More than 92% of the adsorbed rHuIFN-α was desorbed in all cases when NaCl was used for elution. The desorbed rHuIFN-α has a specific activity of 3.5×10^8 IU/mg as inhibition of the cytopathic effect of MDBK cells. This result showed that the specific activity of HuIFN-α remained almost constant during the adsorption–elution steps. Note that there was no dye release in this case which shows that Cibacron Blue F3GA molecules are attached to the beads surface by strong chemical bond. With the elution data given above we concluded that NaCl is a suitable elution agent especially for the Cibacron Blue F3GA carrying beads, and allows repeated use of the dye affinity adsorbents developed in this study.

To investigate the reusability of the Cibacron Blue F3GA-attached beads, the adsorption–elution cycles of rHuIFN-α were repeated 10 times using the same poly(GMA) beads. Adsorption capacities for beads did not change noticeably during the 10 repeated adsorption–elution operations. This is an important feature indicating the possibility of recycling dye-affinity beads for reuse in the protein separation process.

To evaluate the effects of adsorption conditions on rHuIFN-α structure, fluorescence spectrophotometry was employed. The fluorescence spectrum of rHuIFN-α samples obtained from the elution step was recorded. The fluorescence spectra of native and heat-denaturated rHuIFN-α were also taken. A clear difference was observed between the fluorescence spectra of native rHuIFN-α and heat-denaturated rHuIFN-α. An appreciable shift was seen in the maximum wavelength of denaturated rHuIFN-α according to the native one. On the other hand, the fluorescence spectrum of the samples withdrawn from the elution step were very close to those of native rHuIFN-α and no significant shift of maximum wavelength was detected in the spectra of these samples relative to that of native rHuIFN-α. It may be concluded that dye-affinity beads can be applied for rHuIFN-α separation without causing any conformational changes and denaturation.

CONCLUSIONS

A wide variety of functional molecules, including enzymes, coenzymes, cofactors, antibodies, amino acid derivatives, oligopeptides, proteins, nucleic acids, and oligonucleotides may be used as ligands in the design of novel adsorbents.[25,31–36] These ligands are extremely specific in most cases. However, they are expensive, because of high cost of production and/or extensive purification steps. In the process of the preparation of specific adsorbents, it is difficult to immobilize certain ligands on the supporting matrix with retention of their original biological activity.[37] Precautions are also required in their use (at adsorption and elution steps) and storage. Dye-ligands have been considered as one of the important alternatives to natural counterparts for specific affinity chromatography to circumvent many of their drawbacks, mentioned above.[38] Dye-ligands are able to bind most types of proteins, especially enzymes, in some cases in a remarkably specific manner. They are commercially available, inexpensive, and can easily be immobilized, especially on matrices bearing hydroxyl groups.[39] Although dyes are all synthetic in nature, they are still classified as affinity ligands because they interact with the active sites of many proteins by mimicking the structure of the substrates, cofactors, or binding agents for those proteins.[40] The triazine dye Cibacron Blue F3GA immobilized to monosize

poly(GMA) beads provided an efficient method to separate rHuIFN-α, showing high binding capacity, assuring a recovery of about 92%. To examine the effects of adsorption conditions on conformational changes of rHuIFN-α molecules, fluorescence spectrophotometry was employed. It appears that the dye-affinity chromatography with modified monosize magnetic beads can be applied for the adsorption of rHuIFN-α without causing any denaturation. Repeated adsorption–desorption processes showed that these dye-attached monosize magnetic beads are suitable for rHuIFN-α adsorption.

