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Polysaccharide hydrogels for protein drug delivery

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Delivery of protein drugs is highly challenging due to the low permeability, short circulatory half-life, rapid proteolysis, low stability, and immunogenicity of the protein drugs. Studies using polysaccharide hydrogels to overcome these problems are reviewed. The different approaches are divided into four classes: (1) polysaccharide microspheres; (2) polysaccharide-conjugated protein drugs; (3) polysaccharide matrix in protein drug delivery; and (4) microencapsulation of protein drugs. Polysaccharide hydrogels will be useful in the development of controlled release formulations for protein drugs.

INTRODUCTION

Recent advances in recombinant DNA technology and other methods have made it possible to produce large quantities of a wide variety of biologically active peptides and proteins (Takakura *et al.*, 1989). The clinical application of those protein drugs to treat chronic diseases, however, still faces many obstacles because of their unique properties, such as large molecular size, short circulatory half-life, and low stability. Although oral administration is the most convenient route of drug delivery, protein drugs cannot be delivered orally. Protein drugs can be easily denatured in the stomach and degraded by enzymes present in the gastrointestinal tract (GI). Furthermore, protein drugs cannot easily be absorbed from the GI tract or through the skin because of their large molecular size. Thus, parenteral administration remains as the only viable way to achieve a systemic therapeutic effect of protein drugs (Tomlinson & Livingstone, 1989).

Due to their high potency, the protein drugs usually act as part of a strictly timed cascade of events, and hence both the timing and method of delivery are critical for their correct use. For this reason, it is desirable to develop advanced ways to deliver protein drugs, if they are to be useful in treating chronic diseases. Research on the advanced delivery systems for protein drugs has been focused on the following issues:

(1) Development of non-parenteral routes for the administration of protein drugs with high bioavailability.
(2) Extension of circulatory half-life and masking immunogenicity of protein drugs by making conjugates with other macromolecules.
(3) Development of controlled release delivery systems.
(4) Enhancement of the stability of protein drugs.

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Hydrogel systems have been used widely in the development of delivery systems for protein drugs, to develop delivery systems for non-parenteral administration and other controlled release protein delivery systems. Hydrogels of natural polymers, especially polysaccharides have been used widely, as have those of synthetic polymers (Pitt, 1990), because of their unique advantages. Polysaccharides are, in general, non-toxic, biocompatible, biodegradable, and abundant. The biodegradable property is especially useful for the release of drugs at a certain time and/or at a certain site in the body. Many polysaccharides possess chemical active functional groups which can be used for further manipulation in making controlled release formulations. This paper reviews polysaccharide hydrogels which have been used to develop delivery systems for protein drugs.

POLYSACCHARIDE MICROSPHERES

Systemically acting peptides and proteins are normally administered by parenteral injection (Edman & Björk, 1992). There are several drawbacks to this route of administration. Firstly, injection must be given by trained staff; secondly, there is a risk of infection; and thirdly, patients may express a phobia for needles and syringes (Björk & Edman, 1990). Extensive research has been devoted to finding alternative routes, including oral, nasal, pulmonal, buccal, rectal, vaginal, and transdermal routes. Among them, the nasal route has attracted the most attention. The nose is well suited to the absorption of drugs since it has a large epithelial surface area due to the presence of numerous microvilli. Furthermore, the subepithelial layer is highly vascularized and the venous blood from the nose passes directly into the systemic circulation bypassing the liver (Illum *et al.*, 1987).

The main problems with nasal protein drug delivery are the low bioavailability caused by the short residence time and low permeability of high molecular weight protein drugs. The permeability, and thus the bioavailability, of protein drugs has been increased by using absorption enhancers, such as surfactant, bile acid, and sodium taurodihydrofusidate (STDHF) (Daugherty *et al.*, 1988; Deurloo *et al.*, 1989; Hirai *et al.*, 1981; Longenecker *et al.*, 1987). Although these absorption enhancers improve the bioavailability, their effects are often associated with adverse reactions in the mucosa. Polysaccharides have also been used to enhance the absorption of protein drugs as listed in Table 1.

Starch is one of the most frequently used polysaccharides in the delivery of protein drugs. Of the many protein drugs, insulin has been used widely as a model, probably because the delivery of insulin is most challenging. Insulin is required only when blood glucose level is increased and should not be delivered when the glucose level is lowered. In addition, insulin delivery requires long-term delivery which requires a simpler means of delivery.

Illum *et al.* (1987) first used degradable starch microspheres (DSM) in nasal drug delivery. The microspheres formed a muco-adhesive gel which was cleared slowly from the nasal cavity. The half-life of clearance of the starch microspheres was prolonged to 240 min as compared to 15 min for the liquid and powder control formulations. Björk and Edman (1988) used this system to study the nasal absorption of insulin in rats (Table 2). Insulin and starch microspheres, with diameters of 45 μm, decreased the blood glucose level within 30–40 min by 40% and 64% with doses of 0.75 IU/kg and 1.70 IU/kg, respectively. The peak insulin level was reached 8 min after dosing. The absolute bioavailability was 30%, whereas the insulin solution had negligible

Table 1. Delivery of protein drugs by nasal administration using polysaccharides

Carrier	Protein	Comments	Ref.
Starch	Insulin	Mucosa adhesive Absolute bioavailability was 30% in rats Time to peak insulin concentration was 8 min	(Björk & Edman, 1988)
Starch + LPC	Insulin	Starch only gave relative bioavailability of 10.7% in sheep Starch plus LPC had relative bioavailability of 31.5%	(Farraj *et al.*, 1990)
Starch	Insulin	The carrier must be able to absorb water and be water-insoluble to promote absorption	(Björk & Edman, 1990)
(a) Epichlorohydrin cross-linked starch (b) Sephadex	Insulin	(a) was more effective than (b) (a) caused no morphology alteration on the mucosa	(Edman and Björk, 1992)
(a) Hyaluronate–sodium (b) Ethyl(hydroxyethyl)cellulose (c) Sephadex (d) DEAE–Sephadex	Insulin	(a), (b), and (c) effectively increased absorption, (d) had no effect	(Rydén & Edman, 1992)
Hyaluronic acid ester	Insulin	Relative bioavailability was 11%	(Illum *et al.*, 1994)
Hyaluronate–sodium	Vasopressin	MW must be larger than 3×10^5 to result in enhancement Bioavailability increased by 2-fold Viscous and bioadhesive solution	(Morimoto *et al.*, 1991)
Starch + LPC	Human growth hormone	Starch only gave relative bioavailability of 2.7% Starch plus LPC had relative bioavailability of 14.4%	(Illum *et al.*, 1990)

LPC, lysophosphatidylcholine.

Table 2. Parameters for two doses of degradable starch microsphere–insulin given intranasally

Dose (IU/kg)	Serum insulin				Blood glucose	
	AUC (μU h/ml)	Peak level (μU/ml)	Peak time (min)	BA (%)	Maximal decrease (%)	Peak time (min)
0.75	66 ± 23	146 ± 64	8.0 ± 1.5	30	40 ± 13	36 ± 12
1.70	165 ± 97	167 ± 46	7.3 ± 2.6	33	64 ± 8	38 ± 8

BA, bioavailability; AUC, area under concentration–time curve (from Björk & Edman, 1988).

effects on the blood glucose level. When sheep were used as animal models for the nasal delivery of insulin, the insulin and starch microsphere system gave 10.7% relative bioavailability compared to that of subcutaneous administration (Farraj *et al.*, 1990). When lysophosphatidylcholine (LPC), a biological absorption enhancer, was added, the relative bioavailability increased to 31.5%. Again, the insulin solution gave negligible relative availability, 1.8%. A comparison study (Björk & Edman, 1990) between the starch microspheres and the starch powder showed that the insoluble starch (molecular weight (MW) 25000) and the microspheres reduced the plasma glucose level to the same extent. On the other hand, the water-soluble starch powder (MW = 11000) did not show any effect. They concluded that the carrier must be water-insoluble for the absorption-promoting effect.