References

1. Sen, G. C.; Lengyel, P. J Biol Chem 1992, 267, 5017.
2. Nyman, T. A.; Tölö, H.; Parkkinen, J.; Kalkkinen, N. Biochem J 1998, 329, 295.
3. Pestka, S.; Langer, J. A.; Zoon, K. C.; Samuel, C. E. Annu Rev Biochem 1987, 56, 727.
4. Scapol, L.; Rappuoli, P.; Viscomi, G. C. J Chromatogr 1992, 600, 235.
5. Novick, D.; Eshar, Z.; Gigi, O.; Marks, Z.; Revel, M.; Rubinstein, M. M. J Gen Virol 1983, 64, 905.
6. Kagawa, Y.; Takasaki, S.; Utsumi, J.; Hosoi, K.; Shimizu, H.; Kochibe, N.; Kobata, A. J Biol Chem 1988, 263, 17508.
7. Anfinsen, C. B.; Bose, S.; Corley, L.; Gurari-Rotman, D. Proc Natl Acad Sci USA 1974, 71, 3139.
8. Seaminathan, S.; Khanna, N. Protein Expr Purif 1999, 15, 236.
9. Karakoc, V.; Yavuz, H.; Denizli, A. Colloids Surf A. 2004, 240, 93.
10. Ma, Z. Y.; Guan, Y. P.; Liu, X. Q.; Liu, H. Z. Langmuir 2005, 21, 6987.
11. Denizli, A.; Yavuz, H.; Arica, Y.; Garipcan, B. J Appl Polym Sci 2000, 76, 115.
12. Takafuji, M.; Ide, S.; Ihara, H.; Xu, Z. Chem Mater 2004, 16, 1977.
13. Ma, Z. Y.; Guan, Y. P.; Liu, H. Z. Polym Int 2005, 54, 1502.
14. Altıntas, E. B.; Denizli, A. Int J Biol Macromol 2006, 38, 99.
15. Piskin, E.; Tuncel, A.; Denizli, A.; Ayhan, H. J Biomater Sci Polym Ed 1994, 5, 451.
16. Gong, B.; Zhu, J.; Li, L.; Qiang, K.; Ren, L. Talanta 2006, 68, 666.
17. Liu, X.; Guan, Y.; Shen, R.; Liu, H. J Chromatogr B 2005, 822, 91.
18. Yalcin, G.; Elmas, B.; Tuncel, M.; Tuncel, A. J Appl Polym Sci 2006, 101, 818.
19. Tuncel, A.; Denizli, A.; Purvis, D.; Lowe, C. R.; Pişkin, E. J Chromatogr 1993, 634, 161.
20. Ma, Z. Y.; Guan, Y. P.; Liu, X. Q.; Liu, H. Z. Langmuir 2005, 21, 6987.
21. Lee, G. Y.; Chen, C. H.; Wang, T. H.; Lee, W. C. Anal Biochem 2003, 312, 235.
22. Ma, Z. Y.; Guan, Y. P.; Liu, X. Q.; Liu, H. Z. J Appl Polym Sci 2005, 96, 2174.
23. Mondal, K.; Gupta, M. N. Biomol Eng 2006, 23, 59.
24. Zhang, S.; Sun, Y. Ind Eng Chem Res 2003, 42, 1235.
25. Denizli, A.; Pişkin, E. J Biochem Biophys Methods 2001, 49, 391.
26. Altıntas, E. B.; Denizli, A. J Chromatogr B 2006, 832, 216.
27. Liu, Y. C.; Stellwagen, E. J Biol Chem 1987, 262, 583.
28. Zhang, S.; Sun, Y. Biotechnol Prog 2004, 20, 207.
29. Subramanian, S. Arch Biochem Biophys 1982, 216, 116.
30. Yu, Y.; Sun, Y. J Chromatogr A 1999, 855, 129.
31. Wu, C. Y.; Suen, S. Y.; Chen, S. C.; Tzeng, J. H. J Chromatogr A 2003, 996, 53.
32. Birch, R. M.; O'Byrne, C.; Booth, R. I.; Cash, P. Proteomics 2003, 3, 764.
33. Yavuz, H.; Denizli, A. Macromol Biosci 2004, 4, 84.
34. Wang, J.; Peng, X.; Liu, D.; Bao, Y.; An, L. Sep Purif Technol 2006, 50, 141.
35. Yavuz, H.; Denizli, A. Macromol Biosci 2005, 5, 39.
36. Uzun, L.; Odabaşı, M.; Arica, M.; Denizli, A. Sep Sci Technol 2004, 39, 2401.
37. Uzun, L.; Yavuz, H.; Say, R.; Ersöz, A.; Denizli, A. Ind Eng Chem Res 2004, 43, 6507.
38. Odabaşı, M.; Denizli, A. J Appl Polym Sci 2004, 93, 719.
39. Ma, Z. Y.; Guan, Y. P.; Liu, H. Z. React Funct Polym 2006, 66, 618.
40. Denizli, A.; Köktürk, G.; Yavuz, H.; Pişkin, E. J Appl Polym Sci 1999, 74, 2803.