Other polysaccharide systems have been evaluated for their ability to deliver insulin by Rydén and Edman (1992). Dextran crosslinked with epichlorohydrin (Sephadex) (Fig. 1) was shown to have a similar enhancing effect on insulin absorption to the starch microspheres, although the starch microspheres were slightly more effective (Edman *et al.*, 1992). DEAE–Sephadex microspheres produced from DEAE–dextran had little absorption enhancing effect. This was suggested to be due to the high ionic interaction between the negatively charged insulin molecule and the positively charged microspheres, resulting in a low release of insulin from the microspheres in the low electrolyte concentration present in the nasal cavity (Rydén & Edman, 1992). Thermogelling ethyl(hydroxyethyl)cellulose solution and viscous sodium hyaluronate solution (Fig. 2) also caused a significant decrease in plasma glucose level when used as vehicle for insulin. Hyaluronic acid ester microspheres were also used in promoting nasal absorption of insulin in sheep (Illum *et al.*, 1994). The mean relative bioavailability of this system was 11% as compared with the bioavailability by the subcutaneous route. The toxicity of the starch microspheres with insulin has been evaluated in rabbits (Edman *et al.*, 1992). After 8 week administration at doses of 10 and 20 mg, twice a day, no nasal mucosa alteration was shown by scanning electron microscopy.

In addition to insulin, vasopressin and human growth hormone have also been delivered by nasal administration using polysaccharides. Viscous sodium hyaluronate solution (Fig. 2) resulted in enhanced nasal absorption of vasopressin (Morimoto *et al.*, 1991). Sodium hyaluronate tends to form gels which are muco-adhesive and this may be the reason for enhanced absorption. Illum *et al.* (1990) studied the nasal absorption of biosynthetic human growth hormone (hGH) in sheep using the same system. As compared to the subcutaneous injection, nasal hGH solution had a relative bioavailability of only 0.1%. The hGH in DSM

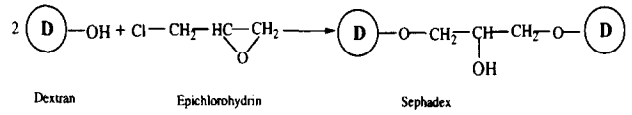


Fig. 1. Crosslinking of dextran (D) with epichlorohydrin.

increased the relative bioavailability up to 2.7%. When LPC was combined with DSM, the relative bioavailability increased up to 14.4%. These studies have shown that the combination of DSM and LPC is a potent nasal delivery system for protein drugs.

The starch microsphere and other polysaccharide delivery systems are effective in enhancing the absorption of protein drugs. There are two possible explanations for this. Firstly, the microspheres used in the above studies are muco-adhesive and this property is expected to decrease the rate of mucociliary clearance from the nasal cavity. The longer contact time between the drug and the absorbing membrane surface should result in enhanced bioavailability. Secondly, the polysaccharide delivery systems may cause a transient widening of the tight junctions between the cells of the nasal epithelia. This is due to the swelling of the dry microspheres and viscous polysaccharides which results in dehydration of the mucosa with a consequent reversible widening of the tight junction between the cells (Illum *et al.*, 1994). While much more information needs to be obtained for the polysaccharide delivery systems, the study to date has clearly shown that the polysaccharide hydrogels are effective in the delivery of protein drugs by nasal administration.

POLYSACCHARIDE-CONJUGATED PROTEIN DRUGS

As mentioned above, the practical use of protein drugs is limited by the short half-life in blood resulting from chemical or biological instability, rapid excretion and immunogenicity. One of the most promising approaches to overcome these problems is chemical modification of protein drugs (Takakura *et al.*, 1989). Naturally occurring glycoproteins are known to be more stable than their carbohydrate-free counterparts (Pazur *et al.*, 1970). Thus, chemical modification of proteins by carbohydrates has been frequently investigated (Armstrong *et al.*, 1972; Azori, 1987; Barker *et al.*, 1972; Benbough *et al.*, 1979; Ferruti & Tanzi, 1985; Foster & Wileman, 1979, Marshall, 1978; Marshall & Humphreys, 1979; Marshall *et al.*, 1977; Poznansky & Juliano, 1984; Sezaki & Hashida, 1984). The conjugated enzymes have been shown to reduce immunogenicity and enhance resistance to proteolysis as well as heat denaturation. Dextrans, due to their excellent physicochemical properties and physiological acceptance, have been most often used for conjugation. Some examples are given in Table 3.

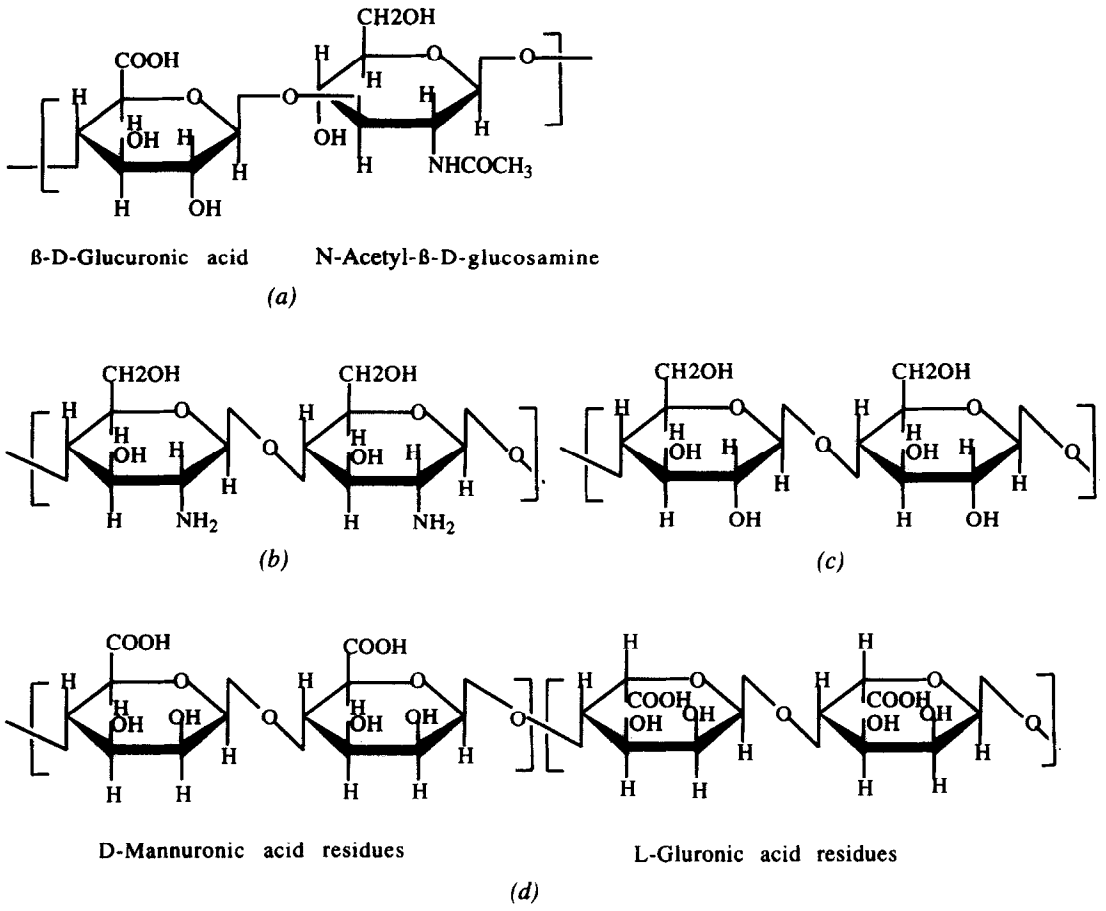


Fig. 2. Structures of (a) hyaluronic acid; (b) chitosan; (c) cellulose; and (d) alginate.

When dextran was bonded covalently to asparaginase, the resulting conjugates showed an increased circulatory half-life and reduced antigen reactivity (Wileman, 1991). It is thought that dextran forms a steric barrier around the enzyme which not only protects the enzyme from degradation but also masks the antigenic determinants from interaction with the immune system. In most cases, the conjugates retained 50–60% of their activity. Soybean trypsin inhibitor was covalently bonded to dextran (MW = 9900) by periodate, cyanogen bromide, carbodiimide, cyanuric chloride, epichlorohydrin, and *N*-succiniimidyl-3-(2-pyridyldithio)pro-pionate (Takakura *et al.*, 1989). The periodate oxidation method (Fig. 3) seems to work well in terms of the modification ratio, yield and activity recovered. More than 60% of the original activity was retained after modification by the periodate oxidation method.

Melton *et al.* (1987) studied the molecular weight effect on the persistence time and activity of the conjugates. Carboxypeptidase G_2, when bonded to dextran with a molecular weight of 4×10^4, showed a 5-fold increase in half-life and a 3.3-fold increase in K_m. On the other hand, bonding to dextran with molecular weight of 1.5×10^5 resulted in a 15-fold increase in half-life and little decrease in activity. In both cases, the resistance to proteolysis was remarkably increased.

POLYSACCHARIDE MATRIX IN PROTEIN DRUG DELIVERY

The extreme susceptibility of proteins to proteolysis and rapid clearance from the bloodstream requires the use of controlled delivery systems. The hydrophilic polymer matrix, due to its high compatibility, is generally preferred. Many studies were focused on the synthetic polymers, such as, polyacrylamide, polyvinylpyrrolidone, poly(hydroxyethyl methacrylate), poly(hydroxyethyl acrylate), poly(methacrylic acid), poly(methoxyethyl methacrylate), poly(methoxy-ethoxyethyl methacrylate), and poly(ethylene glycol) (Albin *et al.*, 1985; Davis, 1972; Heller *et al.*, 1983; Sato *et al.*, 1984; Sefton & Nishimura, 1980; Torchinin *et al.*, 1977). Recently, polysaccharide hydrogels have also been used widely. Polysaccharide hydrogels have their own advantages. One of the advantages is that polysaccharide systems are susceptible to enzymatic digestion in the body.

Kamath *et al.* (1993) made dextran hydrogels by gamma-irradiating the functionalized dextran. Dextran was reacted with glycidyl acrylate in an aqueous system (Fig. 5). The degree of functionalization could be controlled by the reaction ratio, reaction time, molecular weight of dextran, stirring rate, etc. The

Table 3. Examples of polysaccharide-conjugated protein drugs

Carrier	Protein	Comments	Ref.
Dextran 7×10^4	Insulin	Prolonged duration of activity in dogs	(Molteni & Scrollini (1974)
Dextran 4×10^4	Insulin	36 times less active in rats than the native form	(Kågedal & Akerström, 1971)
Dextran 2×10^6	Insulin	Activity shown after subcutaneous administration in rats	(Armstrong *et al.*, 1972)
Dextran 9.9×10^3	Soybean trypsin inhibitor	63% activity was recovered	(Takakura *et al.*, 1989)
Dextran	Asparaginase	50–60% activity was recovered Prolonged circulatory half-life and masked immunogenicity	(Wileman, 1991)
Dextran $4–15 \times 10^4$	Carboxypeptidase G2	Increasing plasma persistence by 5–15-fold The conjugates were resistant to proteolysis by trypsin and chymotrypsin Little loss of activity	(Melton *et al.*, 1987)
Sephadex	Insulin	Prolonging the action time by 3-fold compared to parent insulin after i.v. injection in dogs	(Torchinin *et al.*, 1977)
Cellulose	Insulin	Maintaining normal glucose level over several days after i.p. injection in diabetized rabbit	(Singh *et al.*, 1981)
Cellulose derivative	Chymotrypsin A	Relative activity towards casein was 26%, towards ester 65%	(Kennedy *et al.*, 1973)

i.v., intravenous; i.p., intraperitoneae.

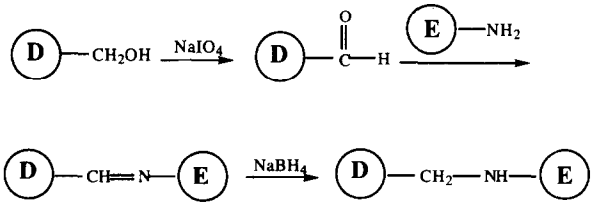


Fig. 3. Covalent bonding of enzyme (E) to dextran (D) by periodate oxidation method.

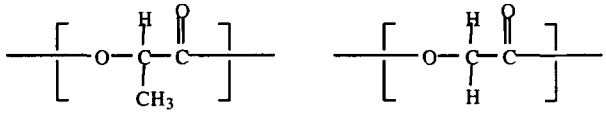
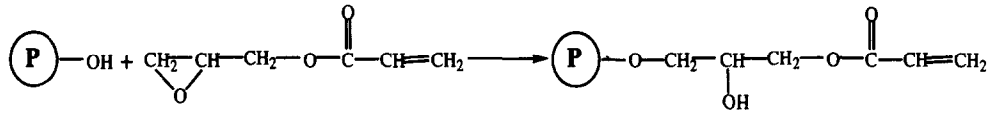


Fig. 4. Structures of two synthetic biodegradable polymers.

functionalized dextran was purified before hydrogel formation, mixed with protein drugs, and exposed to gamma-irradiation to form hydrogels. This method enables hydrogel preparation without using external crosslinking agents which may also react with proteins. It also allows loading of a high amount of protein drugs into the hydrogels, since proteins are mixed before hydrogel formation (Kamath & Park, 1994). Since the protein drugs are exposed to gamma-irradiation during hydrogel formation, the effect of gamma-irradiation on the bioactivity of protein drugs has to be examined. When invertase was used as a model drug, the decrease in bioactivity was only by a few percent after hydrogel formation. Thus, this approach can be used to prepare protein-containing hydrogel systems without losing much of the bioactivity of the incorporated protein drugs. The same approach has been used to prepare hydrogels using gelatin, albumin, and starch.

Hydroxypropylcellulose matrix has been used to deliver insulin. Ishida prepared an oral mucosal dosage form of insulin (Ishida *et al.*, 1981). The core base contained cacao butter, insulin and additive. The peripheral base contained a mixture of hydroxypropyl cellulose and carbopol-934. This dosage form could stick tightly to the oral mucosa of beagle dogs for 6 h. However, even with the promotion of sodium glycocholate, only 0.5% insulin was absorbed when compared with intramuscular injection. Lehr *et al.* (1992) found that the bioadhesive property of chitosan



Glycidyl acrylate

Fig. 5. Modification of polysaccharides (P) with glycidyl acrylate.

(Fig. 2) was almost as good as poly(acrylic acid). This property was used to improve the intestinal absorption of 9-desglycinamide 8-arginine vasopressin in rats (Rental *et al.*, 1993). A starch gel system containing prostaglandin E_2 for local administration was developed by Harris (Harris *et al.*, 1980). The matrix of a cross-linked starch polymer provided a stabilizing milieu for the labile prostaglandin E_2. The gel offered advantages over existing preparations in terms of chemical and microbiological stability, homogenicity, and dosage safety. Papini *et al.* (1993) characterized the diffusion properties of biodegradable hyaluronate ester membrane. Peptides and proteins ranging from 294 to 66 000 Da were tested. They found that the logarithm of the apparent diffusion coefficient (log D_{app}) was linearly related to the logarithm of solute molecular weight and to the square of the solute radius. These membranes may be useful in the controlled release of protein drugs.

MICROENCAPSULATION OF PROTEIN DRUGS

The microencapsulation technique has been used widely in protein drug delivery. Microspheres provide longer circulatory half-lives and better stability. They are also used in drug targeting, protein immobilization and oral vaccination. Both non-degradable and biodegradable synthetic polymers have been used widely in microencapsulation for the delivery of proteins (Chang & Chang, 1974). The most widely used biodegradable synthetic polymers are polylactic acid, polyglycolic acid and their copolymers (Fig. 4) (Chang, 1976; Hora *et al.*, 1989; Kenley *et al.*, 1987; Ogawa *et al.*, 1988; Ruiz *et al.*, 1989; Sanders *et al.*, 1984, 1985, 1986). Polysaccharides have also been used widely in the preparation of microcapsules for the delivery of proteins and cells. Some examples of the polysaccharides used to make microspheres for protein drug delivery are listed in Table 4.

Hyaluronane derivatives of ethyl alcohol and benzyl alcohol are known to be biocompatible, biodegradable, and non-immunogenic. Nerve growth factor (NGF) was physically incorporated into the hyaluronane derivative microspheres (Ghezzo *et al.*, 1992). The hyaluronane hydrogel was able to protect, carry, and release NGF in its pharmacologically active form. Due to the protein/matrix interaction, however, only 4–11% of the total NGF loaded within the microsphere was recovered.

Edman *et al.* (Artursson *et al.*, 1984; Edman *et al.*, 1980) prepared polyacryldextran and polyacrylstarch microspheres by chemical derivatization of dextran and starch with glycidyl acrylate (Fig. 5). The functionalized polysaccharides were further copolymerized with bisacrylamide. In the case of polyacryldextran, up to 40% (w/w) proteins, such as human serum albumin, immunoglobin G, and bovine carbonic anhydrase, were incorporated in the microspheres. Without significant loss of bioactivity, the thermostability and hydrolytic stability were considerably increased. Approximately 5–10% enzyme activity was retained even after 30 min at 100°C. Similar results were obtained with polyacrylstarch. The microspheres were targeted to the lysosome. All the incorporated lysozyme was released in 9–12 weeks.

Igari *et al.* (1990) prepared a system which combined the advantages of microspheres and liposomes to deliver protein drugs. Liposomes containing vitamin B_{12} and poly-L-lysine were coated with phospholipase A_2 and then embedded in alginate. The presence of phospholipase A_2 resulted in a pulsatile release of the

Table 4. Examples of polysaccharide microspheres

Carrier	Protein	Comments	Ref.
Hyaluronate ester	NGF	NGF was protected, carried, and released in active form	(Ghezzo *et al.*, 1992)
Polyacryldextran	Carbonic anhydrase etc.	Better temperature and pH stability Little loss in activity	(Edman *et al.*, 1980)
Polyacrylstarch	Carbonic anhydrase, lysozyme, etc.	Targeted to lysosome Loaded up to 40% (W/W) protein Better stability with little activity loss	(Artursson *et al.*, 1984)
Ca-alginate	Poly(*L*-lysine), liposome, phospholipase A2	Pulsatile release manner Maximized the advantages of alginate microsphere and liposome	(Igari *et al.*, 1990)
Ca-alginate	Rifamycin oxidase	Mechanically too weak	(Banerjee, 1993*a*)
k-Carrageenan	Rifamycin oxidase	Immobilized enzyme was more stable and reusable	(Banerjee, 1993*b*)
Cellulose acetate phthalate (CAP)	Vaccine	Targeted to enteric mucosal immune system Better pH, temperature and enzyme stability	(Lee & Tzan, 1992)
(a) Cellulose triacetate (b) Ethyl cellulose (c) CAP	Vaccine	No or little absorption was observed compared to synthetic polymer systems	(Eldridge *et al.*, 1990)

incorporated drug. At 10°C, the system had no release for 30 days, and it could be activated simply by exposure to 37°C.

Current oral vaccine research is focused on targeting of the antigen to the Peyer's patches in the intestine which lead to the induction of IgA. The practical application of this oral immunization has been hampered by the denaturation and degradation of the vaccine in the GI tract through acid in the stomach as well as proteolytic enzymes of the gut. Lee *et al.* encapsulated *Mycoplasma hyopneumoniae* vaccine with cellulose acetate phthalate (Lee & Tzan, 1992). The microspheres showed much better temperature and pH stability. Resistance to proteolytic enzymes such as trypsin was greatly enhanced. Eldridge *et al.* (1990) made microspheres consisting of ethyl cellulose, cellulose acetate phthalate or cellulose triacetate (Fig. 2). *Staphylococcal Enterotoxin* B vaccine was incorporated but no or little uptake by the Peyer's patch was observed. However, the microspheres consisting of synthetic polymers, such as polylactide and polyhydroxybutyrate, effectively delivered and released the vaccine in the gut-associated lymphoid tissue. Recently, Bowersock *et al.* (1995) used alginate gels (Fig. 2) to deliver vaccines. The vaccine-containing alginate microspheres were taken up by the Peyer's patches and resulted in the production of antibodies.

CONCLUSION

Although advances in biotechnology have stimulated the development of controlled delivery systems for protein drugs, many obstacles to their practical applications still exist. Current synthetic polymers employed in protein drug delivery have several drawbacks in the design of desirable therapeutic systems. Low biodegradability and biocompatibility are two critical problems. Although the rapid progress of material sciences could partially solve these problems via the introduction of biodegradable polymers such as lactide/glycolide copolymers, polyanhydrides and poly(ortho esters), their hydrophobicity, possible toxicity after degradation, heterogeneous degradation, heterogeneous release, and long degradation time are still problematic.

Natural polymers such as proteins and polysaccharides are often considered as attractive substitutes when synthetic polymers meet their deadlock. In particular, polysaccharides have useful properties in the design of drug delivery systems in that they are relatively more stable, more biocompatible and more economical than proteins. Chemical modification can also provide greater flexibility of drug delivery. Hence, it is possible to control the degradation time and hydrophilicity as well as mechanical properties of delivery systems. As summarized in this review, delivery techniques for protein drugs based on polysaccharides are promising. However, much more work needs to be done for successful clinical applications of these systems.

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